Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **12(1),** 1-13, February **(2022)** *Res. J. Chem. Sci.*

Wound healing activity of *Mangifera indica* seed extracts in Wistar Rats

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Available online at: www.isca.in, www.isca.me
Received 25th September 2020, revised 8th December 2021, accepted 30th January 2022

Abstract

Mangifera indica is among the plants of high medicinal value in ethno medicine's system in Nigeria that is used in treating many ailments. Different parts of M. indica plant have been widely used but the activity of its seed extracts for healing of wound has not been documented scientifically hence the need for precise information about this so as to be able to expand the use of the plant and thereby include its integration into modern medical healthcare systems. Present study investigated the activity of hexane and methanolic seed extracts of M. indica for healing of wounds using Wistar rats. Phytochemical analysis of the seed extracts was determined so as to know their various phytoconstituents. The extracts were examined for antimicrobial activity on multiple drug resistant bacteria (P. aeruginosa: PA, S. aureus: SA, B. subtilis: BS and E. coli: EA) and fungi (A. niger: AN and C. albicans: CA) using agar technique of pour plate and surface plate dilution respectively. The effect of M. indica seeds for healing of wound was evaluated by incorporating the hexane and methanol extracts into paraffin in 5% and 10% (v/v) concentrations. 35 Wistar rats which were distributed into 5 groups of n=7 in each group, were given water plus feed continually for the study that lasted for a period of 21 days. The weight, area of wound (mm^2) and wound closure percentage were noted on a four-day basis with epitheliasation of each wound measured from the 16th to 20th day of study period. Blood samples with skin and tissues (liver, kidney, heart, lung and spleen) of animals from each group were then subjected to haematological and histopathological analyses respectively. Phytochemical analysis revealed the existence of various chemical constituents including tannins, glycosides plus phenols. M. indica methanol extract recorded MIC value of 12.5% against SA, BS, CS and PA while that of AN was 25%. MIC value for M. indica hexane extract was 12.5% against SA, 25% against BS, CA, EC and PA and 50% against AN. Group 3 rats that were treated with 5% (v/v) methanol extract ointment formulate of M. indica seeds had the fastest epithelialisation time with healing time of 16.74±0.18 days as compared with control rats (group 1) that had ointment for treatment. Almost all the test rats treated with both methanol and hexane extracts displayed epithelialisation times that were better and faster than those of the control rats. The control and test rats showed no significant differences for their histopathological and haematological analyses results. This is an indication that the extracts contain chemical constituent that accelerated the wound healing process. M. indica seed extract exhibited good efficacy for wound healing and might therefore serve as a good replacement for conventional ointment used in treatment of wound.

Keywords: *M. indica*; phytochemical, antimicrobial, histopathological, haematological, wound healing.

Introduction

Mangifera indica trees are largely cultivated in tropical West Africa. The seeds have been found to show some antibiotic properties[1]. It belongs to the genus *Mangifera* and a flowering plant family *Anacardiaceae*. It consists of many tropical fruiting trees that is usually cultivated for edible fruit of which most of the species exist in nature in wild form. *M. indica* can be seen majorly in tropical and equatorial Africa, preferably in deep, loamy-sandy and well drained soils. Mango tree, a native to northern India, the foot of Himalayas, and naturalized in West Africa, does not tolerate stagnant water, frequent or prolonged floods. It is often cultivated and is very common in more of less humid tropical areas provided it is protected from live stock in its early stage[1]. In Nigeria, because of the stringent properties of its barks and leaves, they are used as a lotion for relieving toothache, sore gums and sore throat or as in infusion in diarrhea and dysentery. The bark, fruit and leaves are known to be useful in the treatment of emetic, dysentery, colic, dlennorhoea, leucorrhoea, wounds, skin disease, rheumatism, diuretics, antipyretic, cough sore throats, asthma, tooth decay, blennorhoea, scurvy, stone, vermifuge, diarrhea, and internal hemorrhage[2]. Several plants have wide usage in medicine, health functional foods and home remedies but only a few have undergone safety evaluation[3,4]. This study evaluates the efficacy of *M. indica* seed extract in healing wound on Wistar rats at 5% and 10 % w/v ointment; it is an updated version of a conference presentation[5] and is a continuation of our efforts in bringing many unconventional seeds into limelight[6-8].

Materials and methods

Plant Material: *M. indica* fruits were bought from a local market in Ibadan, a city in Oyo State of Nigeria. The weight of the fruits was taken before the experiment started and used to calculate the followings:

$$\% \text{ W/W of seed in fruit} = \frac{\text{total weight of seed}}{\text{total weight of fruit}} \times 100$$

$$\% \text{ W/W of kernel in seed} = \frac{\text{total weight of kernel}}{\text{total weight of seed}} \times 100$$

$$\% \text{ W/W of kernel in fruit} = \frac{\text{total weight of kernel}}{\text{total weight of fruit}} \times 100$$

Preparation of Seed Extracts: The collected seeds were dried and grinded to coarse powder with an electric blender to increase extent of extraction of the oil. Approximately 500g of dried powdered samples were put in 2 aspirator bottles, 1L of methanol was added to one and 1L hexane added to the other and left at 30°C for 5 days with continuous stirring to ensure proper mixing with the solvent after which it was filtered[9]. The filtrate was then distilled to recover the solvent from the oil for further analysis. The extracts, labelled as *M. indica* methanolic extract (MIME) and *M. indica* hexane extract (MIHE), were stored in universal bottle and placed in a refrigerator at 4°C until needed for usage.

Phytochemical Analysis: Different chemical test was carried out qualitatively on each extract to check for the presence of phytoconstituents/secondary metabolites like terpenoids, phenols, glycosides, tannins[10]. Anthocyanins were examined using technique employed by Ayoola *et al.*[11] and Musila *et al.*[3].

Antimicrobial activity and Minimum inhibitory concentrations (MIC) of the seed extracts: Organisms used for test: The fungal tested organisms used for this study were the Multi Drug Resistant (multi) clinical isolates of *AS* and *CA* while the bacterial tested ones were *BC, BS, EC* and *PA*. The pure bacterial and fungal strains were obtained from Faculty of Pharmacy in University of Ibadan, Nigeria and cultured in a single day at 37°C and 28°C in nutrient agar (Oxoid, Hampshire, UK) and potato dextrose agar (Oxoid) respectively.

Assay of Antimicrobial activity of MIME and MIHE: MIME and MIHE antimicrobial activity were evaluated against a few pathogenic bacteria such as *BS, EC, PA, SA* and fungal isolates like *AN* and *CA* using pour plate and surface plate methods for pathogenic bacteria and fungi strains respectively. Graded concentrations of MIME and MIHE at 12.50%, 25.00%, 50.00% and 100.00% were prepared so as to be able to estimate their MIC against the bacteria and fungi strains. The negative and positive control for the study was -N-hexane and methanol, gentamycin for bacteria strains and Tioconazole for fungi counterpart following the method of Sarker *et al.*[12].

MIC of MIME and MIHE: MIC is defined as the lowest concentration of extracts that inhibit the visible growth of organisms on agar surface or turbidity in micro well broth. MIC of MIME and MIHE for the strains of bacterial and fungal organisms was estimated following the method outlined by Ayoola *et al.*[11].

Experimental animals: 35 female pale-skinned Wistar rats, with weights ranging from 110g to 245g, chosen for the investigation, were kept up in polypropylene confines and had admittance to food and water not obligatory. All research protocols were in compliance with University of Ibadan Ethics Committee on Research in Animals (15/0208/UI/ECRA) as well as international accepted principles for experimental animal use and care.

Assessment of rats' weight: In all the groups, the weight of each rat was noted at the beginning of the wound healing study, then once weekly and finally at the day of sacrificing the animals[13].

Estimation of MIHE and MIME wound healing activity: Formulation of ointment- extracts: 5% and 10% (v/v) MIHE and MIME seeds were prepared by mixing 2.5 ml and 5ml of each extract in yellow soft paraffin collected from store unit in Chemistry Department of University of Ibadan following the methods of Ajayi *et al.*[6] and Carter[14].

Wound excision model: The wound healing activity of MIHE and MIME was looked into with the use of excision wound model. The rats were distributed into five groups of n=7 each and the extracts/ointments formulated applied topically on each of them once a day. The animals were anaesthetized prior to and during the creation of experimental wounds with ketamine hydrochloride (100mg/kg b. wt) i.p.[15]. Rats were then perpetrated with extraction twisted by the strategy portrayed by Anusha[16]. The dorsal hide of the dorsolateral flank zone was shaved with scissors. After injury region arrangement with 70% liquor, the skin from the foreordained shaved zone was extracted to its full thickness to acquire an injury zone of around 200 mm^2 utilizing forceps, a careful cutting edge and scissors. Excision wounds were made on the dorsal thoracic area 1.5cm from the vertebral section on one or the other side.

Haemostasis was accomplished by smudging the injury with a q-tip absorbed ordinary saline. The injury on the creatures was left open and treated utilizing the figured concentrates the treatment for animals in group 1 was ointment base (control), groups 2 and 3 ones received 5% (v/v) of MIHE and MIME ointments while those of the ones in groups 4 and 5 were given 10% (v/v) of MIHE and MIME ointments respectively.

The animals were closely inspected on one-on-one basis for any infection so as to exclude any infected one from the study.

Estimation of Wound healing activity: To evaluate the wound healing ability of the prepared formulations, the following parameters were monitored: i. Wound area (mm^2) of the excision wound, ii. Rate of wound contraction and epithelialisation time (excision wound), iii. Histopathological studies of healed tissues.

Wound healing was estimated by tracing the wound on the first, 4th, 8th, 12th, 16th and 20th post wounding days. Wound closure was measured at regular intervals to calculate the percentage wound closure and epithelialisation time which indicates the formation of new epithelial tissue to cover the wound was determined[17].

Rate of wound contraction: The rate of wound compression was estimated as rate decrease of size at each 4-day stretch. Consistent reduction in the wound size was noted by utilizing clear paper and a sharpie; the injury region was measured graphically to screen the level of wound contraction which showed new epithelial tissue to cover the injury. The percentage wound contraction was determined using the following formula:

$$\text{Percentage wound contraction} = \frac{Ao - At}{Ao} \times 100$$

Where A_o = Initial area of wound at day "0'' of the experiment, A_t = Area of wound at day "t'' of experiment.

Period of epithelialisation was given by the number of days required for filling of the scar without any residual of the raw wound.

Hispathological analysis: The skin, liver, heart, spleen, lungs and kidney were gathered on the 21st day of the analysis from all the five gatherings of rats and prepared for histological examination to decide the example of set down for collagen for the skin and to check the impact of effective utilization of concentrates on interior organs. The skin and inside organs examples from the treated creatures were gathered in 10% cradled formalin[6,18], exposed to segment and 6 mm thickness areas of each stained with hematoxylin and eosin[19]. After this, the stained slides were pictured for histological changes under a light magnifying lens[20].

Haematological analysis: The method of Dacie and Lewis[21] was followed in determining the haemoglobin concentration (Hb), red blood cell (RBC), packed cell volume (PCV) and white blood cell (WBC) of the blood samples of rats from the different groups. In order to do this, 3ml of blood was collected into heparinized vials by cardiac puncture and stored at 10°C for analysis the same day. The results obtained were used to calculate the mean corpuscular haemaglobin (MCH), mean corpuscular haemaglobin concentration (MCHC) and mean corpuscular volume (MCV)[22-24]. Two-thirds of microhaemocrit capillary tubes were filled to mark with well mixed venous blood and sealed with plasticine at one end after which they were placed in microhaematocrit centrifuge. The tubes, after having the safety cover securely screwed on, were centrifuged for 5 mins at 10,000 revolutions per mins. Micro-haemocrit reader was then used to read the volume of the RBC.

$$\text{MCH} = \frac{Hbx\ 10}{RBC} \quad \text{MCHC} = \frac{Hbx\ 100}{PCV}$$

$$\text{MCV} = \frac{\text{PCV x 10}}{\text{RBC}}$$

Statistical analysis: Statistical analysis was performed on each group and ANOVA test (IBM SPSS version 20) was used to compare the mean value of each treatment. Significant differences between the means of parameters were determined by using the Dunnett T test ($P < 0.05$) and "Bonferroni posttests for grouped data. The results represented means and standard deviation of four replicated determinations.

Results and discussion

Weight of seed samples: The seeds of the plant used for this study was evaluated on weight per weight basis and used for determination of percentage fruits, seeds and kernels composition. As seen from Table-1, the mango fruit, seed and kernel have an average weight of 124.66±5.32 g, 28.04±3.05 g and 23.86±2.93 g respectively.

Phytochemical analysis: The presence of various chemical constituents such as glycosides, phenols and tannins were revealed from the result of the phytochemical analysis of MIHE and MIME (Table-1). Phenols and tannins were detected in MIME but glycosides were in both MIME and MIHE. According to Scortichini[25] and Sasidhran *et al.*[26], terpenoids, due to their astringent and high antimicrobial activities are believed to increase the efficiency of process of healing wound and is assumed as the one playing crucial role in wound contraction.

Antimicrobial activity: Result for antimicrobial activity of MIHE and MIME against fungal and bacterial is shown on Table-2. The concentrates from the seeds displayed great antimicrobial activity against all the tried microorganisms; *SA, EC, AN, CA* and *BS* diseases could be well dealt using MIHE and MIME. Medicinal plants have been utilized in people's medication and are found to be valuable in injury care, wound mending advancement and limiting agony/uneasiness and scarring of the patient[27]. MIHE and MIME effectively inhibited the growth of all organisms at different concentrations when compared to the negative and positive controls. *EC* and *PA* were more susceptible than *BS, SA* and *CA and AN* for both seed extracts. MIME had MIC of 12.5% against *BS, PA* and *SA* including *CA* and MIC of 25% against *AN*. It inhibited the growth of *EC* at all concentrations. 12.5% was the MIC of MIHE against *SA*, 25% against *EC, BS, PA* and *CA* and 50% against *AN*. Within the bacteria group, the highest activity displayed by MIME was against *E.coli* with 20.00±0.00mm inhibition diameter zone, the next was *BS* and *SA* (18.00±0.00mm). MIHE had inhibition zone in diameter of 16.00±0.89mm, 16.00±1.00 mm and 16.00±0.00mm against *EC, PA* and *SA* respectively and then *BS* (14.00±0.00mm). Among the fungi group, MIME was most active against *CA* (16.00±0.03mm) while *CA* and *AN* were maximally inhibited by MIHE with 14.00±0.00 mm and 14.00±0.03mm inhibition diameter zone respectively.

Minimum inhibitory concentration (MIC): MIC of MIHE and MIME indicated that the extracts have good antibacterial and antifungal activities (Table-2). Further assay was prepared with different concentrations of the seed extracts against the tested organisms and they all proved to be sensitive. MIME inhibited *EC and SA* from growing at a concentration of 25%; and *BS* and *PA* (bacteria) and *AN* and *CA* (fungi) at 50% concentration. On the other hand, the growth of all bacteria tested organisms was inhibited by MIHE at 50% concentration. The leaf extract of *E. singampattiana* was reported by Pavendan and Sebastian[28] to also be effective on *CA*.

Effects of seed extracts on weight of rats: At the beginning of the experiment, group 2 rats had the highest average body weight (243.57±34.67g), while that of group 1 was the lowest (128.00±18.35g) (Table-3). It was observed that the weight of the test and control rats increased throughout study period; the slight drop in the weight of rats in some groups could be as a result of adjustment or reaction to wound creation but it later improved over time with the highest average body weight still recorded for group 5 (263.33±15.37g) and the lowest for group 3 (163.00±2.45g). In the sub-chronic studies carried out by Musila *et al.*[3], there was no significant difference observed in weight gain of the test and control animals, this is an indication that there was no major toxic impact on the rats by the extracts. In like manner, MIME and MIHE ointments had no deleterious effect on the health state and body weight of the animals and they effectively healed the wounds.

Estimation of MIHE and MIME wound healing activity: Contraction of wound and time of epithelialisation: The rate at which the unhealed area during the healing process is reduced is referred to a wound contraction. It can then be deduced that the formulation that contracts wound the fastest is the best one for medication[29]. The fastest healing of wound was observed in group 3 that was treated with 5% (v/v) MIME ointment with the complete healing coming around 16.74±0.18 days as compared to group 1 17.83±2.34 days which was treated with the control (Table-4). Groups 2 and 4 rats treated with 5% and 10% of MIHE ointments got healed at 16.93±1.68 and 16.76±0.30 days respectively while the healing time for group 5 rats treated with 10 % MIME ointment was 17.77±1.13 days (Figure-1). Almost all groups had a better and faster epithelialisation time than the control base group which showed the fact that the extracts actually have an effect in accelerating the wound healing process. Natural products from plant sources have ability to heal wound due to their wound healing effects and serves as agent to the healing of wounds and this is more reliable due to their availability in almost everywhere, they are not toxic, the side of effects of these natural products are minimal and very efficient by preparing them as crude in formulations[30]. According to Sugana *et al.*[31], based on studies carried out on rats using *Centella asciatia* and *Terminalia chebula*, extracts from the two plants are highly effective in wound healing.

Seed extracts' effect on animal tissues: The primary organs that are usually affected by metabolic reaction caused by toxicant of experimental rats are heart, kidney, liver, lungs and spleen. After sacrificing the animals, these organs were taken and their weight noted for all animals in the groups to see if the seed extracts had any adverse effect on them. The weight of an organ is an important indication of the pathological and physiological state in Wistar rats; it helps in knowing if the organ has been injured or not. Liver, which is susceptible to damage caused by any large variety of chemicals, is the key organ that metabolises and detoxifies xenobiotics[32]. Different organs have varied weight (Table-5) but no toxic effect was seen on the weight of the internal organs of each rat in all the groups *vis-a-vis* that of their body. The seed extracts seemed to have no negative impact on the organs' weight.

Haematological experiment: Functions of extracts from plant that has to do with blood can be evaluated using haematological parameters. One of the most vulnerable targets of harmful compounds is haemopoietic system; it is a significant bindex of status in both humans and animals physiologically and pathologically[13]. Haematological analysis result of blood of animals in all the groups is given on Table-6. There was no significant effect of MIME and MIHE on the parameters examined in the rats' blood from all the groups for the period of study. This is similar to the one for *P. eldarica* extract; that it had no negative effect on most haematological parameters evaluated in sub-acute study[19].

Histopathological analysis: Histopathology of the wound scar area: Histological assessment of parts of the rats' skin gathered from the injury zones that were treated for 21 days with MIME and MIHE balms uncovered the presence of a developed granulation tissue in virtually all the profundity of the dermis for wound model extraction (Figure-2). According to the definition of Chattopaddhya *et al.*[33], healing of wound is complex in nature and a dynamic process that involves tissue structure restoring back to the previous state. It is dependent on the tissue repairing ability, type and extent of damage *vis-à-vis* overall host's health status.

Histopathology of internal organs: The result for the histopathological analysis carried out on the organs displayed that none of the organs of group 4 rats treated with 10 % MIHE had any lesion (Table-7). Ajayi and Ifedi[22] gave report in literature that the heart, kidney and liver of the control and test rats fed with *P. longifolia* seed flour did not show any lesion indicating that the seed flour was probably harmless to these organs. Similarly, lesion was not seen in the heart of all the animals, except the ones from group 5 that received 10% MIME (Figure-3). As a matter of fact, all the organs of group 5 rats had some slight lesion. This is in line with the report by Nath and Yadav[35] that various toxicological changes that are treatment related were noticed in the liver and kidney of animals that were given 800 mg/kg dose of extract for a period of 14 days.

Table-1: Weight (g) and Phytochemical constituents of seeds.

Sample	Total weight			Average weight			Percentage weight (%)		
	Fruit	Seed	Kernel	Fruit	Seed	Kernel	Seed/fruit	Kernel/seed	Kernel/fruit
Mango	1246.6	280.4	238.6	124.66±5.32	28.04±3.05	23.86±2.93	20.14	60.71	12.22
Phytochemical constituents of the seed extracts									
Phytochemical				*M. indica* seed extracts			Methanol		
Tannins				Hexane			+		
Alkaloids				-			-		
Terpenoids				-			-		
Steroids				-			-		
Glycosides				+			+		
Phenols				-			+		
Anthocyanins				-			-		

Table-2: Antimicrobial activities and MIC of seed extracts against pathogenic microorganisms.

Conc.%	Zone of inhibition diameter (mm). Test organisms.						
	S. aureus	*E. coli*	*B. subtilis*	*P. aerugonisa*	*C. albicans*	*A. niger*	Groups
100	18.00±0.00[a]	20.00±0.00[a]	18.00±0.00[a]	18.00±0.00[a]	16.00±0.03[a]	14.00±0.00[a]	MIME
50	14.00±0.00[a]	18.00±0.00[a]	14.00±0.00[a]	14.00±0.01[a]	14.00±0.00[a]	12.00±0.00[a]	
25	12.00±0.09[a]	14.00±0.00[a]	12.00±0.00[a]	12.00±0.03[a]	12.00±0.00[a]	10.00±0.00[a]	
12.5	10.00±0.00[a]	12.00±0.00[a]	10.00±0.00[a]	10.00±0.00[a]	10.00±0.00[a]	-	
6.25	-	10.00±0.00[a]	-	-	-	-	
100	16.00±1.00[a]	16.00±0.89[a]	14.00±0.00[a]	16.00±0.00[a]	14.00±0.00[a]	14.00±0.03[a]	MIHE
50	14.00±0.00[a]	14.00±0.30[a]	12.00±0.00[a]	14.00±0.00[a]	12.00±0.00[a]	12.00±0.00[a]	
25	12.00±0.00[a]	10.00±0.00[a]	10.00±0.02[a]	10.00±0.00[a]	10.00±0.00[a]	-	
12.5	10.00±0.00[a]	-	-	-	-	-	
6.25	-	-	-	-	-	-	
	Minimum Inhibition Concentration (MIC)						
100	-	-	-	-	-	-	MIME
50	-	-	+	+	+	+	
25	+	+	+	+	+	+	
12.5	+	+	+	+	+	+	
100	-	-	-	-	-	-	MIHE
50	+	+	+	+	+	+	
25	+	+	+	+	+	+	
12.5 %	+	+	+	+	+	+	

*Values are expressed as mean ± SD of three experiments. Data with different superscript letters along the same column are significantly different ($p<0.05$) using one ANOVA followed by Dunnett's test, +ve control = Gentamicin (for bacteria), 70% Triconazole (for fungi). -ve control = hexane and methanol (Solvent of dilution).

Table-3: Experimental animals' body weight[£].

Groups	Weight (g)[$]				
	Day 0	Day 4	Day 8	Day 12	Day 16
1*	128.00±18.35^{c*}	139.33±16.43^{def*}	132.67±22.53^{de*}	154.75±21.26*	167.33±7.71^{def*}
2	243.57±34.67^{a}	237.33±36.38^{a}	228.67±32.71^{ab}	219.00±49.53^{bc}	258.33±11.73^{a}
3	134.00±22.80^{c}	140.00±16.26^{b}	143.33±25.72^{cde}	148.00±25.78^{cd}	163.00±2.45^{def}
4	195.71±43.64^{b}	200.67±15.17^{ab}	181.67±26.13^{bcde}	176.66±17.44^{bc}	198.33±18.19^{bcd}
5	235.86±28.00^{ab}	225.33±16.32^{a}	255.67±26.96^{a}	254.00±24.91^{a}	263.33±15.37^{a}

*****Control was used in conjunction with other experiments (8). [£]Values are expressed as mean ± SD for groups of four animals each. [$]Data with different superscript letters along the same column are significantly different ($p<0.05$) using one ANOVA followed by Dunnett's test.

Table-4: Effect of topical application of seed extracts on wound healing area, period of epithelialisation and % wound contraction[£].

Groups	Wound healing area (mm ^{2})					Epithelialisation Time (day)
	Day 0	Day 4	Day 8	Day 12	Day 16	
1*	200±0.00^{a*}	193.00±7.59^{a*}	160.00±10.00^{a*}	96.00±4.76^{ab*}	37.00±4.73^{a*}	17.83±2.34^{ab*}
2	200±0.00^{a}	190.00±19.15^{a}	143.00±17.00^{ab}	103.33±3.86^{ab}	30.00±8.16^{ab}	16.93±1.68^{bc}
3	200±0.00^{a}	196.67±4.71^{a}	90.00±10.00^{de}	37.00±12.47^{cd}	6.70±2.36^{d}	16.74±0.18^{bc}
4	200±0.00^{a}	196.67±4.71^{a}	113.00±33.00^{bcde}	43.00±9.43^{cd}	8.30±2.36^{d}	16.76±0.30^{bc}
5	200±0.00^{a}	196.67±2.36^{a}	100.00±10.00^{cde}	56.00±4.76^{c}	20.00±8.16^{c}	17.77±1.13^{ab}
	Percentage wound healing (%)					
	Day 4		Day 8	Day 12	Day 16	
	3.50±4.26^{a*}		20.00±5.00^{e*}	52.00±2.38^{cd*}	81.50±2.36^{d*}	
	5.00±3.07^{a}		28.50±8.95^{de}	48.33±2.90^{cd}	85.00±5.00^{cd}	
	1.67±1.35^{a}		55.00±5.00^{ab}	81.50±7.67^{ab}	96.65±1.18^{a}	
	1.67±1.36^{a}		43.50±16.43^{abcd}	78.50±4.72^{ab}	95.85±1.18^{a}	
	1.67±1.18^{a}		50.00±0.00^{abc}	72.00±2.38^{b}	90.00±5.00^{b}	

*****Control was used in conjunction with other experiments (8). [£]Values are expressed as mean ± SD for groups of four animals each. Data with different superscript letters along the same column are significantly different ($p<0.05$) using one ANOVA followed by Dunnett's test.

Table-5: Weight of tissues collected from the experimental animals (g)[£.]

Animal groups	Liver	Kidney	Heart	Lung	Spleen
1*	5.20±0.60^{a*}	0.94±0.10^{a*}	0.76±0.38^{a*}	1.39±0.14^{a*}	1.13±0.16^{a*}
2	7.99±0.72^{a}	1.38±0.21^{a}	0.88±0.08^{a}	3.18±1.14^{a}	1.21±0.43^{a}
3	5.49±0.41^{a}	0.88±0.13^{a}	0.55±0.24^{a}	1.38±0.28^{a}	0.75±0.20^{a}
4	6.55±1.48^{a}	1.09±0.14^{a}	0.75±0.19^{a}	2.04±0.19^{a}	1.06±0.15^{a}
5	8.11±1.00^{a}	1.33±0.28^{a}	0.86±0.05^{a}	2.12±0.53^{a}	0.91±0.11^{a}

*Control was used in conjunction with other experiments (8). [£]Values are expressed as mean ± SD of four animals. Data with different superscript letters along the same column are significantly different ($p<0.05$) using one ANOVA followed by Dunnett's test.

Table-6: Result of haematological analysis of the experimental rats.

Group	PCV	Hb	RBC	WBC	PLA	LYM	NEUT	MON	EOS	Absolute Count				mc MCH	MCHC	MCV
	(%)	(g/dl)	(L)	($x10^3$ L)	($x10^4$L)	(%)	(%)	(%)	(%)	LYM ($x10^3$L)	NEU ($x10^3$L)	MON ($x10^3$L)	E0S ($x10^3$L)	(Pg) (Pg)		(Fl)
1*	41.00	13.63	6.79	7.96	19.98	70.25	25.25	1.75	2.75	5.59	2.01	0.14	0.22	20.07	33.24	60.38
	±2.12[a]	±0.67[a]	±0.42[a]	±1.32[a]	±1.24[a]	±3.86[a]	±3.30[a]	±0.50[a]	±1.25[a]	±0.93[a]	±0.33[a]	±0.02[a]	±0.04[a]	±0.04[a]	±0.06[a]	±0.17[a]
2	37.75	12.78	6.39	5.51	12.83	66.25	29.00	2.25	2.50	3.65	1.60	0.12	0.14	20.00	33.85	59.07
	±3.35[a]	±0.97[a]	±0.72[a]	±0.41[a]	±2.68[a]	±7.68[a]	±8.04[a]	±0.96[a]	±0.58[a]	±5.09[a]	±2.33[a]	±0.02[a]	±0.01[a]	±0.07[a]	±0.07[a]	±0.27[a]
3	40.00	13.33	6.71	8.51	21.73	68.00	27.00	2.25	2.75	5.79	2.30	0.19	0.23	19.87	33.33	59.61
	±0.00[a]	±0.27[a]	±0.04[a]	±0.77[a]	±1.70[a]	±3.74[a]	±4.00[a]	±0.96[a]	±0.96[a]	±2.54[a]	±1.08[a]	±0.02[a]	±0.03[a]	±0.04[a]	±0.02[a]	±0.01[a]
4	38.50	12.88	6.55	5.86	19.38	61.25	35.00	1.75	2.00	3.59	2.05	0.10	0.12	19.66	33.50	58.78
	±0.87[a]	±0.55[a]	±0.14[a]	±0.92[a]	±4.67[a]	±3.77[a]	±4.24[a]	±0.96[a]	±0.82[a]	±2.31[a]	±1.48[a]	±0.02[a]	±0.02[a]	±0.01[a]	±0.01[a]	±0.05[a]
5	33.75	11.18	5.41	5.96	12.04	62.75	33.25	2.75	1.25	3.74	1.98	0.16	0.075	20.67	33.13	62.38
	±3.77[a]	±1.43[a]	±0.90[a]	±0.50[a]	±4.41[a]	±3.86[a]	±4.35[a]	±0.50[a]	±0.96[a]	±2.42[a]	±1.45[a]	±0.01[a]	±0.01[a]	±0.09[a]	±0.03[a]	±0.30[a]

*Control was used in conjunction with other experiments (8). [£]Values are expressed as mean ± SD for groups of four animals each. Data with different superscript letters along the same column are significantly different ($p<0.05$) using one ANOVA followed by Dunnett's test, *****PVC=Packed cell volume, Hb= Heamoglobin, RBC= Red blood cell, WBC= White blood cell, MONO=Monocyte, EOS=Eosinophil, LYM=Lymphocyte, NEUT=Neutrophil, MCH=Mean corposcular haemoglobin¸ MCHC=Mean corpuscular haemoglobin concentration, MCV= Mean corpular haemaglobin.

Table-7: Result of histophathology of organs. *Control was used in conjunction with other experiments (8).

Groups	Internal organs				
	Heart	Kidney	Liver	Lungs	Spleen
1*	No visible lesion. Cardiomyocytes appear normal.	No visible lesion. Glomeruli and tubules appear normal.	The hepatic plates are closely-packed [normal]. There is moderate Kupffer cell hyperplasia.	There are locally extensive foci of moderate proliferative thickening of the inter-alveolar spetae.	There are multiple fairly large and well-spaced PALSs. There is moderate congestion of the splenic sinuses and sinusoids.
2	No visible lesion. Cardiomyocytes appear normal.	There are a few foci of mild sloughing off of renal tubular epithelium. There is mild congestion of renal blood vessels.	The hepatic plates are closely-packed [normal].	No visible lesion.	There are multiple fairly large and well-spaced PALSs. There is moderate congestion of the splenic sinuses and sinusoids.
3	No visible lesion. Cardiomyocytes appear normal.	No visible lesion seen in glomeruli and tubules.	The hepatic plates are closely-packed. No visible lesion	The airways are clear. No visible lesion.	There are multiple fairly large and well-spaced PALSs. There is moderate congestion of the splenic sinuses and sinusoids.
4	No visible lesion. Cardiomyocytes appear normal.	Normal	Normal	Normal	There are multiple fairly large and well-spaced PALSs. There is moderate congestion of the splenic sinuses and sinusoids.
5	There are multiple foci of degeneration of cardiomycotes as well as increased amounts of fibrous connective tissue. There is moderate congestion of coronary blood vessels.	There are few foci of intraluminal proteinaceous tubular casts.	There are few foci of mild vascuolar change of hepatocytes. There are random foci of single-cell hepatocellular necrosis Random foci of single-cell hepatocellular necrosis.	There are few foci of mild proliferative thickening of the Inter-alveolar spetae. The airways and alveoli are clear.	There are mulitiple fairly large and coalescing PALS. There is moderate congestion of the splenic sinuses and sinusoids.

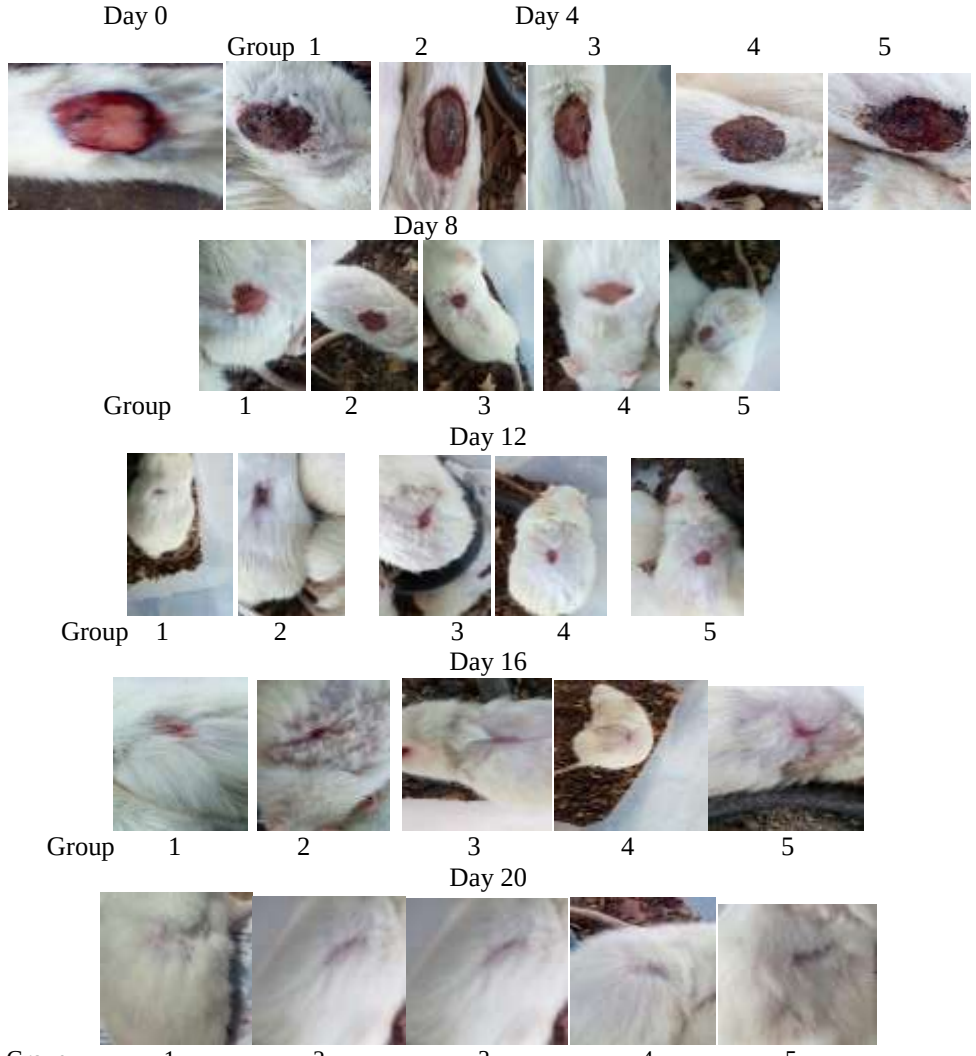
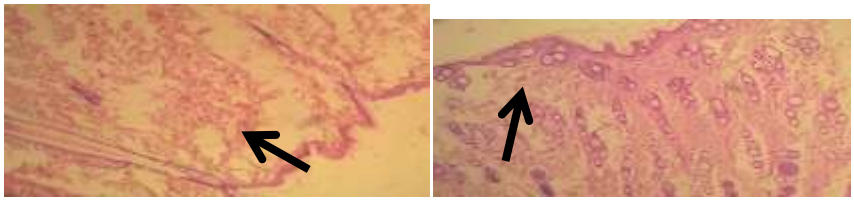


Figure-1: Photograph showing various stages of wound healing activity of MIHE and MIME. Control was used in conjunction with other experiments[8].



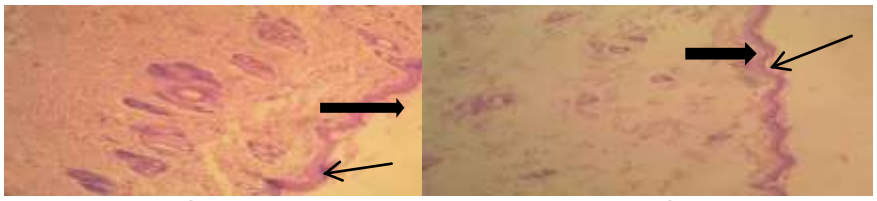
A i A ii

Figure-2A i & ii: Photomicrograph of healed wound skin area for group 1 treated with control base ointment (H&E x100): (i) The epidermis (arrow) is keratinized stratified squamous. NVL in dermis. (ii) Epidermis is keratinizing. There are numerous sebaceous glands (arrows) in the dermis. Control was used in conjunction with other experiments (8).

B i B ii

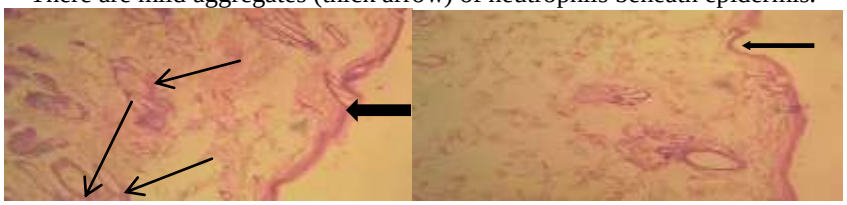
Figure-2B i & ii: Photomicrograph of healed wound skin area for group 2 treated with 5 % v/v of MIHE ointment (H&E x100):

(i) There is mild ballooning degeneration (arrow) of epidermal cells. (ii) The epidermis is mildly thickened.



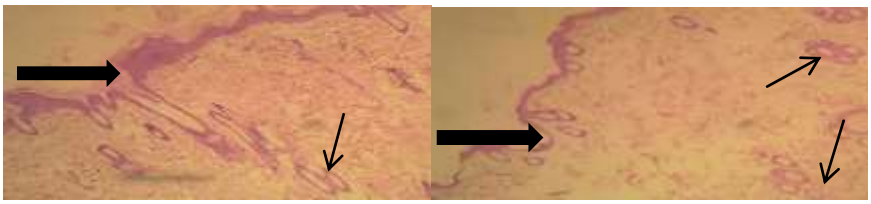
Ci C ii

Figure-2C i & ii: Photomicrograph of healed wound skin area for group 3 treated with 5 % v/v of MIME ointment (H&E x100): (i) There is mild thickening of the epidermis. (ii) There are a few foci of ballooning degeneration (thin arrow) of epidermal cells. There are mild aggregates (thick arrow) of neutrophils beneath epidermis.



D i D ii

Figure-2D i & ii: Photomicrograph of healed wound skin area for group 4 treated with 10 % v/v of MIHE ointment (H&E x100): (i) There are foci of discontinuing of epidermis (thick arrow). There are numerous hair follicles (thin arrows). (ii) There are foci of thinning out of epidermis.



E i Eii

Figure- 2E i & ii: Photomicrograph of healed wound skin area for group 5 treated with 10% v/v of MIME ointment (H&E x100): There appears to be re-epithelialization (thick arrows) at previously ulcerated margins. There are numerous hair follicles (thin arrows).

Figure-2: Photomicrograph of healed wound skin area for all test groups.

Figure-3.1a: Photomicrograph of heart for group1 animals treated with control base ointment (H&E x400).

Figure-3.1b**:** Photomicrograph of heart for group 2 animals treated with 5% v/v MIHE ointment (H&E x400).

Figure-3.1c: Photomicrograph of heart for group 3 animals treated with 5% v/v MIME ointment (H&E x400).

Figure-3.1d: Photomicrograph of heart of rats treated with 10 % v/v MIHE ointment (H&E x 400).

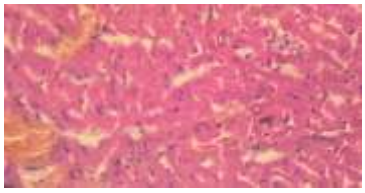


Figure-3.1e: Photomicrograph of heart of rats treated with 10 % v/v MIME ointment (H&E x 400).

Figure-3.1: Photomicrograph of heart for all groups. Control was used in conjunction with other experiments[8].

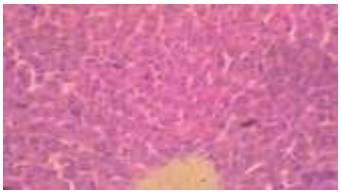


Figure-3.2a: Photomicrograph of liver of group 1 animals treated with control base ointment (H&E x400).

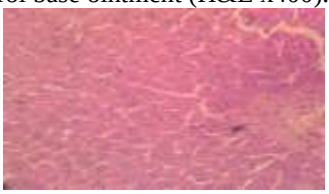


Figure-3.2b: Photomicrograph of liver of group 2 animals treated with 5% v/v MIHE ointment (H&E x400).

Figure-3.2c: Photomicrograph of liver of group 3 animals treated with 5% v/v MIME ointment (H&E x400).

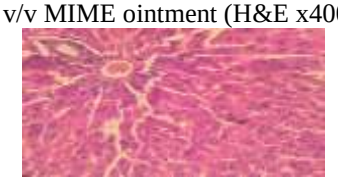


Figure-3.2d: Photomicrograph of liver of group 4 animals treated with 10% v/v MIHE ointment (H&E x400).

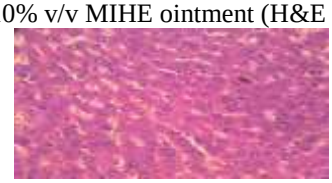


Figure-3.2e: Photomicrograph of liver of group 5 animals treated with 10% v/v MIME ointment (H&Ex400).

Figure-3.2: Photomicrograph of liver for all groups. Control was used in conjunction with other experiments (8).

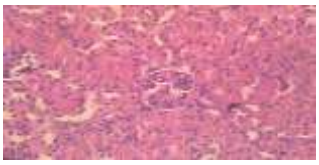


Figure-3.3a: Photomicrograph of kidney of group 1 animals treated with control base ointment (H&E x400).

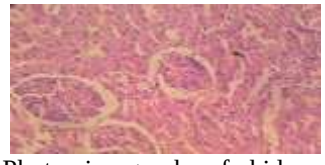


Figure-3.3b: Photomicrograph of kidney of group 2 animals treated with 5% v/v MIHE ointment (H&E x400).

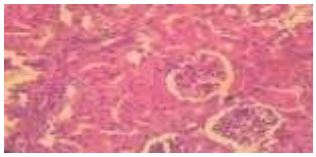


Figure-3.3c: Photomicrograph of kidney of group 3 animals treated with 5% v/v MIME ointment (H&E x400).

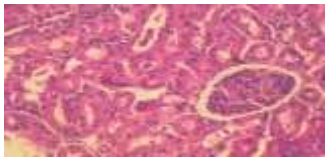


Figure-3.3d: Photomicrograph of kidney of group 4 animals treated with 10% v/v MIHE ointment (H&E x400).

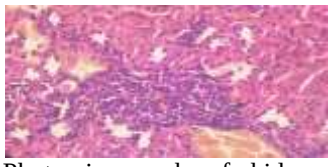


Figure-3.3e: Photomicrograph of kidney of group 5 animals treated with10% v/v MIME ointment (H&E x400).

Figure-3.3: Photomicrograph of kidney for all groups. Control was used in conjunction with other experiments (8).

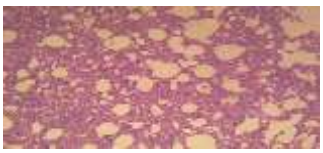


Figure-3.4a: Photomicrograph of lungs of group1 animals treated with control base ointment (H&E x100).

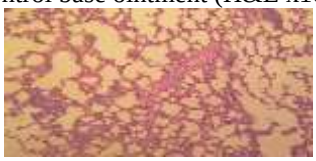


Figure-3.4b: Photomicrograph of lungs of group 2 animals treated with 5% v/v MIHE ointment (H&E x100)

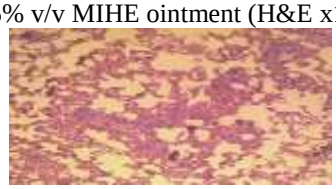


Figure-3.4c: Photomicrograph of lungs of group 3 animals treated with 5% v/v MIME ointment (H&E x100).

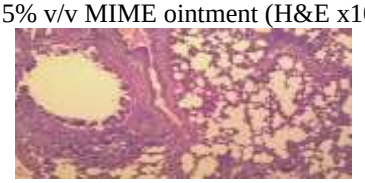


Figure-3.4d: Photomicrograph of lungs of group 4 animals treated with 10 % v/v MIHE ointment (H&E x100)

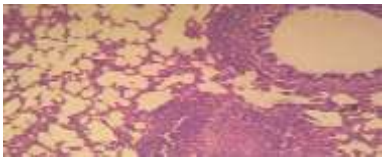


Figure-3.4e**:** Photomicrograph of lungs of group 5 animals treated with 0% v/v MIME ointment (H&E x100).
Figure-3.4: Photomicrograph of lungs for all groups. Control was used in conjunction with other experiments (8).

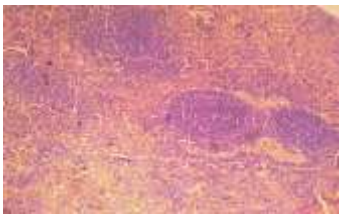


Figure-3.5a: Photomicrograph of spleen of group 1 animals treated with base ointment (control) (H&E x100).

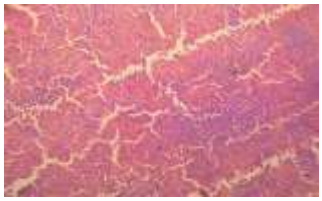


Figure-3.4b: Photomicrograph of spleen of group 2 animals treated with 5% v/v MIHEointment (H & E x 400).

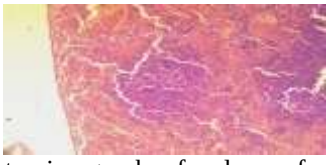


Figure-3.4c: Photomicrograph of spleen of group 3 animals treated with 5% v/v MIME ointment (H&E x100).

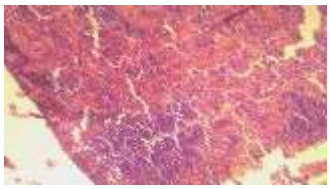


Figure-3.5d: Photomicrograph of spleen of group 4 animals treated with 10% v/v MIHE ointment (H&Ex100).

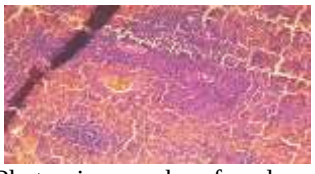


Figure-3.5e: Photomicrograph of spleen of group 5 animals treated with10% v/v MIME ointment (H&E x100).
Figure-3.5: Photomicrograph of spleen for all groups. Control was used in conjunction with other experiments (8).

Conclusion

The histopathological and hematology results, alongside the injury constriction rate and epithelialisation time, showed the injury mending capability *M. indica* seeds. *M. indica* seeds may accordingly be helpful inhealing of wounds.

Acknowledgement

Chemistry Department, Faculty of Science and Veterinary Pathology unit, Faculty of Veterinary both in University of Ibadan, Ibadan, Nigeria are appreciated by the authors for making their equipment available for this study.

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