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Research Article

Healing Potentials of Nigerian Bee Propolis on Meticillin-Resistant Staphylococcus Aureus Infected Skin Wound of Wistar Rats

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ABSTRACT

This study evaluated the effects of Nigerian Bee Propolis extract on Methicillin Resistance Staphylococcus Aureus (MRSA) infected skin wounds of albino rats. Two full thickness circular wounds were created each on the dorsum of eighteen (18) healthy adult male albino rats with mean body weight of 126±7.09g. Each wound was contaminated with 108 colony forming unit of MRSA. The rats were then randomized into three (3) treatment groups (n=6) with topical application of Propolis extract (PE Group), Silver sulphadiazine (SS Group) and untreated Control (UT Group). Gross wound healing indices (exudation, edema, hyperemia, wound contraction), histopathologic (granulation, angiogenesis, fibroplasia, epithelialization) and immunologic healing indices were evaluated using standard methods. Bacteria clearance was through culture and quantification. The wound surface exudation and edge edema and hyperemia were prominent in all the groups from day 0 to 6 but persisted in the untreated group till day 12. Wound contraction was gradual in all the groups from day 0-18, it was higher between days 0 and 3 in the PE and SS than the UT group ($P<0.05$). Complete wound closure occurred on SS (day 15), and PE (day 16). The histopathological changes observed showed neutrophils regressed on day 6 to 18 in all groups and was faster in PE and SS groups ($P<0.05$). Platelets reduction was ` gradual from days 3 to 18 in all groups and was absent from days 6 to 18 in the PE group. Nigerian Bee propolis has a profound bacteria clearance and healing effect on wound infected with methicillin resistant staphylococcus aureus (MRSA) comparable to silver sulphadiazine and therefore recommended for infected wound treatment.

Keywords: *Wound healing, MRSA clearance, Nigerian bee propolis.*

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INTRODUCTION

Wound healing is an essential physiological process that consists of cells and their products geared towards restoration of tissue integrity and functions (Shaw and Martin, 2009; Eyarefe *et al*., 2017). This process is divided into predictable phases that include: inflammation, repair and tissue remodelling (maturation) (Ning Xu *et al*, 2016). Wound healing is influenced by various factors including: host (Wall *et al.*, 2002), wound characteristics (Rosique *et al.*, 2015), closure methods and applied agents (Oguwike *et al.*, 2013). Investigation into agents with perceived healing potentials, especially, natural products have been intense in recent times, due to increasing challenge of bacteria resistance to antimicrobials (Eyarefe *et al.*, 2014).

Wounds are susceptible to microbial contamination and infection (White *et al*., 2006). Host tissues show evidence of erythema, warmth, exudation, pain, tenderness, or induration as clinical evidence of infection and damage to tissues (Moore and Cowman, 2007). Wound infection prolongs healing process (Talan *et al*., 2011). Several microbial organisms including *Staphylococcus aureus, Escherichia coli*, *klebsiella pneumonia*, *Actinobacter baumannii*, *Pseudomonas aeruginosa* etc have been isolated from infected wound. *Staphylococcus aureus* is an important cause of wound infection in human beings and animals. Methicillin-resistant S. aureus (MRSA) is of particular concern due to limited treatment options and their zoonotic potentials. It is also responsible for the majority of all superficial skin infections of surgical patients (Lowy, 2003).

With current trends in bacterial resistance to antibiotics, consideration is being given to topical antimicrobial therapy as adjunct to systemic antibacterial in infected wound management (Eyarefe *et al*., 2018). Topical antimicrobials have traditionally been formulated for wound management (Patel *et al*., 2008). Natural products such as *Aloe vera* (Vinson *et al*, 2005), Bannana leaves (Joshua *et al*, 2013), Cocoa, Tree barks (Patel, 2014; De Armas *et al*, 2015), Honey

(Molan, 2011; Eyarefe & Oguntoye, 2016), and Pineapple juice (Howat and lewis, 2002) have been used for wound treatment. Their efficacies have been investigated with measurable indices of wound healing: gross, histopathologic, immunologic and immunohistochemical examinations (Gupta and Kumar 2015).

Bee propolis or bee glue is a resinous material produced by honeybees by mixing plant exudates with wax, pollen, salivary secretions and bee enzymes (Simone-Finstrom *et al*, 2010). The precise composition of propolis varies with the geographical location, botanical origin and bee species (Toreti *et al*., 2013). In Nigeria the predominant trees that bees use to make propolis are Mahogany, African Nut and black hyedua tree and fruit trees such as mango, cashew and coconut trees (Ibrahim, 2013). Common content of propolis includes fatty and phenolic acids and esters, substituted phenolic esters, flavonoids, terpenes, ϐ-steroids, aromatic aldehydes, alcohols, derivatives of sesquiterpenes, naphthalene, stilbenes, aliphatic acids, aromatic acids, carbohydrates, amino acids, ketones, chalcones, dihydrochalcones, terpenoids, vitamins, and inorganic substances (Popova, 2003). There is a dearth of literature on the pharmacologic potentials of the Nigeria bee propolis. Only a few studies on its antidiabetic and antihyperglycemic properties have been conducted (Mikhail, 2013; Babatunde, 2013). Recently, its wound healing potentials have been investigated (Eyarefe *et al*., 2018). However, its antibacterial potentials especially, against MRSA infected wounds is yet to be known. The prevalence of wound cases and surgical site infections in veterinary clinics and hospitals in Nigeria (Eyarefe *et al.,* 2011) and the limitations of modern methods of wound management due to antimicrobial resistance, biocompatibility issues and cost, especially in poor resource settings, has led to an increasing interest in the healing potentials of natural products. This study therefore investigated the healing potential of the Nigeria bee propolis on wounds infected with methicillin-resistant *Staphylococcus aureus* in order to provide information on its potentials as a possible non-antibiotic, natural antimicrobial agent for management of methicillin resistant *Staphylococcus aureus* (MRSA) infected wounds.

MATERIALS AND METHODS

Ethical clearance: Ethical approval was obtained from the Animal Care and Use Ethical Committee, University of Ibadan.

Experimental Animals: Eighteen (18) adult, male Albino rats (Wistar strain) were involved in the study. They were housed at the experimental animal unit of the faculty of veterinary medicine in well ventilated cages, fed with rat concentrate feed and Water ad-libitum, and exposed to 12hours of light and dark period. They were stabilized for the period of two weeks before commencement of the study.

Study design: Two excisional wounds were created on the dorsum of each rat under anesthesia and each of the wounds were contaminated with Staphylococcus aureus (10^8 Colony forming units (CFU). The rats were randomized into 3 groups as follows: Group PE= Propolis Extract; Group SS = Silver sulphadiazine; Group UT= Control, with 6 rats in each group.

Propolis extraction: Crude Bee propolis was obtained from the Apiary unit, Department of Crop Science and Protection, University of Ibadan and stored in a dark polythene container. The propolis was extracted by maceration at room temperature, with occasional shaking in 100 ml of solvent (ethanol 80% v/v). The extracts were obtained after 7 days of maceration, and filtered. The residue was disposed while the filtrate was concentrated to remove the ethanol. After the ethanol has been completely evaporated, the propolis jelly formed was reconstituted into 10% solution with propylene glycol and then stored in a dark bottle for use (Idenize *et al*., 2004; FAO, 2016).

Preparation of MRSA inoculum: The bacteria were obtained from the Department of Microbiology, University College Hospital, University of Ibadan. The microorganism was subcultured in nutrient agar and transported in tryptic soy broth.

Anaesthesia and wound creation: Each rat was premedicated with an intramuscular injection of 2% Xylazine HCL at a dose of 5mg/kg body weight and 5% Ketamine at a dose of 35mg/kg via the quadriceps group of muscles (Eyarefe and Amid, 2010). Following anesthesia, the dorsum (back) of each rat was prepared for aseptic surgery. Two full thickness excisional skin wound were created on the dorsum of each rat as previously described (Eyarefe *et al*, 2018). Each wound area was measured on a graph sheet, contaminated with 10^8 colony forming unit (CFU) of Meticillin-resistant staphylococcus aureus (MRSA), and rats were returned to their cages following recovery from anesthesia for further monitoring of wounds.

Confirmation of infection: After 48 hours following wound contamination, the presence of infection was confirmed by wound hyperemia, edge edema and exudation. Swab samples of wound exudate were also obtained for culture as previously described (Khoo *et al*., 2010), in MacConkey and Chocolate agar and the bacterial colonies were morphologically identified and further confirmed using Gram staining, catalase and coagulase tests (Shaikh, 1994).

Wounds treatment: Wound treatment commenced 72hours after inoculation. Rats were randomised into treatment groups: Propolis Extract (PE), Silver sulphadiazine (SS), Control/Untreated (UT). Propolis extract (0.1ml) was applied to wounds in PE group, Silver sulphadiazine cream was applied copiously on the entire wound surface in SS group, while wounds in control group were left untreated. Treatment was done once daily for 18 days and the rate of healing was monitored daily.

Monitoring with gross wound healing indices: Each animal was evaluated for gross wound healing indices (wound surface exudation, edge edema, surface hyperemia and rate of wound contraction for two weeks as previously described by Eyarefe *et al*, (2014). Wound size (surface area of the wound in (cm3)

was measured every three days using a transparent graph sheet. The evaluated wound size was employed to calculate the percentage of wound contraction, taking initial size of wound as 100% by using the following formula:

% Wound contraction = initial wound size – specific day wound size/initial wound size × 100.
A pictorial capture of each wound was also made on each day of observation.

Evaluation of wound bacterial clearance: Swab samples were collected from the wound exudates at days 3, 5 and 7 for bacterial culture and quantification of colony as earlier described (Talan *et al*., 2011).

Histopathological evaluation of wound tissues: Following euthanasia of 3 animals (one animal per group) at day 3, 6, 9, 12, 15, 18 with Ketamine (70 mg/kg) and Xylazine (15 mg/kg), indepth excision of entire wound region with liberal margins of the surrounding skin, including the underlying connective tissues and fascia above the dorsal muscles were excised and fixed in 10% formalin and cut in 5-µm thick serial sections, and processed for histopathological examinations. Sections were qualitatively assessed under the light microscope and the histologic parameters (Leukocyte count, fibroblast count, granulation tissue, vascularization, fibroelastic tissue and epithelialization) were studied. The leucocyte and fibroblast count were expressed as mean and standard deviation while the histomorphologic parameters (granulation tissue, vascularization, fibroelastic tissue and epithelialization) were assessed and scored as described by Akriti and Pramod, (2015) with the semi-quantitative four-point scale scoring system.

Immunohistochemistry of the wound samples: The staining was performed using the avidin-biotin peroxidase complex kit (M IHC Select Detection System, HRP/DAB, Merck, Germany LOT: 2775482) with slight modification of the procedure. Briefly, thin sections (4µm) of the skin were cut in triplicates, floated and mounted on APES charged glass slides. Each tissue section was deparaffinized before placed in citrate buffer solution (10 mM citric acid, pH 6.0) for antigen retrieval in microwave for 8 minutes. The sections were incubated in 70% methanol with 3% H_2O_2 for 10 min to inhibit endogenous peroxidase activity, and they were then washed three times in phosphate buffered saline (PBS). The sections were then treated with blocking solution for 10 min. After draining of blocking serum, each of the sections were individually incubated with primary antibodies (monoclonal antibody to EGF at a dilution 1:150 in PBS at 4˚ C overnight in a humidified chamber. After washing three times with PBS, the sections were treated with biotinylated anti-goat polyvalent secondary antibody for 10 min. Then the sections were washed three times in PBS and treated with the peroxidase-conjugated streptavidin for another 10 min. After another PBS bath, the sections were incubated with 3, 3-diaminobenzidine (DAB). After colour change, the sections were washed in tap water and then counterstained with Mayer's haematoxylin. The slides were mounted with cover slips and DPX for examination and grading of staining intensities.

The photomicrographs were taken with a computer enabled digital camera (Amscope MU900) attached to the microscope (Olympus CX21FS1). The images were quantified for staining intensity using reciprocal intensity on the open source Fiji (ImageJ) software. The optical density of the staining intensities was calculated using the formular;

OD= log10 (max reciprocal intensity/ mean reciprocal intensity)

RESULTS

The gross appearances of the wounds were hyperaemia, oedematous with sharp outline at day 0 (DO) till day 3 when there was deposition of scab on signs of contraction. At day 6 (D6), the outlines of the wounds showed remarkable shrinkage (contraction), exudation with scab. At day 12 (D12), the wounds showed remarkable contraction and closure in the PE and SS treatments compared with the UT group (Plate 1).

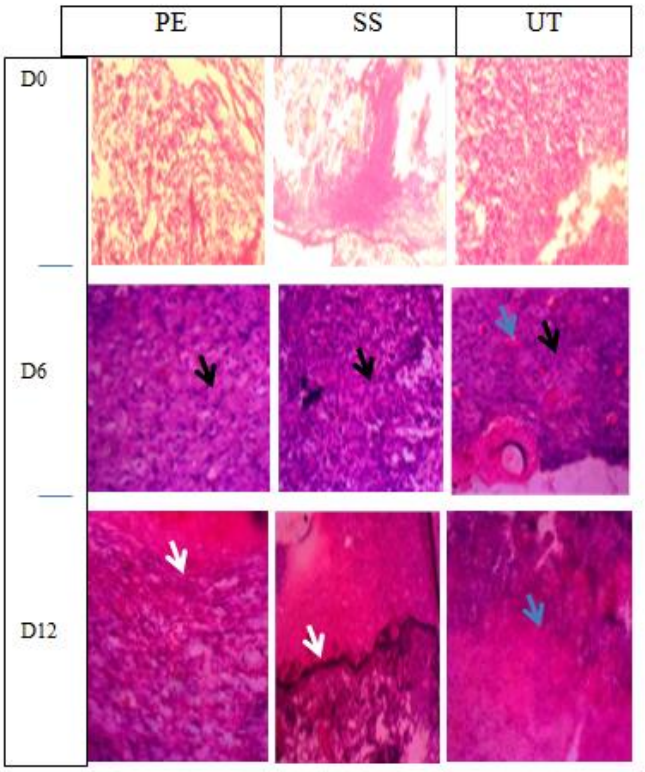

Plate 1.
Gross appearance of the wounds across the three groups from day 0, 6, & 12. The wounds are hyperaemic, reflective (oedematous) with sharp outline at day 0 (DO). At day 6 (D6), the oulines of the wounds show remarkable shrinkage (contraction), exudative with scab (granulation tissue). At day 12 (DO), the wounds show remarkable contraction.
PE= Propolis extract; SS= Silver Sulphadiazine; UT= Untreated wounds

Wound exudation: Wound exudation was prominent in all the groups, but reduced gradually from day 0 to 6. It was lesser in the SS (16%) and PE (25%) at day 7 compared to the UT (66%) with the trend being SS < PE < UT ($p<0.05$) (Plate 1).
Wound edge edema: Wound edge edema was prominent in all the groups from days 0 to 3. It was less in the PE (33%)

and SS (33%) at day 4 compared to the UT (75%) group ($p<0.05$). It was absent in the PE and SS groups from day 5, but present in the UT groups (75%) up to day 12 (16%) (Figure 2).

Wound surface hyperaemia: Wound surface hyperemia was prominent in PE from days 0 (100%) to 3 (83%), but was mild from day 5 (16%) to 6 (8%). Hyperemia was prominent in SS from days 0 (100%) to 3 (91%), mild from days 4 (41%) to 6 (33%). In the UT, hyperemia was prominent from days 0 (100%) to 3 (66%), but it became less prominent from days 5 (33%) to 11 (16%) (Figure 3).

Wound contraction: Wound contraction was gradual in all the groups from days 0-18. It was higher at day 9 in the PE (37.66±12.25) and SS (46.10±11.91) compared with the UT (15.73±9.51) group (P=0.000). The trend continued until day 15 when healing was complete in the SS group (Figure 4).

Bacteria clearance from wound: The rate of bacterial (methicillin resistance staph aureus) clearance in the propolis extract group (997.50±34.65) and the silver sulphadiazine group (911.00±26.87) was significantly higher than that of the untreated group (1502.50±10.61) ($P< 0.05$). Propolis extract and Silver suphadiaxine reduced dense growth of methicillin resistance staph aureus on the culture plate at day 3. Untreated wounds allowed dense growth of methicillin resistance staph aureus on the culture plate. At Day 6, PE and SS treatment allowed only sparse S. aureus colonies on the plates, while untreated wound allowed dense growth of methicillin resistance S. aureus on the culture plate. At Day 9, PE and SS inhibited growth of S. aureus colony, but untreated wounds still showed dense growth of MRSA with some contaminants on the culture plates (Fig 2).

Table 1:
Percentage of wound exudation, edema and hyperaemia in the first two weeks of treatment across the three groups

Days	Wound Exudation			Wound Edge Edema			Wound surface hyperaemia		
	PE	SS	UT	PE	SS	UT	PE	SS	UT
0	12/100	12/100	12/100	12/100	12/100	12/100	12/100	12/100	12/100
1	12/100	12/100	12/100	12/100	12/100	12/100	12/100	12/100	12/100
2	12/100	12/100	12/100	11/91	12/100	12/100	12/100	12/100	12/100
3	12/100	12/100	12/100	8/66	9/75	9/75	10/83	11/91	8/66
4	9/75	8/66	10/83	4/33	4/33	9/75	6/50	2/16	6/50
5	9/75	7/58	10/83	0	0	7/58	2/16	2/16	3/25
6	7/58	6/50	9/75	0	0	5//41	1/8	1/8	3/23
7	3/25	2/16	8/66	0	0	5/41	0	0	2/16
8	0	1/8	6/50	0	0	5/41	0	0	2/16
9	0	0	5/50	0	0	4/33	0	0	2/16
10	0	0	5/25	0	0	4/33	0	0	2/16
11	0	0	4/33	0	0	2/16	0	0	2/16
12	0	0	4/33	0	0	2/16	0	0	0
13	0	0	1/8	0	0	0	0	0	0
14	0	0	1/8	0	0	0	0	0	0

N = number of wounds that showed exudate
% = percentage of animals that showed wound wetness (exudation)
PE= Propolis extract; SS= Silver Sulphadiazine; UT= Untreated group

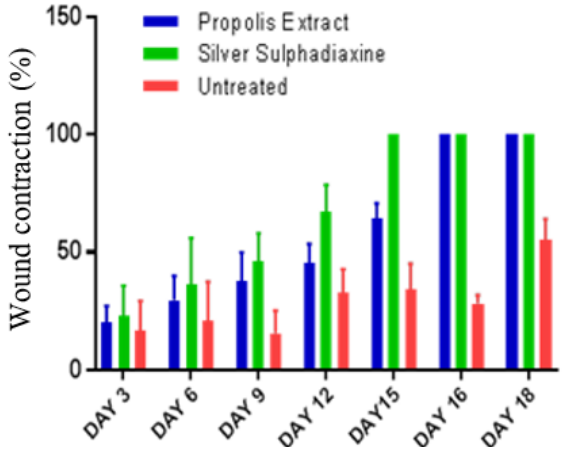

Figure 1
Wound contraction rate in the different treatment groups of rats with cutaneous wound.

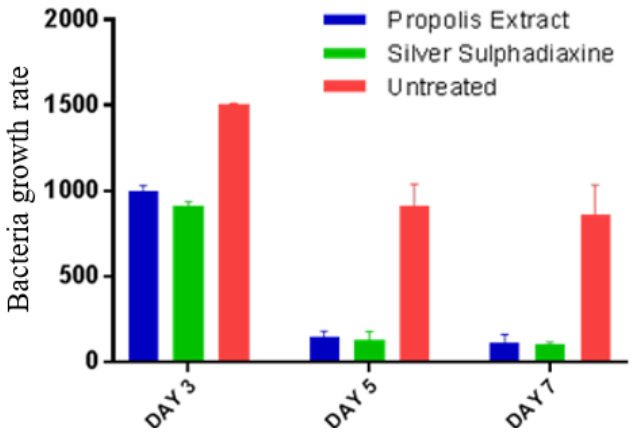

Figure 2
Methicillin resistance staph aureus clearance quantification across the three treatment groups

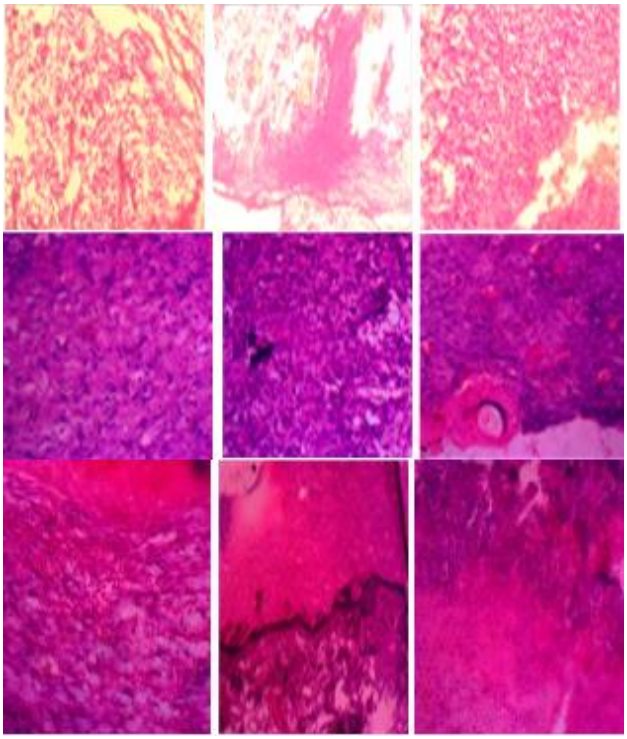

Plate 2:
Histologic wound healing indices across the three groups from day 0, 6, & 12. PE showing Inflammatory stage with diffuse cellular infiltrates (black arrow) and healing response (white arrow). SS showing Inflammatory stage with diffuse acute cellular inflammation (black arrow) and healing response (white arrow). UT showing early inflammatory stage with marked wound hyperaemia (blue arrow) and acute inflammatory cells (black arrow).
PE= Propolis extract; SS= Silver Sulphadiazine; UT= Untreated

Inflammatory cells response*:* The number of neutrophils in tissues reduced from days 6 to 18 in all groups but it was remarkable in the PE and SS treated wounds compared to the UT wounds on days 9 and 12 ($p<0.05$). Platelets reduced gradually from days 3 to 18 in all the wounds but was absent from days 6 to 18 in the PE treated wounds. PE treatment showed numerous acute inflammatory cells and granulation tissue and remarkable hyperaemia deposition at day 3, and fibroblasts at day 9 (Table 2). The epidermis also showed hydropic degeneration of keratinocytes at day 6, but there was complete healing at day 15. SS treatment showed numerous acute inflammatory cells and granulation tissue on the wound at day 3, fibroblasts at day 6, wound maturation with well-formed collagen (connective tissue) from day 9 to 12. UT wounds showed numerous acute inflammatory cells and granulation tissue on the wound at day 3 to 9, before appearance of fibroblasts, collagen at day 12 through to day 18 (Figure 4).

The wound granulation tissue comprised of budding capillaries and type III collagen fibers at day 12 in the SS and PE compared to the UT treatment (Table 3). Capillary budding/angiogenic blood vessels were high ($P<0.05$) in the UT group on day 9 and 12 when compared to the PE and SS groups. The SS groups showed a faster rate of epithelialization followed by PE and least in UT treatment ($P<0.05$). The amount of fibro elastic tissue at day 9 was higher in the PE than in SS and UT treatments ($P<0.05$).

Table 2:
Inflammatory cells and Fibroblast response on days 3, 6, and 9 across the three groups

Histologic parameters	PE	SS	UT
DAY 3			
Macrophages	6.50±0.70[a]	5.50±3.53[a]	2.50±0.70[a]
Monocytes	31.00±16.97	30.00±15.55	36.50±9.19
Fibroblasts	7.00±4.24[a]	5.00±4.24[a]	7.50±0.70[a]
Neutrophils	132.00±8.48[a]	130.50±14.84[a]	126.00±22.62[a]
Mast Cell	3.00±1.41[a]	2.50±0.70[a]	0.00±0.00[a]
Eosinophils	5.50±4.94[b]	1.00±1.41[a]	0.00±0.00[a]
Platelets	11.00±1.41[a]	12.00±2.82[b]	4.50±0.70[a]
DAY 6			
Macrophages	22.50±14.84[a]	17.00±7.07[a]	23.50±3.53[a]
Monocytes	72.50±14.84[a]	80.00±1.41[a]	78.00±14.14[a]
Fibroblasts	31.00±14.14[a]	42.50±9.19[a]	58.00±48.08[a]
Neutrophils	59.50±48.79[b]	30.50±31.81[a]	38.00±48.08[b]
Mast Cell	0.00±0.00[a]	0.50±0.70[a]	1.00±1.41[a]
Eosinophils	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Platelets	0.00±0.00	1.50±2.12	1.50±2.12
DAY 9			
Macrophages	11.00±4.24[b]	7.00±1.41[a]	6.50±2.12[a]
Monocytes	65.00±4.24[b]	21.50±12.02[a]	13.00±7.07[a]
Fibroblasts	70.00±2.82[b]	25.00±29.69[a]	13.00±4.24[a]
Neutrophils	26.00±2.82[a]	34.00±39.59[a]	84.00±36.76[b]
Mast Cell	1.50±0.70[a]	1.00±1.41[a]	2.00±0.00[b]
Eosinophils	0.00±0.00[a]	1.00±1.41[a]	2.50±3.53[a]
Platelets	0.00±0.00	3.00±4.24[b]	1.50±2.12
DAY 12			
Macrophages	12.00±0.00[b]	8.50±2.12[a]	7.50±2.12[a]
Monocytes	25.00±4.24[a]	16.50±2.12[a]	44.00±2.82[b]
Fibroblasts	90.00±59.39[b]	95.50±38.89[b]	9.50±9.19[a]
Neutrophils	5.00±4.24[a]	2.50±0.70[a]	25.50±4.94[b]
Mast Cell	0.00±0.00[a]	0.00±0.00[a]	1.50±0.70[b]
Eosinophils	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Platelets	0.00±0.00[a]	0.00±0.00[a]	10.00±2.82[b]
DAY 15			
Macrophages	6.50±2.12[a]	8.00±0.00[a]	5.00±1.41[a]
Monocytes	16.0±1.41[a]	21.00±7.10[a]	18.00±2.82[a]
Fibroblasts	52.5±10.61[a]	139.00±9.90[b]	34.00±2.82[a]
Neutrophils	4.0±2.83[b]	2.00±2.82[a]	3.00±1.41[a]
Mast Cell	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Eosinophils	0.00±0.00[a]	0.50±0.70[a]	0.00±0.00[a]
Platelets	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
DAY 18			
Macrophages	6.00±2.82[a]	6.00±0.00[a]	7.00±1.41[a]
Monocytes	19.00±4.24[a]	14.00±1.41[a]	15.00±21.21[a]
Fibroblasts	67.00±43.84[b]	40.00±2.82[a]	100.00±56.00[b]
Neutrophils	3.00±1.41[a]	5.50±3.53[a]	2.00±2.82[a]
Mast Cell	0.00±0.00[a]	1.00±1.41[a]	0.00±0.00[a]
Eosinophils	0.00±0.00[a]	0.00±0.00[a]	0.50±0.70[a]
Platelets	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

There is significant difference in values with superscript 'b' across the groups and no significantly difference with values with superscript 'a'
PE= Propolis extract; SS= Silver Sulphadiazine; UT= Untreated group

Table 3:
Histologic Wound Healing Indices on days 3, 6, 9, 12, 15 and 18 across the groups

Histologic parameters	PE	SS	UT
DAY 3			
Granulation tissue	1.50±0.71[b]	1.00±0.00[b]	2.00±0.00[b]
Vascularization	2.50±0.71[b]	2.00±0.00[a]	2.00±0.00[a]
Fibro-elastic tissue	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Epithelialization	1.00±1.41[a]	0.00±0.00[b]	1.00±0.00[a]
DAY 6			
Granulation tissue	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]
Vascularization	1.50±0.71[a]	2.00±0.00[b]	1.50±0.71[a]
Fibro-elastic tissue	2.00±0.00[b]	1.50±0.71[a]	1.00±0.00[a]
Epithelialization	1.50±0.71[a]	1.00±1.41[b]	2.00±0.00[b]
DAY 9			
Granulation tissue	1.50±0.71[a]	1.50±0.71[b]	1.00±0.00[a]
Vascularization	1.00±0.00[a]	1.00±1.41[a]	2.50±0.70[b]
Fibro-elastic tissue	3.00±0.00[b]	2.00±1.41[a]	1.50±0.71[b]
Epithelialization	1.00±0.71[a]	1.00±1.41[a]	0.50±0.71[a]
DAY 12			
Granulation tissue	1.00±0.00[a]	2.50±0.71[a]	1.00±0.00[a]
Vascularization	0.50±0.71[a]	0.00±0.00[a]	2.00±0.00[b]
Fibro-elastic tissue	3.00±0.00[a]	2.50±1.70[a]	2.50±0.71[a]
Epithelialization	1.50±0.71[b]	2.00±0.00[b]	0.50±0.71[a]
DAY 15			
Granulation tissue	3.00±0.00[a]	3.00±0.00[a]	3.00±0.00[a]
Vascularization	2.50±0.71[b]	0.00±0.00[a]	0.00±0.00[a]
Fibro-elastic tissue	2.50±0.71[a]	2.50±1.70[a]	2.00±0.00[a]
Epithelialization	1.00±1.41[b]	2.00±0.00[b]	1.50±0.71[b]
DAY 18			
Granulation tissue	1.00±0.00[a]	2.00±0.00[b]	2.50±0.71[b]
Vascularization	0.50±0.71[a]	1.50±2.12[a]	1.00±1.41[a]
Fibro-elastic tissue	2.50±0.71[a]	2.50±1.70[a]	2.00±1.41[a]
Epithelialization	1.50±0.71[a]	1.51±0.71[a]	1.50±0.71[a]

There is significant difference in values with superscript 'b' across the groups and no significantly difference with values with superscript 'a'
PE= Propolis extract; SS= Silver Sulphadiazine; UT= Untreated group

Expression of epidermal growth factor: Immunohistochemical evaluation of the intensity of epidermal growth factor (EGF) was high in the SS and PE treatments from days 4-12 than UT treatment (P=0.001) (Figure 5).

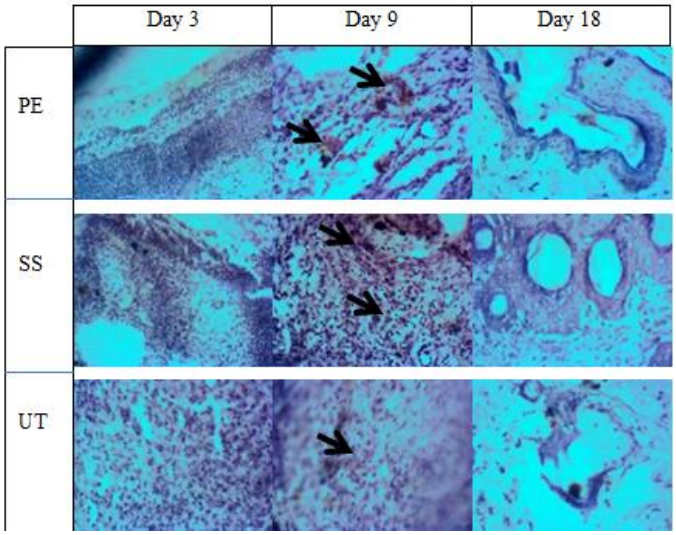

Plate 3
Immunohistochemical expression of EGF (arrows) on the healing wounds across the three groups from day 3, 9& 18
PE= Propolis extract;
SS= Silver Sulphadiazine;
UT= Untreated treatment

DISCUSSION

This study has futher confirmed the antibacterial properties and accelerated the healing potentials of Nigerian Bee propolis on cutaneous wound in rats. The use of Silver sulphadiazine as positive control was because of its established bactericidal effect against MRSA (Miller *et al*., 2012).
The indices evaluated were conventional for assessment of topical agent efficacy as previously established in previous studies which includes, gross (wound exudation, wound edge edema and wound hyperemia), immunologic, and histopathologic (granulation tissue, angiogenesis, fibro-elastic tissue and epithelialization) evaluation (Eyarefe *et al*, 2014 ; Akriti *et al*, 2015 ; Patrick, 2016, Eyarefe *et al*, 2018).

The prominence of wound surface exudation, edema and hyperemia in all the groups for the first six days was due to the enhanced acute local inflammatory response, but gradually phased out in the SS and PE treatments. The early complete wound closure in the PE as well as SS treatments was related to the contraction of the wound and possibly enhanced fibroblastic response and re-epithelization. These indices are indications of tissue response to injury (Rosique *et al*, 2015).
The rate of bacterial clearance in the PE treatment was also directly proportional to healing of the wound. This may not be unrelated to the presence of flavonoids in propolis. Its anti-edema and anti-hyperaemic effects can be ascribed to the anti-inflammatory and antimicrobial properties of flavonoids such as quercetin, naringenin and Caffeic acid phenethyl ester (CAPE). They reduce the production and release of histamine and other inflammatory mediators such as interleukins and tumor necrosis factor-α (de Moura, 2011). Terpenoids are phytochemicals that have been shown to possess antibacterial and antifungal properties (Mirzoeva *et al*, 1996). Anthraquinones and Cardiac glycosides which are present in this propolis sample have been used to treat inflammation and edema (Khan *et al*, 2011). The in vitro antibacterial activity of propolis has been verified against both Gram-positive and Gram-negative bacteria and this is as a result of synergism between propolis compounds, mainly pinocembrin and galangin flavonoids (Cushnie *et al*, 2005).

Local signs of acute inflammation to injury in clean wounds are wound edge edema and hyperemia and could be signs of wound infection and evidence of debridement challenges when these signs progress beyond day 3 of injury (Eyarefe *et al*, 2014, Patrick, 2016). This observation may provide a rationale for the prolonged wound exudations and hyperemia observed between days 5 to 12 in the untreated group. The reduction in edema and surface hyperemia seen in wounds treated with Silver sulphadiazine is in line with the use of silver sulphadiazine as a drug of choice in treating burn

wounds due to its wide spectrum of bactericidal activity against both gram-positive and gram-negative organisms (Miller *et al*, 2012). More so, the wound contraction rate could be traced to the ability of propolis to influence the production of Transforming growth factor- alpha and beta 1 (TGF-α & TGF-β1) by immune cells which stimulates cell growth, mobilization of fibroblasts and epithelial migration (Simona *et al*, 2015). Some of the fibroblast transform to myofibroblasts, which is mainly responsible for wound contraction (Patrick, 2016). Wound contraction was slower in the untreated group due to inadequate production of granulation tissues as a result of extended inflammatory and debridement phases (Rosique *et al*, 2015).

Our findings showed that Nigerian Bee propolis extract increased the rate of wound healing and re-epithelialization of full thickness skin wounds infected with methicillin resistance staphylococcus aureus in rats due to its immunomodulatory, anti-inflammatory, antioxidant and inhibition of bacterial growth and tissue regenerative properties. Propolis is known to possess a number of pharmacological properties such as: anti-inflammatory, anti- acceleration of regenerating processes of damaged cartilages and bones (Park *et al*, 2002), immunomodulatory (Qiao, 1991), antimicrobial (Silva *et al*., 2008) antioxidant (Cushnie and Lamb, 2005), as analgesic (Demestre, 2008), as an anti-inflammatory agent (Fearnely, 2001) and antitumoral agent (Gavanji, 2015) properties.

In conclusion, Nigerian Bee propolis has a profound healing effect on wound infected with meticillin resistant staphylococcus aureus (MRSA) comparable to silver sulphadiazine and therefore, it is recommended for treatment of infected and non-infected wound

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