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Novel topical combo-therapeutic formulation integrating honey, neomycin and bacitracin for diabetic wound management

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Abstract

This study evaluated the healing effects of honey as a topical therapy for diabetic ulcers singly and in combination with bacitracin and neomycin (Cicatrin®), formulated as ointments in experimental rats.

Antimicrobial evaluation of the test agents against Vancomycin and Oxacillin resistant *Staphylococcus aureus* (VORSA) and *Pseudomonas aeruginosa* was done by the cup plate agar-diffusion technique using the Checkerboard method. Subsequently, the optimized combination was formulated into an ointment and administered as single therapy and in combination to hyperglycemic rats made diabetic by subcutaneous injection of alloxan (130 mg/kg) and inflicted with wounds. Administration was done daily on wounds for 21 days while infected wounds had the pus from them evaluated for presence of VORSA and *Pseudomonas aeruginosa*.

The triple combo-therapeutics formulation had improved anti-bacterial activity, in comparison with the individual formulations, with the ratio (1:9) of Cicatrin®: Honey respectively giving the best activity against VORSA. Also, the triple combo-therapeutics exhibited positive wound contraction and size reduction. Furthermore, clinical signs of infection were absent at the end of the follow-up period in the rats administered the combo-therapeutic agents while other groups of rats administered the bland ointment, and the individual agents were infected with either *Pseudomonas aeruginosa* or VORSA. In addition, the triple combo-therapeutics formulation exhibited good physicochemical stability throughout the treatment duration and beyond (28 days), with insignificant ($p > 0.05$) changes in pH and spreadability.

The triple combination therapeutics formulation showed superior effect to the singly administered agents (honey and Cicatrin®) in the management of diabetic wounds

Keywords: Honey; Cicatrin®; Diabetic wound; Vancomycin-oxacillin resistant *Staphylococcus aureus* (VORSA); *Pseudomonas aeruginosa*

1. Introduction

Diabetes mellitus (DM) is a critical public health challenge with rising cases in sub-Saharan Africa and the world at large. World Health Organization (WHO) estimated that the prevalence of diabetes in the African Region varies between countries from 8.7 % and 8.5 % in males and females respectively [1-4]. In Nigeria, this varied from 0.65 % in the North

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to 11 % in the south [3,5]. It is estimated that by the year 2030 over 500 million adults will be affected by DM [6] with the increase projected to be higher in Africa and Asia where there is rapid epidemiological transition [7]. Using data from 14 countries, WHO estimated that by 2030, Nigeria with a projected population of 5.5 % of the world population would have 5,316,000 cases [8].

Studies have shown that diabetic patients have up to a 25 % risk of developing foot ulcer [9] and thus diabetic patients should be examined at least annually for possible predisposing factors to ulcer. Once an ulcer has developed, there is an increased risk of wound progression that may ultimately lead to amputation. Diabetic patients are susceptible to the development of foot ulcers. These have been attributed to various underlying factors such as neuropathy, ischemia, and an elevated blood pressure [10]. Foot ulcers and amputations are implicated mostly in morbidity, disability as well as psychological and physical effects and costs. An infected ulcer can lead to development of cellulitis and osteomyelitis [11].

The repair of skin lesions is one of the most complex biological processes in humans, occurring throughout an orchestrated cascade of overlapping biochemical and cellular events. To stimulate the regeneration process and prevent the wound to fail the healing, traditional therapies and natural products have been used with promising results [12-15]. Although these products are in general, less expensive than the modern treatments, they can be sensitive to the geographic location and season, and exhibit batch-to-batch variation, which can lead to unexpected allergic reactions, side effects, and contradictory clinical results. Honey is a carbohydrate rich syrup produced by bees, primarily from floral nectars. The use of honey for dressing of local wound is due to its antibacterial activity. The type of honey and its source affect greatly its effect on tissue repair [16]. Like modern dressings, honey is easy to apply, painless and comfortable, harmless to the tissue. The work was aimed at investigating the synergism of honey and commercially available Cicatrin® (neomycin-bacitracin) against selected microorganisms (*Pseudomonas aeruginosa* and vancomycin-oxacillin resistant *Staphylococcus aureus*) and its effect on diabetic wound healing.

Several researches have been conducted on the use of honey as wound healing agents in both laboratory and clinical settings with good results [17-19]. Manuka and Medi Honey have been employed in the treatment of ulcers, infected wounds and burns [20,21] while Revamil honey has been explored in wound dressings for treating burn wounds [22]. In addition, combination therapies have been experimented with honey and some other naturally occurring substances e.g., Honey and ginger [23]; Honey and *Origanum vulgare* L. essential oil [24]; Honey and Lemon juice [25]; Honey and Garlic [26]. However, there is no published literature on the combination of honey with commercially available synthetic combinations of antibacterial agents. The choice of this combination is therefore hinged on the possibility of obtaining a product with a broader spectrum of antibacterial activity against pathogens that commonly infest wounds thus providing a better cover for wound healing.

2. Material and methods

2.1. Materials

Honey (South-Eastern Nigeria brand), Cicatrin®, Ointment base (Vaseline®)

2.1.1. Test organism

Vancomycin and oxacillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains were isolated from clinical samples from the Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria the organisms were identified microscopically and with biochemical tests, catalase, coagulase, and oxidase test.

2.1.2. Culture media

Mueller-Hinton agar (MHA), Mannitol salt agar and cetrimide agar were prepared according to manufacturer's guide. The media were sterilized using an autoclave at 121 °C for 15 min. Other reagents used include 0.5 McFarland turbidity standard from 1 % anhydrous barium chloride, 1 % sulfuric acid.

2.1.3. Animals

Twenty albino rats of both sexes aged 10 weeks and weighing 120 - 160 g were bought from the Veterinary Medicine Department of the University of Nigeria, Nsukka, Enugu State, Nigeria and placed into five groups of four based on treatment schedule. The rats were allowed to acclimatize for seven days with free access to food and water, and adequate 12-hour light and dark cycles. Animal experiments were carried out in accordance with the guidelines of the

Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka and the National Institute of Health (NIH) guide for care and use of laboratory animals (Pub No: 85-23 Revised 1985).

2.2. Methods

2.2.1. Antimicrobial studies

Antibacterial activity of honey and Neomycin-Bacitracin (Cicatrin®) were evaluated by the agar-diffusion method [27] to determine their minimum inhibitory concentrations. An inoculum equal to 0.5 McFarland turbidity standard was prepared from each isolate and streaked on the surface of MHA (Oxoid, Difco, USA). An 8 mm sterile cork borer was used to make a well in the MHA. Using a sterile distilled water, graded concentrations (100, 50, 25, 12.5, 6.25, 3.125 %) of honey and (1000, 500, 250, 125, 62.5, and 31.25 µg/mL) of Cicatrin® were prepared and added into each well in the culture plates seeded with the organism. The culture was then incubated at 37 °C for 24 h and antimicrobial activity determined by measuring the zone of inhibition (ZOI) around each well (excluding the diameter of the well). Each concentration was tested in triplicate.

2.2.2. In vitro synergism study

The combination ratios were carried out by adopting the Checkerboard assay as described by Okore [28] for the evaluation of the combination effects of Neomycin sulfate + Bacitracin Zinc (Cicatrin®) and Honey. Here, the individual MICs were used in preparing the stock solution of each of the agents. Separate solutions of the two agents were prepared with water for injection, each solution containing twice the MIC of the individual agent.

Thereafter, the solutions were combined in different ratios, adopting the continuous variations model (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 10:0 of the Honey and Cicatrin® MICs respectively). Each combination was then diluted two-fold serially up to 5 dilutions in sterile pyrex test tubes. An aliquot of 80 µL corresponding to 0.08 mL of each of the serial dilutions was transferred into a corresponding well in a sterile agar plate previously seeded with 0.5 McFarland standard of the test organism. The plates were incubated at 37 °C for 24 h. The fractional inhibitory concentration (FIC) of each extract is the minimal inhibitory concentration in the combination divided by the independent MIC of the extracts. The sum of the FICs of both extracts gave the FIC index. This is expressed as in equation below:

$$\text{FIC Index} = \frac{A'}{A''} + \frac{B'}{B''} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 1$$

Where, A' and B' represent minimal concentrations of extracts A and B having inhibitory effects when acting together, while A" and B" stand for the respective MICs of the extracts. The FIC Index is interpreted as synergism if its value is less than 1.0; Additivity if it is equal to 1.0; Indifference if more than 1.0 < 2.0; and antagonism if more than 2.0.

2.2.3. Formulation of the combo-therapeutic ointment

For the formulation, honey 38.0 % w/w, cicatrin powder 1.0 % w/w, methyl paraben 0.2 %w/w, propyl paraben 0.1 % w/w, glycerine 10.0 % w/w, ascorbic acid 0.1 % w/w and petroleum jelly 50.6 % w/w were used. The dry reagents were first weighed out, triturated together and then dispersed in glycerine. Petroleum jelly was dissolved and added to the glycerine mixture with continuous stirring after which honey was added and the mixture homogenized further for 30 min before dispensing into the container and then labelled appropriately and allowed to cool. A bland ointment containing petroleum jelly 89.6 % w/w, glycerine 10.0 % w/w, methyl paraben 0.2 % w/w, propyl paraben 0.1 % w/w, and ascorbic acid 0.1 % w/w was prepared.

2.2.4. Characterization of ointment

The ointment was characterized using the following parameters: pH, spreadability, colour, texture, and consistency.

2.2.5. pH determination

The pH of the preparation was determined in triplicate using a pH meter (Jenway 6505, USA) after calibration with standard buffers.

2.2.6. Spreadability

This was done using the method of [29]. A 0.5 g of the ointment was placed on one slide and another slide was used to cover it on the upper side. The initial diameter of spread was taken. The weight of 50 g was then placed on the covering upper slide for one minute after which the weight was removed, and the diameter of spread determined. The procedure

was performed in triplicate and repeated using the 100 g weight. The spreadability was then calculated using the formula:

$$\frac{increase\ in\ diameter}{initial\ diameter} \text{X} \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 2$$

2.2.7. Evaluation of colour, texture, and consistency

This was done by observing the formulated ointments visually.

2.2.8. Induction of diabetes mellitus

The initial blood glucose level of the rats was determined prior to the induction of diabetes and used as baseline. Alloxan monohydrate dissolved in distilled water was administered to each rat at a dose of 130 mg/Kg intra-peritoneally. The blood glucose level was determined 48 h post-administration with the aid of a glucometer [30].

2.2.9. Excision of wound

This was performed using the wound excision model as reported by [31]. The animals were anaesthetized with 10 mg/Kg ketamine hydrochloride, and the furs on the desired injury site shaved off. The shaved portion was disinfected with 70 % alcohol before wound excision. The wound was left undressed and open to the environment with no local or systematic antimicrobial agent administered for 24 h. This was to ensure a stable size of the wound site and to mimic a typically exposed wound prior to treatment.

2.2.10. Treatment of wound

The rats were placed in five groups. Group 1 was administered the bland ointment, group 2 the Cicatrin®, group 3 the honey- Cicatrin® combination, group 4 the honey and Cicatrin® applied individually, and group 5, honey alone. The various treatment formulations were applied once daily for 21 days.

2.2.11. Evaluation of wound infection and healing

Pus from wound sites were extracted on days 4, 8, and 12, and cultured to identify prevalent organisms such as resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* that could cause wound infections. The wound diameter was measured on days 3, 6, 9, 12, 15, 18 and 21 of treatment and compared to the baseline wound diameter.

The degree of wound healing was calculated using the equation below.

$$\frac{X-Y}{X} \text{X} \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Where X is diameter on day 0 and Y wound diameter on corresponding days.

2.3. Data analysis

The results were analyzed using statistical package for social sciences (SPSS) version 22 and presented as mean ± SEM. Significance between honey-Cicatrin® ointment and other treatments evaluated were determined using students t-test and one-way analysis of variance (ANOVA). Difference between means were considered significant at $p < 0.05$

3. Results

3.1. Combined activity of the test agents (Cicatrin® and honey)

Preliminary antimicrobial evaluation of honey against *VORSA* showed ZOI of 13.33 ± 0.33 and 19 ± 0.58 mm at a concentration of 100 and 50 % respectively while its zone of inhibition for *Pseudomonas aeruginosa* at both concentrations (100 and 50 %) were 16.33 ± 0.67 and 13.33 ± 0.33 mm respectively (Table 1). In addition, the Cicatrin® showed inhibition of VORSA up till a concentration of 31.25 µg/ml (Table 2). For *Pseudomonas aeruginosa*, Cicatrin® had no ZOI even at the highest concentration, (Table 2). From the results obtained, 62.5 µg/ml Cicatrin® was combined with 100 % honey for the combined activity using checkerboard method.

Table 1 Antimicrobial activity of honey against VORSA and *Pseudomonas aeruginosa*

Honey concentration (%)	Zone of Inhibition (mm)	
	VORSA	***Pseudomonas aeruginosa***
100.00	13.33 ± 0.33	19.00 ± 0.58
50.00	11.00 ± 0.58	11.33 ± 0.67
25.00	-	-
12.50	-	-
6.25	-	-
3.13	-	-

Key: Vancomycin-Oxacillin Resistant *Staphylococcus aureus (VORSA)*n = 3; Data presented in values of Mean ± SEM

Table 2 Antimicrobial activity of Cicatrin® against VORSA and *Pseudomonas aeruginosa*

	Zone of Inhibition (mm)	
Cicatrin concentration (µg/mL)	**VORSA**	***Pseudomonas aeruginosa***
1000.00	16.33 ± 0.67	-
500.00	13.33 ± 0.33	-
250.00	12.00 ± 0.00	-
125.00	11.67 ± 0.33	-
62.50	11.00 ± 0.00	-
31.25	10.33 ± 0.33	-

Key: Vancomycin-Oxacillin Resistant *Staphylococcus aureus (VORSA)* n = 3; Data presented in values of Mean ± SEM

3.2. Results of Checkerboard Analysis

The results of the checkerboard analysis are presented in Tables 3 and 4. From the results observed, combinations of both agents (Cicatrin® and Honey) at lower concentration ratios of honey showed no activity against *Pseudomonas aeruginosa.* At higher concentration ratios, there was additive effect, while a synergistic effect was observed at a ratio of 1: 9 for Cicatrin® and honey respectively (Table 3). For activity against VORSA, it was observed that the ratios containing the agents alone (10:0, and 0:10) had nil FIC while all the combinations of the two agents showed synergistic activity (Table 4).

Table 3 Combined antibacterial activity of Cicatrin® and Honey against *Pseudomonas aeruginosa*

Volume ratios (ml)	Two-fold dilutions						
[A:B]	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**FIC**	**Inference**
10:0	+	+	+	+	+	nil	Nil
9:1	+	+	+	+	+	-	-
8:2	+	+	+	+	+	-	-
7:3	+	+	+	+	+	-	-
6:4	-	+	+	+	+	2.00	Additive
5:5	-	+	+	+	+	2.00	Additive
4:6	-	+	+	+	+	2.00	Additive
3:7	-	-	+	+	+	1.00	Additive
2:8	-	-	+	+	+	1.00	Additive
1:9	-	-	-	+	+	0.50	Synergistic
0:10	-	-	+	+	+	Nil	Nil

Key: A = Cicatrin (62.5 µg/mL); B = Honey (100%); FIC = Fractional Inhibitory ConcentrationT_1 (2-fold dilution of volume ratio); T_2 (2-fold dilution of T_1); T_3 (2-fold dilution of T_2); T_4 (2-fold dilution of T_3); T_5 (2-fold dilution of T_4); + = growth; - = No growth.

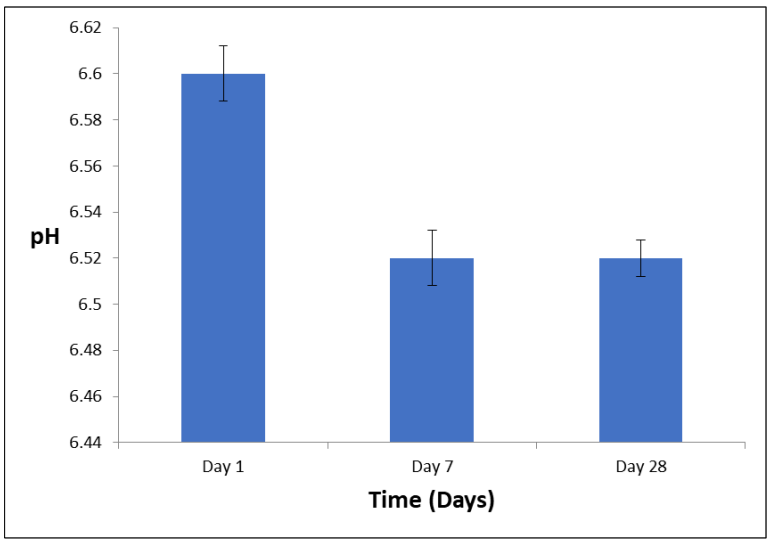
Table 4 Combined antibacterial activity of Cicatrin® and honey against vancomycin-oxacillin resistant Staphylococcus aureus (VORSA)

Volume ratios (ml)	Two-fold dilutions						
[A:B]	T1	T2	T3	T4	T5	FIC	Inference
10:0	-	-	-	+	+	nil	Nil
9:1	-	-	-	+	+	0.50	Synergistic
8:2	-	-	-	+	+	0.50	Synergistic
7:3	-	-	-	+	+	0.50	Synergistic
6:4	-	-	-	+	+	0.50	Synergistic
5:5	-	-	-	+	+	0.50	Synergistic
4:6	-	-	-	+	+	0.50	Synergistic
3:7	-	-	-	+	+	0.50	Synergistic
2:8	-	-	-	+	+	0.50	Synergistic
1:9	-	-	-	+	+	0.50	Synergistic
0:10	-	-	-	+	+	Nil	Nil

Key: A = Cicatrin (62.5 µg/mL); B = Honey (100%); FIC = Fractional Inhibitory Concentration T_1 (2-fold dilution of volume ratio); T_2 (2-fold dilution of T_1); T_3 (2-fold dilution of T_2); T_4 (2-fold dilution of T_3); T_5 (2-fold dilution of T_4); + = growth; - = No growth.

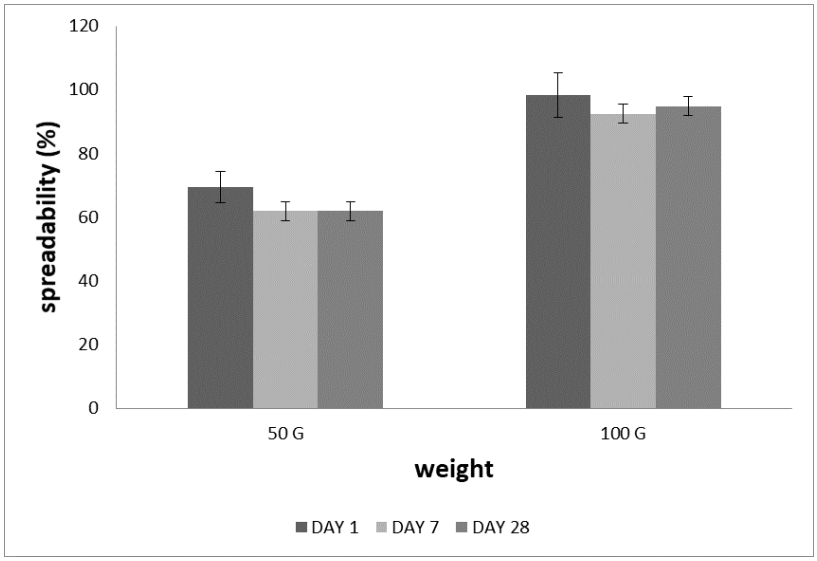
3.3. Physicochemical characteristics of the formulation

The pH range of the formulation throughout the study was 6.8 ± 1.0 (Fig. 1). The spreadability profile of the formulation using the three-weight category showed a slight increase over the course of the 28 days study but the formulation was still very spreadable (Fig. 2). Other parameters, such as consistency, colour and texture were unchanged throughout the course of the study.



n = 3; Data presented in values of Mean ± SEM

Figure 1 pH evaluation of formulation



n = 3; Data presented in values of Mean ± SEM

Figure 2 Spreadability evaluation of formulation

3.4. Induction of Diabetes

The results of diabetes induction in the various groups are presented in Table 5. From the results obtained, it was seen that the post induction blood glucose values of animals in all the groups showed a three-fold increase indicating a hyperglycaemic state.

Table 5 Results of induction of hyperglycaemia in experimental rats

Group	Basal (mg/dL)	Post induction (mg/dL)
1	53.50 ± 3.10	163.50 ± 8.39
2	58.00 ± 2.94	160.50 ± 1.04
3	54.50 ± 2.22	157.25 ± 3.12
4	54.50 ± 1.25	160.75 ± 7.50
5	55.00 ± 2.38	158.75 ± 4.19

n = 4; Data presented in values of Mean ± SEM

3.5. Wound healing evaluation results

Wound diameter for group 1 rats reduced by an average of 0.07 ± 0.02 cm every three days, while groups 2 and 5 reduced by 0.12 ± 0.10 cm with significant difference ($p < 0.05$) recorded on days 6, 9 and 12 (Table 6). For group 3 rats, which received Cicatrin® and honey combination, the process of wound healing was significantly high with wounds completely healed by day 21. Such significant healing process was also recorded for the group that received honey and Cicatrin® but administered individually (group 4).

Group 3 and 4 rats all healed with absence of pus exudates which could be adduced to a lack of wound infection. In addition, groups 1, 2, and 5 showed presence of pus exudates, which presented as whitish viscous liquid on the surface of the injury and further microbial evaluation through culturing identified the organisms involved in each case (Table 7).

Table 6 Result of Wound Healing Evaluation

Groups	Wound diameter (cm)							
	Day 0	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 21**
1	1.125 ± 0.08	1.3 ± 0.07 ns	1.4375 ± 0.09 ns	1.475 ± 0.09 *	1.55 ± 0.05 **	1.55 ± 0.06 **	1.525 ± 0.09 *	1.475 ± 0.05 *
2	1.45 ± 0.13	1.175 ± 0.12 ns	1.25 ± 0.22 ns	1.1125 ± 0.23 ns	1.0375 ± 0.22 ns	0.7125 ± 0.03 *	0.6125 ± 0.05 **	0.475 ± 0.03 **
3	1.375 ± 0.08	0.975 ± 0.05 **	0.8375 ± 0.04 **	0.7125 ± 0.04 **	0.4875 ± 0.03 **	0.2 ± 0.04 **	0.05 ± 0.02 **	0.00 ± 0.00 **
4	1.175 ± 0.06	1.025 ± 0.05 ns	0.925 ± 0.03 **	0.8375 ± 0.04 **	0.7125 ± 0.01 **	0.5875 ± 0.03 **	0.35 ± 0.06 **	0.175 ± 0.06 **
5	1.175 ± 0.03	1.05 ± 0.05 ns	1.0125 ± 0.05 ns	0.95 ± 0.05 ns	0.8375 ± 0.06 **	0.775 ± 0.07 **	0.65 ± 0.10 **	0.4 ± 0.09 **

KEY: ns- no significance; P > 0.05; *- severe 0.05 > P > 0.01; **- very severe P < 0.01
Group 1(bland ointment); Group 2 (Cicatrin®); Group 3 (Honey-Cicatrin® combination); Group 4 (Honey and Cicatrin® applied individually); Group 5 (Honey alone).
n = 4; Data presented in values of Mean ± SEM

Table 7 Result of Wound Infection Evaluation

	Day 4		**Day 8**		**Day 12**	
Group	**Pus**	**Organism**	**Pus**	**Organism**	**Pus**	**Organism**
1	+	*P. aeruginosa* *S. aureus*	++	*P. aeruginosa* *S. aureus*	+++	*P. aeruginosa* *S. aureus*
2	+	*P. aeruginosa* *S. aureus*	+	*P. aeruginosa* *S. aureus*	+	*P. aeruginosa*
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	+	*S. aureus*	+	*S. aureus*	+	*S. aureus*

Key: +++ (severely present); ++ (moderately present); + (mildly present); - (not present); Group 1(bland ointment); Group 2 (Cicatrin®); Group 3 (Honey-Cicatrin® combination); Group 4 (Honey and Cicatrin® applied individually); Group 5 (honey alone). n = 4; Data presented in values of Mean ± SEM

4. Discussion

The test organisms were successfully isolated, and viability verified using media that were specific for their growth. *Pseudomonas aeruginosa* was confirmed biochemically because it produced a green colour on cetrimide agar. In addition, with oxidase test, it produced a purple complex that resulted from its oxidase enzyme reacting with the reagent tetramethyl p-phenylene diamine dihydrochloride [32-34]. Vancomycin-oxacillin resistant *Staphylococcus aureus* (VORSA) grown on mannitol salt agar were confirmed using catalase test where the catalase enzyme of the *S. aureus* reacted with hydrogen peroxide to give water and bubbles. This was important to differentiate it from *Streptococcus* species that would have interfered with this study. In addition to catalase, coagulase test was also used to verify that the *Staphylococcus* strain is in fact pathogenic in nature and the clumping in plasma cells on contact with the organism proved this [35].

The antimicrobial susceptibility tests showed that the honey alone was effective against both organisms but had no activity below 50 % concentration which was in accordance with White [36] in his work on the benefits of honey in wound management. However, Agbagwa and Frank-Peterside [37] in their work had similar activity for the honey tested at 100 % against *Staphylococcus aureus* but a lower zone of inhibition (ZOI) at 50 %, while their samples were less effective against *Pseudomonas aeruginosa* at 100 and 50 % concentrations of honey. Conversely, The Cicatrin®

(neomycin-bacitracin) while showing extensive activity against *VORSA* had no effect against *Pseudomonas aeruginosa.* This inactivity of bacitracin and neomycin (components of the Cicatrin®) is supported by literature evidence [38, 39].

In-vitro evaluation of the honey-Cicatrin® combination using checkerboard assay method was employed to establish the best combination ratio that produced significant desirable activity. While the combination performed excellently against the *VORSA* strain, it had lower effect against the *Pseudomonas aeruginosa*. It was also observed that the higher the quantity of Cicatrin® in the formulation, the lower the activity of the formulation against the *Pseudomonas aeruginosa*. There is no literature evidence until date on the effectiveness of neomycin or bacitracin in the treatment of VORSA; however, Blanchard et al. [40] showed that there was improved activity against methicillin resistant *Staphylococcus aureus* when neomycin was combined with mupirocin ointment.

Honey is a natural substance whose wound healing effect has been variously explored [21, 41, 42]. This effect is attributed to several factors including an acidic nature that ensures killing of bacteria and prevents biofilm formation on wounds [43]; ability to reduce protease activity thus providing a suitable environment for increased fibroblast activity and wound healing; and an osmotic effect that makes growth of microorganisms difficult in its surrounding [44]. These among other factors make it a suitable substance for wound healing. From the results obtained in the wound healing study, it can be deduced that the rats of group 3 (Honey-Cicatrin® combination) showed better wound healing processes that culminated in the maximum closure of wound diameter on day 21 of study unlike other groups. It can also be deduced that co-administration of honey and Cicatrin® (Group 4) showed an improved healing process when compared to the individual treatments (Groups 2; Cicatrin alone) and (Group 5; honey alone) respectively. An initial increment in average wound diameter was noticed in rats of group 1 as they presented with impaired wound healing processes which eventually started on day 12. It was also noticed that the rats of group 2 showed a slight increase in average wound diameter on day 9, which may be attributed to the aggressive display of the rats towards each other that ultimately caused injuries sustained by scratches and bites.

The effectiveness of adding honey to the treatment regimen was evident in the absence of pus in rats in groups 3 and 4 that were administered a combination of honey and Cicatrin®. Factors affecting wound healing can be classified as local and systemic, with infections grouped under the localized causes. Other plant substances have also shown good promise in the management of wounds [Honey has been widely used in the management of diabetic foot ulcers [17]. This work provides preliminary but useful evidence on the possibility of combining honey with other commercially available synthetic agents for a synergistic effect.

5. Conclusion

Diabetic wound management has been managed over the years with synthetic drug substances. The emergence of resistance strains to these synthetic substances thus necessitates the exploration of alternative remedies especially from natural sources. A combination of the natural agent-Honey- and the synthetic agent –Cicatrin® (Neomycin-Bacitracin) - gave an improved antimicrobial effect than when co-administered on the same wound. Diabetic wound healing process was improved by limiting the risk of infection to injury. The formulation also showed a better activity against *Vancomycin-Oxacillin Resistant Staphylococcus aureus* (VORSA). This finding will thus provide a basis for future research into the possibility of other combinations of honey and synthetic wound healing agents especially in incidences where resistance patterns have been established against the synthetic agents.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Statement of ethical approval

Animal experiments were carried out in accordance with the guidelines of the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka and the National Institute of Health (NIH) guide for care and use of laboratory animals (Pub No: 85-23 Revised 1985)

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