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Full Length Research Paper

Wound Healing Potential of Natural Honey in Diabetic and Non-Diabetic Wistar Rats

Eyarefe O.D[1]., Ologunagba F. M[1] and Emikpe B.O[2]

[1]Department of Veterinary Surgery &Reproduction and Pathology, University of Ibadan, Ibadan, Nigeria

ABSTRACT

The cutaneous wound healing effects of natural honey were compared in diabetic and non-diabetic rats. Thirty adult male Wistar rats (159g ± 31.5) where randomized into alloxan diabetics (n=15) and non-diabetic (n=15) groups. A 6mm full thickness biopsy punch wound was created on the nape of each rat under 2% xylazine (5mg/kg) and 5% ketamine (35mg/kg) anaesthesia. The wounds were contaminated with *Staphylococcus aureus* (10^8 Colony Forming Unit (CFU). Each group was then randomised into three subgroups: A [control, n=5], B (n=5) amikacin (0.8mg) and C (n=5) natural honey (0.1 ml) topical treatments. Wounds were evaluated at day: 3, 5, 7, 9, 11, 13, and 15 for wetness, oedema, hyperemia, granulation tissues, and contraction. Wounds of animals in the diabetic group showed significantly less ($p>0.05$) wetness compared with the non-diabetic group at days 3-5 ($p=0.002$) and 5-9, $p=0.002$ while the amikacin subgroup of the non-diabetic group showed non-significant but notable level of wetness at days 5-7. A significantly ($p>0.05$) more wound edge oedema was shown by the diabetic than the non-diabetic group at days 3-5 ($p=0.000$) with the trend being; control > amikacin > honey. The percentage of animals with wound edge oedema between days 3-5 in the non-diabetic group was honey (20%), amikacin (20%) and control (60%); and diabetic group was honey (40%), amikacin (100%) and Control (100%). Between days 5-7, 80% of wounds in both groups where without wound edge oedema except the control subgroups. Wounds of rats in the diabetic group were significantly more hyperaemic ($p>0.05$) at days 5-9 ($p=0.001$) and days 9-15 ($p=0.000$), with the trend being control > amikacin = honey. Wounds of rats in the non-diabetic groups healed with significantly more ($p < 0.05$) granulation tissues at days 5-9 ($p=0.001$) (honey = amikacin > control). Control group at days: 5-9 ($p=0.001$), 9-15 ($p=0.000$); amikacin at days: 5-9 ($p=0.002$), 9-15 ($p=0.005$); and honey at days: 5-9 ($p=0.005$) and 9-15 ($p= 0.001$). Differences in wound diameter (a reflection of wound contraction) were not significantly notable in both subgroups ($p > 0.05$) at various days of measurement except at days 3-5($p=0.008$). Honey and amikacin were effective in enhancing cutaneous wounds healing in wistar rats studied. In diabetic rats, honey showed a promising result when compared to amikacin hence honey could be recommended for wound management of diabetic patient in human and animal hospitals.

Keywords: Honey, amikacin, alloxan, diabetes, wound healing.

INTRODUCTION

Honey is an ancient remedy for the treatment of infected wounds which has recently been reemphasized (Molan, 2004). In laboratory studies, it has been shown to have inhibitory effects against a wide range (70 species) of gram-positive and gram-negative aerobic and anaerobic bacteria including Methicillin Resistant *Staphylococcus aureaus* (MRSA) (Krisztina et al, 2007). It's antifungal actions against some yeast, Aspergillus, and Penicillium species as well as (Carlos

*Corresponding author:
E-mail: odeyarefe@gmail.com

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et al., 1996) its immune-boosting (Abuharfeil et al., 1999), antioxidant (Frankel et al., 1998) and tissue regenerating properties (Eyarefe et al., 2008) have also been emphasized in literatures. The current prevalence of antibiotic-resistant microbial species has encouraged a re-evaluation of the therapeutic uses of honey especially in management of chronic infected wounds including diabetic ulcers (Clayton and Elasy, 2009).

Diabetes is a metabolic disorder characterised chronic hyperglyceamia (ADA. 2011), and affected over 170 million people worldwide by the year 2000 (Clayton and Elasy, 2009). An estimation of 336 million sufferers by the year 2030 has been projected (Wild et al., 2004). A study have shown that diabetic patients have 25% life time risk of developing foot ulcers and possesses impaired rate of wound healing (Galkowskaet al., 2006). The multifactor foot ulcers are associated with hypoglycaemia induced neuropathy and ischaemia (Simmon and Feldman, 2002) with smoking, hypertension and hyper-lipidaemia as risk factors that often result in patients' development of Peripheral Arterial Disease (PAD) (Armstrong and Lavery, 1998). The ulcers often progresses, and lead to foot amputation in 85% of cases (Reiber et al., 1999). It has been postulated however, that amputation could be prevented in most diabetic if adequate attention is paid to wound care through the use of topical antimicrobial dressing agents (Clayton and Elasy, 2009) and parenteral antibiotics (Lipsky and Hoey, 2009).

Although the efficacy of natural honey in management of chronic wounds abounds in literature (Lipsky and Hoey, 2009), its efficacy when topically applied in management of infected wounds of diabetic patients need to be elaborated. This study therefore investigated the effects of honey as topical agents on *Staphylococcus aureaus* infected cutaneous wounds of diabetic and non-diabetic wistar rats.

MATERIALS AND METHODS

Experimental animals

Adult male rats (mean body weight (159g ± 31.5)were bred, housed in well ventilated cages and exposed to 12 hour light:12 hour dark period. They were fed on rodent chow and had access to water *ad libitum.* Animals'vital parameters including temperature, weight, and fasting blood glucose level were assessed and found to be consistent with good health status before the commencement of the study (Eyarefe and Amid, 2010).

Study design

Thirty adult male rats (average body weight 159g ± 31.5), diabetics (n=15) and non-diabetic (n=15) groups, had a 6mm full thickness biopsy punch wound created on the nape of each rats under anaesthesia, and wounds were contaminated with *Staphylococcus aureaus* (10^8Colony Forming Unit (CFU). Each group was then further randomised into three subgroups: honey treatment (0.1ml) (n=5), 0.8 % topical amikacin treatment (0.8mg) (n=5) and control (n=5) (untreated).

Induction of diabetes

Diabetes was induced after overnight fasting of rats (Chinaka et al., 2012) by intra-peritoneal injection (160mg/kg body weight) of a freshly prepared alloxan monohydrate in normal saline. Hyperglycaemia was monitored from 48 hours post-alloxan injection (Ashok et al., 2007) by a drop of whole blood obtained from the tail vein on glucose auto-analyser (ACCU-CHECK Active® Germany). Fifteen rats with consistent blood glucose level above 135mg/dl were considered diabetic (Ashok et al., 2007) and enlisted into the diabetic groups.

Anaesthesia and wound creation

Each rat was anaesthetized with an intramuscular injection of 5% Ketamine (Rottexmedica, Germany) (35.0 mg/kg) and 2% Xylazine (Arendonik, Germany) (5.0 mg/kg) via the quadriceps group of muscles as earlier described (Eyarefe and Amid, 2010). Following anaesthesia, the dorsum [nape] of each rat was prepared for aseptic surgery by shaving and sterilization with chlorhexidine and alcohol. A full-thickness punch biopsy wound was created using a 6mm skin biopsy punch (Kai Industries Co. ltd, Germany) on the dorsum [nape] of each rat.

Each wound was inoculated with 10^8 colony forming unit (CFU) of MRSA *Staphylococcus aureaus* earlier isolated and prepared for the purpose as described by Shaikh et al., 2007. Rats were returned to their cages following recovery from anaesthesia for further monitoring of wounds.

Confirmation of infection

The wounds were left untreated for 48 hours, following which infection (Yan-Tenget al., 2010) was confirmed using catalase and coagulase tests, with Gram stain technique, as earlier described (Shaikh et al., 2007).

Application of Topical Antibacterial agents

Following wound infection, 2 drops (0.1 ml) of 0.8% amikacin was applied to wounds in the amikacin subgroup based on the Minimum Inhibitory Concentration (MIC) for the organism, earlier determined by antibiotics sensitivity test as described by (Moore et al., (2007). Two drops (0.1ml) of natural honey was applied topically to each wound of rat in the honey subgroup of both diabetic and non-diabetic

groups. Wounds of rats in control subgroups were left untreated.

Gross evaluation of wound

The wound of each animal was evaluated every other day (i.e., day 3, 5, 7, 11, 13, 15) for 15 days and scored as described by Yan-Teng et al., 2010 using wetness/dryness, colour, granulation tissues and wound edge oedema as healing parameters. Wound was either wet or dry. Wound edge edema was either present or absent. Wound colour (Hyperemia) was either severe or moderate or absent (0). Granulation tissue was graded as high, low or absent (0). Wound size (mm) was measured by taking the diameter of wound with a digital venial caliper (Globetronics & Co. ltd, Germany).

Data Analysis

Mean of parameter were compared with Analysis of Variance (ANOVA), Duncan's multiple range, and student t test, and probability of $p < 0.05$ was considered significant

RESULTS

Wound wetness (exudation) evaluation

Wounds of animals in the diabetic group showed significantly less ($p>0.05$) wetness compared with the non-diabetic group at days 3-5 ($p=0.002$) and 5-9, $p=0.002$. Animals in the amikacin subgroup of the non-diabetic group also showed non-significant but notable level of wetness at days 5-7.

Wound edge oedema

Wounds of rats in the diabetic group had significantly more ($p>0.05$) wound edge oedema than the non-diabetic group at days 3-5 ($p=0.000$) with the trend being; control > amikacin> honey. The percentage of animals with wound edge oedema between days 3-5 in the non-diabetic group was honey (20%), amikacin (20%) and control (60%); and diabetic group was honey (40%), amikacin (100%) and Control (100%). Between days 5-7, 80% of wounds in both groups where without wound edge oedema except the control subgroups, Table 2.

Table 1:
Evaluation of Wetness/Dryness of Wounds in Diabetic and Non-Diabetic Group at Different Postoperative Days

	NON-DIABETIC GROUP [n=15]						DIABETIC GROUP [N=15]					
	Honey [n=5]		Amikacin [n=5]		Control [n=5]		Honey [n=5]		Amikacin [n=5]		Control [n=5]	
DAY	**N**	**%**	**n**	**%**	**n**	**%**	**N**	**%**	**n**	**%**	**n**	**%**
0	5	100	5	100	5	100	5	100	5	100	5	100
3	5	100	5	100	5	100	1	20	0	0	5	100
5	1	20	2	40	1	20	0	0	0	0	0	0
7	1	20	2	40	1	20	0	0	0	0	0	0
9	1	20	1	20	1	20	0	0	0	0	0	0
11	1	20	0	0	1	20	0	0	0	0	0	0
13	0	0	0	0	1	20	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0

n= Number of animals. %= percentage of animals with wet wounds.

Table 2:
Evaluation of Wound Edge Edema in Diabetic and Non-Diabetic Group at Different Postoperative Days

	NON-DIABETIC GROUP [n=15]						DIABETIC GROUP [N=15]					
	Honey[n=5]		Amikacin[n=5]		Control[n=5]		Honey[n=5]		Amikacin[n=5]		Control[n=5]	
DAY	**N**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	0	0	0	0	0	0	0	0	0	0	0	0
3	5	100	5	100	5	100	5	100	5	100	5	100
5	1	20	1	20	3	60	3	60	5	100	5	100
7	1	20	1	20	3	60	0	0	0	0	1	20
9	1	20	1	20	1	20	0	0	0	0	1	20
11	0	0	0	0	0	0	0	0	0	0	1	20
13	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0

n= Number of animals. %= percentage of animals with oedema of wound edge.

TABLE 3:
Evaluation of Degree of Wound Hyperemia In Diabetic and Non-Diabetic Group at Different Postoperative Days

	NON-DIABETIC GROUP [n=15]												DIABETIC GROUP [n=15]											
	Honey [n=5]				Amikacin [n=5]				Control [n=5]				Honey [n=5]				Amikacin[n=5]				Control [n=5]			
	S. hyp.		M.hyp		S. hyp		M. hyp		S. hyp		M.hyp		S. hyp		M.hyp		S. hyp		M.hyp		S.hyp		M.hyp	
Day	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	5	100	0	0	5	100	0	0	5	100	0	0	4	100	0	0	5	100	0	0	5	100	0	0
3	5	100	0	0	5	100	0	0	3	60	2	40	3	75	1	25	4	100	0	0	5	100	1	0
5	2	40	3	60	1	20	5	100	1	20	4	80	1	25	3	75	4	100	0	0	4	80	1	20
7	1	20	2	40	1	20	4	80	0	0	4	80	1	25	3	75	3	75	1	25	1	20	4	80
9	1	20	4	80	0	0	5	100	0	0	4	80	0	0	4	100	1	25	3	75	1	20	4	80
11	0	0	1	20	0	0	0	0	0	0	1	20	0	0	1	25	0	0	1	25	0	0	5	100
13	0	0	0	0	0	0	0	0	0	0	1	20	0	0	0	0	0	0	0	0	0	0	1	20
15	0	0	0	0	0	0	0	0	0	0	1	20	0	0	0	0	0	0	0	0	0	0	1	20

n= number of animals with moderate hyperaemia (M. hyp) and severe hyperaemia (S. Hyp). %= percentage of animals with S. hyp or M. hyp

Table 4:
Granulation Tissue Evaluations in Diabetic and Non-Diabetic Group at Different Postoperative Days

	NON-DIABETIC GROUP [n=15]													DIABETIC GROUP [n=15]								
	Honey[n=5]				Amikacin [n=5]				Honey[n=5]				Honey [n=5]				Amikacin [n=5]				Control [n=5]			
	H.gra		L.gra		H. gra		L.gra		H. gra		L.gra		H. gra		L.gra		H. gra		L.gra		H. gra		L. gra	
Day	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	1	20	0	0	1	20	0	0	5	100	0	50	2	50	4	100	0	0	4	80	1	20
7	5	100	0	0	5	100	0	0	4	80	1	20	5	25	3	75	0	0	4	100	0	0	5	100
9	1	20	4	80	1	20	4	80	1	20	4	80	1	0	1	25	0	0	1	25	0	0	1	20
11	0	0	1	20	0	0	0	0	0	0	1	20	0	0	1	25	0	0	1	25	0	0	0	0
13	0	0	1	20	0	0	0	0	0	0	2	40	0	0	1	25	0	0	1	25	0	0	0	0
15	0	0	1	20	0	0	0	0	0	0	2	40	0	0	0	0	0	0	0	0	0	0	0	0

n= Number of animals with high granulation (H.gra) and low granulation (L. gra). %= percentage of animals with M.GRA and H. GRA.

TABLE 5:
Mean (±Standard Deviation) Of Wound Diameter (Mm) In Diabetic And Non-Diabetic Group At Different Postoperative Days

	NON-DIABETIC GROUP [n=15]				**DIABETIC GROUP [N=15]**	
Day	**Honey[n=5]**	**Amikacin[n=5]**	**Control[n=5]**	**Honey[n=5]**	**Amikacin[n=5]**	**Control[n=5]**
0	9.66±0.72	9.46±0.57	9.58±0.35	8.97±1.04	8.61±0.82	8.12±0.84
3	7.59±0.76	7.00±1.16	7.29±0.37	6.99±0.32	7.41±0.62	6.07±0.86
5	6.02±0.75	6.09±1.23	6.54±0.61	5.52±0.39	5.42±1.45	3.82±0.66
7	4.94±0.42	5.64±1.95	5.14±1.08	4.19±0.97	4.03±1.47	2.77±0.59
9	4.54±0.95	3.65±1.73	3.41±1.00	2.99±1.10	1.58±1.73	0.59±1.33
11	2.56±1.74	2.19±1.77	2.09±2.13	1.10±2.20	1.07±2.14	0.53±1.19
13	0.86±1.93	0.43±0.96	1.73±2.39	0.55±1.12	0.99±1.99	0.53±1.19
15	0.52±1.17	0.00±0.00	1.33±1.95	0.00±0.00	0.00±0.00	0.00±0.00

Hyperaemia evaluation
Wounds of rats in the diabetic group were significantly more hyperaemic ($p>0.05$) than those in the non-diabetic groups at days 5-9 ($p=0.001$) and days 9-15 ($p=0.000$), with the trend being control > amikacin =honey. The degree of hyperaemia between diabetic and non-diabetic group was not significant ($p > 0.05$) in the various days of observation, Table 3.

Granulation tissue evaluation
Wounds of rats in the non-diabetic groups healed with significantly more($p < 0.05$) granulation tissues than the diabetic groups at days 5-9 ($p=0.001$) (honey = amikacin > control).Control group at days: 5-9 ($p=0.001$), 9-15 ($p=0.000$); amikacin at days: 5-9 ($p=0.002$), 9-15 ($p=0.005$);and honey at days: 5-9 ($p=0.005$) and 9-15 ($p= 0.001$)(Table 4).

Wounds contraction evaluation
Differences in wound diameter (a reflection of wound contraction) were not significantly notable in both diabetic and non-diabetic subgroups ($p > 0.05$) at various days of measurement except at days 3-5($p=0.008$) (Table 5).

DISCUSSION

The study showed that natural multifloral honey and amikacin are effective in management of infected wounds of diabetic and non-diabetic rats. The honey used in this study has been proven to enhance bowel anastomotic wound healing in non-diabetic dogs (Eyarefe et al., 2012) and improved healing of traumatic injuries in a mona monkey (Eyarefe and Oguntoye, 2012).

Wound wetness, edge oedema, colour (hyperaemia), granulation and size were used as wound healing indices in this study (Khoo et al., 2010). Acute wound wetness is a reflection of tissue response to injury and a local sign indicative of wound infection (Willi and Chandra, 2004). The wetness observed in the amikacin subgroup could also be due to toxic high MIC of amikacin against *Staphylococcus aureaus which* could evoke soft tissue responses resulting in observable protracted wound. The wound dryness in the diabetic groups on the other hand, may be associated with hyper-glycaemia induced dehydration associated with frequent urination and in-commensurate fluid intake (Hilton et al., 2004).

A significant level of wound dryness in the honey subgroup of the diabetic group (Table 1) reveals a unique wound healing characteristic of honey. Honey's viscosity encourages pulling of moisture/lymph from wound tissue which results in dryness and contraction (Molan, 2004). This osmotic action also lifts debris from the wound bed, removes malodour and stimulates tissue regeneration (Molan, 2004).
Wounds of rats in the diabetic group had significantly more wound edge oedema between day 3 and 5. There was however lesser wound edge oedema in the honey subgroup of the diabetic group (Table 2, 3). Wound edge oedema and hyperaemia are local signs of wound infection and evidences of debridement challenges (Harold and Marjana, 2007).

In diabetic patients, impedance of cellular responses characteristics of the inflammatory phase of wound healing and macrophage function has been reported (Maruyama et al., 2007). This events result in prolong wound debridement, repair and maturation (Harold and Marjana, 2007) as observed in this study. However, the lesser wound edge oedema of the honey subgroup may be associated with the widely reported antibacterial (Cooper et al., 2002), and anti-inflammatory (Molan and Betts, 2000) properties of honey coupled with it's monocytic cell activities (Tonks et al., 2003) and effective debridement process (Eyarefe and Oguntoye, 2012).

Granulation tissue lay down was slow in all the diabetic groups but very effective in honey and amikacin subgroups (Table 4). It was however significant in the non–diabetic group (Table 4). This

observation could be due to the fact that diabetics is associated with decreased and impaired growth factor production (Galkowska et al., 2006), angiogenic responses, collagen accumulation, epidermal barrier function, and reduced quantity of granulation tissue build up in wounds (Falanga, 2005). However, the honey subgroup showed a better granulation tissue response and this could be due to it's wound nourishing effects that aid in infection clearance and granulation tissue lay-down in wounds (Eyarefe and Oguntoye, 2012).

Wound contraction was remarkable although not statistically significant in the diabetic group compared with non-diabetic group (Table 5). Although diabetics impair keratinocytes and fibroblasts migration and proliferation, and the number of epidermal nerves resulting in poor wound epithelization and contraction, the honey subgroup in the diabetic group showed a remarkable contraction equivalent to amikacin (Table 5).

Comparing honey with the use of aminoglycosides which are known to be effective against staphylococcus infection when administered parenterally (Lipsky et al., 2009), the use of the antibiotics topically was marked with toxic responses which delayed wound healing. Natural honey on the other hand, as observed in this study, it's topical application was non-harmful even in diabetic rats (Fasanmade and Alabi, 2008, Erejuwa et al., 2010, 2012).

Honey being available and cost effectiveness (Eyarefe et al., 2012) confers an obvious economic advantage on the management of wound. Natural multifloral honey could therefore be recommended for management chronic wounds including diabetic foot ulcers especially poor resource settings.

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