Evaluation of the Wound Healing Activity of Mangifera indica Seed Extract in Wistar Rats

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ABSTRACT

Relevance of Study: *Mangifera indica* is among the plants of high medicinal value in ethno medicines system in Nigeria for the treatment of various ailments. The plant different parts have been used extensively but the scientific investigation of wound healing activity of the seed is lacking which makes it necessary to have a precise record of the full medicinal use of the seed extract of this plant to expand its use and to include their integration into modern medical healthcare systems. This study was carried out to investigate the wound healing activity of the seed extract of this plant using wistar rats.

Materials and Method: Phytochemical analysis was determined to know the various phytoconstituents in the seed extract. The antimicrobial activity of the extract was examined on multiple drug resistant bacteria viz: *S. aureus*, *E. coli, B. subtilis, P. aeroginosa* and fungi viz: *C. albicans, A. niger* using agar technique of pour plate and surface plate dilution. . The wound healing effect *M. indica* seed extract was studied by incorporating the hexane and methanol extracts into paraffin in concentrations of 5 % and 10 % (v/v). 35 albino rats divided into five groups containing 7 animals per group were used for the experiment which lasted for a period of 21 days. The rats which had free access to water were fed *ad-libitum* and the weight was taken on a four day basis. Wound healing activities of the extracts were studied by measuring the wound area (mm^2) and percentage of wound closure on the 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th} day. The wound epitheliasation was noted from the 16^{th} to 20^{th} of the experiment. Histopathological analysis of the control and test groups' skin tissues, liver, kidney, heart, lung and spleen were carried out while haematological analysis of the blood parameter was evaluated at the end of the experiment.

Results: Phytochemical analysis revealed the presence of various chemical constituents such as tannins, glycosides and phenols. *M. indica* methanol extract was found to have MIC value of 12.5 % against *S. aureus, B. subtilis*, P. *aeroginosa,* and *C. albicans* while MIC value of 25 % was recorded against *A. niger*. *M. indica* hexane extract was found to have MIC value of 12.5 % against *S. aureus*, MIC of 25 % against *E. coli, B. subtilis, P. aeroginosa* and *C. albicans* and a MIC of 50 % against *A. niger*. The fastest epithelialisation time was observed in group 3 which was treated with 5 % (v/v) methanol extract ointment formulate of *Mangifera indica* with the healing time of 16.74±0.18 days as compared with group 1 (control) treated with the ointment. Almost all the groups treated with both methanol and hexane extract showed a better and faster epithelialisation time than the control group. The results of the histopathology and haematology analysis showed no significant difference between the test and control groups. This is an indication that the extracts contain chemical constituent that accelerated the wound healing process.
Conclusion: *M. indica* seed extract was found to have good efficacy for wound healing and might therefore serve as a good replacement in treatment of wound.

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

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1. INTRODUCTION

Mangifera indica are largely cultivated in tropical West Africa. The seeds have been found to show some antibiotic properties (1). Mango is a juicy stone fruit (drupe) belonging to the genus *Mangifera*, consisting of numerous tropical fruiting trees, cultivated mostly for edible fruit. The majority of these species are found in nature as wild mangoes. They all belong to the flowering plant family *Anacardiaceae*. It is found majorly in tropical and equatorial Africa, preferably in deep, loamy-sandy and well drained soils. Mango tree does not tolerate stagnant water, frequent or prolonged foods. It is native to northern India, the foot of Himalayas. Cultivated and very common in more of less humid tropical areas provided it is protected from live stock in its early stage (1).

Mango is naturalized in West Africa. The barks and leaves have stringent properties and are used in Nigeria as a lotion to relieve in toothache, sore gums and sore throat or as in infusion in diarrhea and dysentery, Mango roots have been found to be good in treatment of insomnia, diabetes, stomatitis–weakness, its bark, fruit and leaves are known to be useful in 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). This study evaluates the wound healing potential of *M. indica* seeds.

2. MATERIALS AND METHOD

2.1 Plant Material

The fruits of *M. indica* were bought from a local market in Ibadan, Oyo State. Fruit samples were weighed before the start of the experiment to calculate the following.

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

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

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

2.2 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 500 g of dried powdered samples were put in 2 aspirator bottles, 1 L of methanol was added to one and 1L hexane added to the other and left at room temperature (30 °C) for 5 days with continuous stirring to allow it to mix properly with the solvent after which it was filtered (3). The filtrate was then distilled to recover the solvent from the oil for further analysis. The extract was stored in universal bottle and refrigerated at 4 °C prior to use.

2.3 Phytochemical Analysis

Different chemical test was carried out on each extracts to check for presence of phytoconstituents. Qualitative test was carried out to detect the presence of secondary metabolite like terpenoids, phenols, glycosides, tannins; anthocyanins were examined using technique of Harborne (4) and Sofowora (5).

2.4 Antimicrobial activity of the seed extracts

2.4.1 Test microorganisms

Multi Drug Resistant (multi)clinical isolates of *Aspergillus niger* and *Candida albicans* were used as the fungal tested organisms. *Pseudomonas aeroginosa*, *Escherichia coli*, *Bacillus subtilis, Bacillus cerus* were used as the bacterial tested organisms. The pure bacterial and fungal strains were obtained from the Department of Pharmacy, University of Ibadan, Ibadan, Nigeria. The bacterial strains were cultured overnight at 37 °C in nutrient agar (Oxoid, Hampshire, UK) while fungal strains were cultured overnight at 28 °C using potato dextrose agar (Oxoid).

2.4.2 Antimicrobial activity assay
The antimicrobial activity of the seed extracts were evaluated against a few pathogenic bacteria such as *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeroginosa*, and the fungal isolates like *Candida albicans* and *Aspergillus niger*.

2.4.2.1 Preparation of graded concentration of the sample
0.5 ml of the sample was weighed and dissolved into 5 mls of the solvent of extraction for proper dissolution; from which 2.5 mls was taken into another 2.5 mls of the solvent and this was taken to the 6^{th} test tube which was the last tube for the extract. The 7^{th} and 8^{th} test tube were negative and positive control (solvent-N-hexane and methanol, gentamycine) for bacteria and Tioconazole for fungi control of the experiment.

2.4.2.2 Pour plate method (bacteria)
An overnight culture of each organism was prepared by taking a loop full of the organism from stock and inoculated each into the sterile nutrient broth of 5 mls each incubated for 18-24 hrs at 37 ^{o}C. From overnight culture 0.1ml of each organism was taken and put into 9.9 mls of sterile distilled water to get 1:100 (10^{-2}) of the dilution of the organism. From the diluted organism (10^{-2}) 0.2 ml was taken into the prepared sterile nutrient agar which was at 45 ^{o}C then aseptically poured into sterile petridishes allowed to solidify for about 45-60 minutes. Using a sterile cork borer of 8 mm diameter the wells were made according to the number of graded concentration of the sample. In each well, the different graded concentrations of the sample were produced, this was done in duplicates. The plates were allowed to stay on the bench for 2 hrs to allow pre-diffusion. The plates were incubated uprightly in the incubator for 18-24 hrs at 37 ^{o}C.

2.4.2.3 Surface plate method (fungi)
A sterile Sabouraud Dextrose Agar (62 g/L) was prepared accordingly and aseptically poured into the sterile plates in duplicates and allowed to set properly 0.2 ml of the 10^{-2} of the agar using sterile spreader to cover all the surface of the agar. The wells were made using a sterile spreader to cover all the surface of the agar. The wells were made using a sterile cork borer of the 8 mm diameter. In each well, the graded concentration of the extract were introduced into the wells including the controls. The plates were left on the bench for 20 minutes so as to allow the extract to diffuse properly into the agar i.e. pre-diffusion. The plates were incubated uprightly in the incubator for 48 hrs at 26-28 ^{o}C.

2.4.2 Minimum inhibitory concentrations (MIC) of the seed extracts
Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the extracts that inhibit the visible growth on agar surface or turbidity in microwell broth. Minimum inhibitory concentrations both for bacterial and fungal strains were measured as reported in literature by Sarker *et al.* (6).

2.5 Experimental animals
35 wistar female albino rats, weighing 110-245 g were selected for the study. Animals were maintained in polypropylene cages with free access to food and water *ad libitum*. All experimental 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 laboratory animal use and care.

2.6 Evaluation of wound healing activity
The excision wound model was used to evaluate the wound healing activity of *M. indica* extracts. The rats were divided into five groups, each containing seven animals and the extracts/ointments formulated were applied topically once a day. The animals in group1 received ointment base (control), groups 2 and 3 animals were treated with 5 % (v/v) of *M. indica* hexane and methanol extract ointments, groups 4 and 5 were treated with 10 % (v/v) of *M. indica* hexane and methanol extract ointments. The animals were anaesthetized with ketamine hydrochloride (100 mg/kg, i.p.) prior to and during infliction of the wound (7). All animals were closely observed for any infection, so that the infected animals can be excluded from the study.

2.7 Ointment- extracts formulation
5 % and 10 % (v/v) hexane and methanolic extracts of *M. indica* seeds were prepared by mixing the extracts 2.5 ml and 5 ml of both extracts in yellow soft paraffin collected from Chemistry Department, University of Ibadan, store unit following the method of Carter (8).

2.8 Wound excision model

The animals were anaesthetized prior to and during the creation of experimental wounds with ketamine hydrochloride (100 mg/kg b.wt) i.p. (7). Rats were then inflicted with excision wound according to the method described by Anusha (9). The dorsal fur of the dorsolateral flank area was shaved with a scissors. After wound area preparation with 70 % alcohol, the skin from the predetermined shaved area was excised to its full thickness to obtain a wound area of about 200 mm^2 using forceps, a surgical blade and scissor. Excision wounds were created on the dorsal thoracic region 1.5 cm from the vertebral column on either side. Haemostasis was achieved by blotting the wound with a cotton swab soaked in normal saline. The wound was left open and all the animals were treated using the formulated extracts and the healing of wound was monitored by tracing the wound on the first, 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th} post wounding days. The wound closure was measured at regular intervals to calculate the percentage wound closure, epithelialisation time that indicates the formation of new epithelial tissue to cover the wound was determined (10).

2.9 Wound healing activity study

To evaluate the wound healing ability of the prepared formulations, the parameters like:

a. Wound area (mm^2) of the excision wound,
b. Rate of wound contraction and epithelialisation time (excision wound)
c. Histopathological studies of healed tissues

2.9.1 Rate of wound contraction

The rate of wound contraction was measured as percentage reduction of size at every 4 day interval. Progressive decrease in the wound size was measured periodically using transparency paper and a maker, and the wound area was measured graphically to monitor the percentage of wound closure which indicates new epithelial tissue to cover the wound. The percentage wound contraction was measured according to 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.

The number of days required for filling of the scar without any residual of the raw wound gave the period of epithelialisation.

2.9.2 Hispatological analysis

The skin, liver, heart, spleen, lungs and kidney were collected on the 21^{st} day of the experiment from all the five groups of animals and processed for histological study to determine the pattern of lay-down for collagen for the skin and to check the effect of topical application of extracts on internal organs. The skin and internal organs specimens from the treated animals were collected in 10 % buffered formalin and were subjected to sectioning and 6 mm thickness sections were stained with hematoxylin and eosin. The stained slides were visualized for histological changes under a light microscope.

2.10 Haematological analysis

For haematological analysis, 3 ml of blood were collected by cardiac puncture into heparinized vials and stored at 10 ^oC for analysis the same day. The packed cell volume (PCV), haemaglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts were determined using standard techniques as described by Dacie and Lewis (11). The differential WBC counts mean corpuscular volume (MCV) and mean corpuscular haemaglobin concentration (MCHC) were calculated (12, 13, 14). Microhaemocrit capillary tubes were filled to two-thirds mark with well mixed venous blood. One end was sealed with plasticine. The sealed tubes were placed in microhaematocrit centrifuge and the safety cover securely screwed on. The sealed capillary tubes were centrifuged for 5 mins at 10,000 revolutions per minutes. The volume of the red blood cell was read on the micro-haemocrit reader.

Mean Corpuscular Volume = $\frac{PCV \times 10}{RBC}$

Mean Corpuscular Haemoglobin = $\frac{Hbx\ 10}{RBC}$

Mean Corpuscular Haemoglobin Concentration = $\frac{Hbx\ 100}{PCV}$

2.11 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

a. Weight of seed samples

The seeds of the plant used for this study was evaluated on weight per weight which was used for determination percentage fruits, seeds and the kernels composition respectively. 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.

3.2 Phytochemical analysis

The result of phytochemical analysis of the extracts indicated the presence of various chemical constituents such as tannins, glycosides and phenols (Table 1). Tannins were detected in methanol extract of *M. indica*, Glycosides were detected in hexane and methanol extracts of *M. indica*; phenols was found in methanol extract of *M. indica.*

Table 1: Weight and Phytochemical constituents of seeds

Sample	Total weight			Average weight			Percentage weight (%)		
	Fruit	Seed	Kernel	Fruit	Seed	Kernel	Seed in fruit	Kernel in seed	Kernel in fruit
Mango (per 1)	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	***Mangifera indica***	
	Hexane	Methanol
Tannins	-	+
Alkaloids	-	-
Terpenoids	-	-
Steroids	-	-
Glycosides	+	+
Phenols	-	+
Anthocyanins	-	-

3.3 Antimicrobial activity

The result for antimicrobial activity of the extracts against fungal and bacterial is shown in Table 2. The seed extracts were found to effectively inhibit the growth of all organisms at different concentration as compared to both negative and positive control. Bacterial (*E. coli* and *P. aeroginosa*) were found to be more susceptible than (*S. aureus and B. subtilis*) and fungi (*C. albicans and A. niger*) for all seed extracts. *M. indica* methanol extract was found to have a MIC of 12.5 % against *S. aureus, B. subtilis, P. aeroginosa, C. albicans* and MIC of 25 % against *A. niger,* it was found to inhibit the growth of *E. coli* at all concentration. *M. indica* hexane extract was found to have a MIC of 12.5 % against S. *aureus*, MIC of 25 % against *E. coli, B. subtilis, P. aeroginosa and C. albicans* and a MIC of 50 % against *A. niger.*

Within the bacteria group, *M. indica* methanol extract showed a maximum activity against E. *coli* giving a zone of inhibition diameter of 20.00±0.00 mm followed by *B. subtilis and S. aureus* with a zone of inhibition of 18.00±0.00 mm; *M. indica h*exane extract showed a maximum activity against E. *coli,* S. *aureus* and P. *aeroginosa* giving a zone of inhibition diameter of 16.00±0.89 mm, 16.00±1.00 mm and 16.00±0.00 mm respectively followed by *B. subtilis* and with a zone of inhibition of 14.00±0.00 mm. Among the fungi group of methanol extract of *M. indica* seeds gave maximum activity against *C. albicans* giving a zone of inhibition diameter of 16.00±0.03 mm and *M. indica* hexane extract gave maximum activity against *C. albicans and A. niger* giving a zone of inhibition diameter of 14.00±0.00 mm and 14.00±0.03 mm respectively.

Table 2: Antimicrobial activities and Minimum Inhibition Concentration 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]	***Mangifera indica***
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]	**methanol**
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]	**extract**
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]	***Mangifera indica***
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]	**hexane extract**
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 %	-	-	-	-	-	-	***Mangifera indica***
50 %	-	-	+	+	+	+	
25 %	+	+	+	+	+	+	**methanol**
12.5 %	+	+	+	+	+	+	**extract**
100 %	-	-	-	-	-	-	***Mangifera indica***
50 %	+	+	+	+	+	+	
25 %	+	+	+	+	+	+	**hexane**
12.5 %	+	+	+	+	+	+	**extract**

*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)

3.4 Minimum inhibitory concentration (MIC)

The MIC of all the seed extracts as shown on Table 2 indicated that the extracts have good antibacterial and antifungal activities. Further assay was prepared with different concentration of the seed extracts against the tested organisms and they all proved to be sensitive. *M. indica* methanol extract inhibited growth of *E. coli and S. aureus* at a concentration of 25 % and bacteria *(P. aeroginosa and B. subtilis) and* fungi (*C. albicans and A. niger*) at a concentration of 50 %; *M. indica* hexane extract inhibited the growth of all bacteria tested organisms at a concentration of 50 %.

3.5 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±34.67 g), while group 1 had the lowest average body weight (128.00±18.35 g) (Table 3). It was observed that there was a body weight increase of the rats throughout the experiment and 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 in group 5 (263.33±15.37 g) and the lowest recorded for group 3 (163.00±2.45 g). This is a positive indication that the extracts of *M. indica* had no adverse effect on the health state and body weight of the animals and they effectively healed the wounds.

Table 3: **Experimental animals' body weight**

Animal groups	Weight (g)				
	Day 0	**Day4**	**Day8**	**Day12**	**Day16**
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. 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 percentage wound contraction

Animal 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 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 two ANOVA followed by Dunnett's test)

3.6 Wound healing activity study

3.6.1 Wound contraction and epithelialisation time

Wound contraction indicates the rate at which the unhealed area during the healing process is reduced. So from this we can deduce that the one that contract the fastest is the best formulation for medication (15). The rate of reduction in wound area of different animal groups by extracts of *M. indica* hexane and methanol extract is shown on Table 4. The fastest healing of wound was observed in group 3 which was treated with 5 % (v/v) methanol extract ointment of *M. indica* with the complete healing coming around 16.74±0.18 days as compared to group 1 17.83±2.34 days which was just treated with the control. Groups 2 and 4 rats which were treated with 5 % and 10 % hexane extract of *M. indica* got healed at16.93±1.68 and 16.76±0.30 days respectively while group 5 treated with 10 % methanol extract of *M. indica* had a healing time of 17.77±1.13 days. 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.

3.8 Effect of seed extracts on the tissues

The internal organs of the experimental rats in all the groups were measured to see if the seed extracts would have an adverse effect on them. There was variation in the weight of the different organs but this is as result of variation in body weight of experimental groups (Table 5). No adverse effect was recorded on the internal organs with respect to the body weight of each rat in all the groups.

Table 5: Weight of tissues collected from the experimental animals

Animal groups	Liver(g)	Kidney(g)	Heart(g)	Lung(g)	Spleen(g)
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. 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 two way ANOVA followed by Dunnett's test)

3.7 Haematological study

Presented on table 6 is the result of the haemotological analysis of the blood of rats in the test and control groups. The seed extracts had no significant or adverse effect on the blood parameters of rats in the different groups during the experiment.

Table 6: Result of haematology analysis of the experimental rats

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

3.9 Histopathological analysis

3.9.1 Histopathology of the wound scar area

The histological examination of the skin samples collected from the wound areas that were treated for 21 days with ointment base *M. indica* seed extract ointments revealed the presence of a mature granulation tissue in almost all the depth of the dermis for excision wound model. The results are shown on the Fig. 4. (See Appendices) According to the definition of Chattopaddhya *et al*. (16), healing of wound is complex in nature and dynamic process which involves tissue structure restoring back to its normal state. Healing depends upon the repairing ability of the tissue, type and extent of damage, and general state of the host's health. It is characterized by haemostasis, re-epithelialisation, granulation, remodeling of the extracellular matrix and scar formation (17, 18).

3.9.2 Histopathology of internal organs

Histopathological studies was carried out on the internal organs of the wistar rats to evaluate the effect of topical application of the control base or seeds extracts ointment on these organs, if any and also to determine if there would be any significant difference in the histology of the organs of test rats as compared to the control base ones (Table 7).

Table 7: Result of histopathology analysis of the organs

Animal 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	NVL. 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 cardiomyocytes as well as increased amounts of fibrous connective tissue. There is moderate congestion of coronary blood vessels	There are a few foci of intraluminal proteinaeceous tubular casts	There are a few foci of mild vacuolar change of hepatocytes. There are random foci of single-cell hepatocellular necrosis	There are a few foci of mild proliferative thickening of the inter-alveolar spetae. The airways and alveoli are clear	There are multiple fairly large and coalescing PALSs. There is moderate congestion of the splenic sinuses and sinusoids

ntrol was used in conjunction with other experiments

4. DISCUSSION

The present investigation described some distinct characteristics of *M. indica* seed extracts activity on excision wound model on rats. 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 (19). Suguna *et al.* (20) also reported that *Centella asciatia* and *Terminalia chebula* have high efficient in healing wound based on studies carried out on rats using extracts of the two plants. Different chemical analyses were performed in this study to review the wound healing activity of *M. indica* seeds as potential wound healing agents.

The result of the phytoconstituents analysis showed that tannins, alkaloids, terpenoids, glycosides, phenols are present in the seed extracts. However further research work on this study is essential to isolate these active compounds that exerted significant pharmacological activities to this studies (7, 21). Scortichini (22) and Sasidhran et *al*. (23) reports terpenoids are believed to increase the efficiency of wound healing process, which is due to their astringent and high antimicrobial activities assumed to be the one playing crucial role in wound contraction. The study revealed that the seed extracts from the seed exhibited favourable antimicrobial activity against all the tested microorganisms; *S. aureus*, *E. coli, A. niger, C. albicans and B. subtilis* infections could be treated favourably with the extracts of *M. indica* seed. Medicinal plants have been used in folk medicine and have been reported to be beneficial in wound care, promoting wound healing, minimizing pain/ discomfort and scarring of the patient (24).

5. CONCLUSION

The histopathological results, haematology results along with the wound contraction rate and period of pithelialisation displayed the wound healing potential of the seed extracts of *M. indica*. *M. indica* seeds might therefore be used for wound healing.

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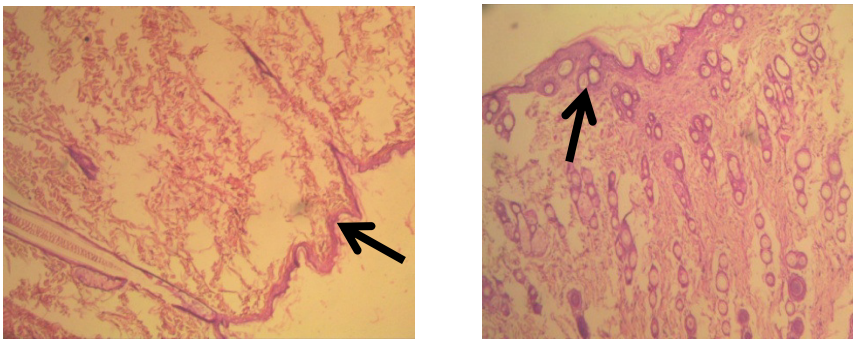
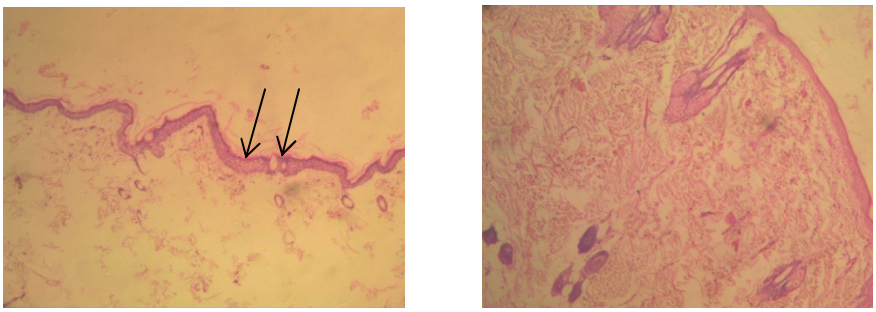
APPENDICES

Figure 3: Photograph showing various stages of wound healing activity of hexane and methanolic extract of *M. Indica* seeds (Excision wound model)



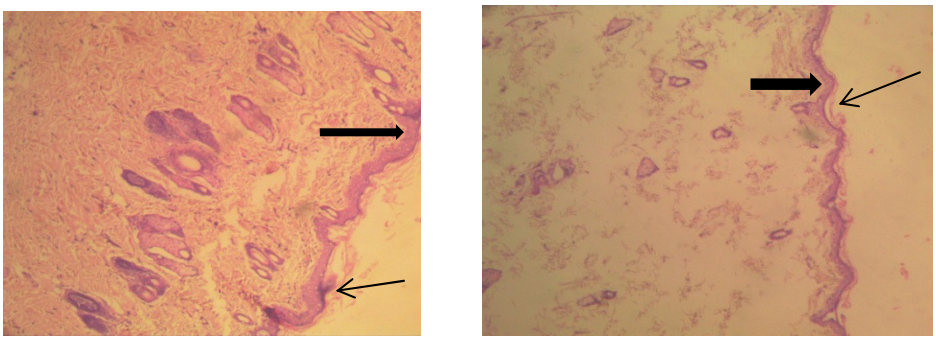
A i A ii

Fig 4A 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.



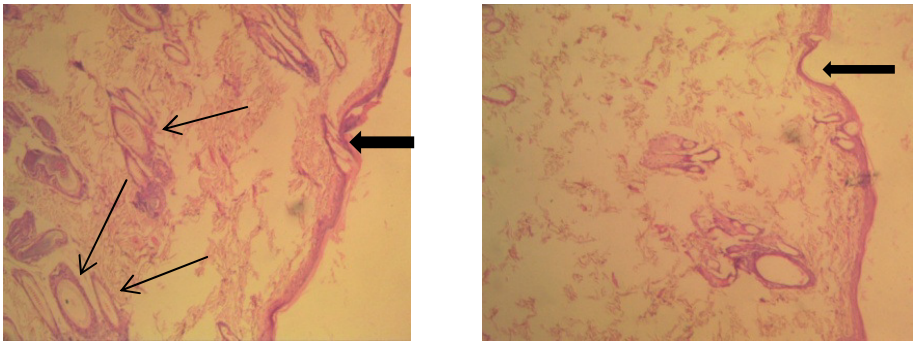
B i B ii

Fig 4B i & ii: Photomicrograph of healed wound skin area for group 2 treated with 5% v/v of *M. indica* hexane extract ointment (H&E x100): (i) There is mild ballooning degeneration (arrow) of epidermal cells. (ii) The epidermis is mildly thickened.



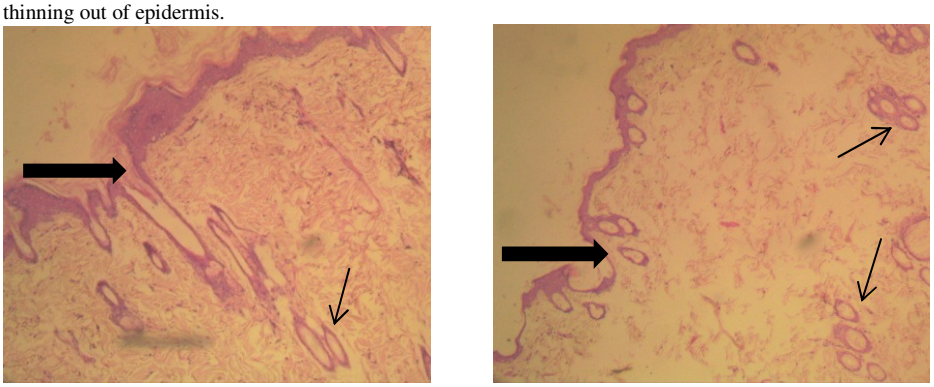
Ci C ii

Fig 4C i &ii: Photomicrograph of healed wound skin area for group 3 treated with 5% v/v of *M. indica* methanol extract ointment (H&E x100): (i) There is mild thickening of the epidermis. 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

Fig 4D i & ii: Photomicrograph of healed wound skin area for group 4 treated with 10 % v/v of *M. indica* hexane extract 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

Fig 4E i & ii: Photomicrograph of healed w ound skin area for group 5 treated with 10% v/v of *M. indica* methanol extract ointment (H&E x100): There appears to be re-epithelialization (thick arrows) at previously ulcerated margins. There are numerous hair follicles (thin arrows).

Fig 4: Photomicrograph of healed wound skin area for all test groups

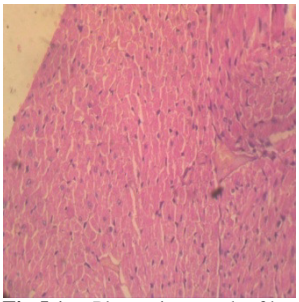


Fig 5.1a: Photomicrograph of heart for group1 animals treated with control base ointment (H&E x400)

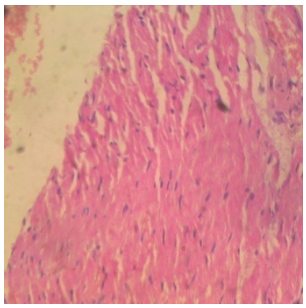


Fig 5.1b: Photomicrograph of heart for group 2 animals treated with 5 % v/v *M. indica* hexane extract ointment (H&E x400)

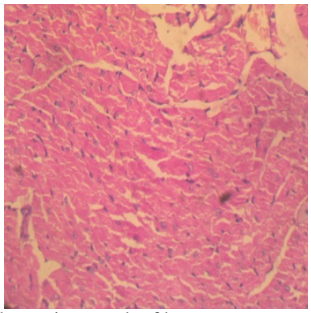


Fig5.1c: Photomicrograph of heart for group 3 animals treated with 5 % v/v *M. indica* methanol extract ointment (H&Ex400)

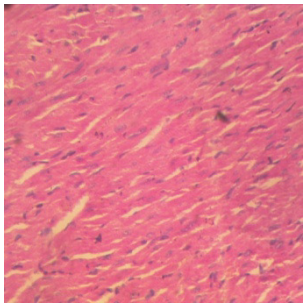


Fig 5.1d: Photomicrograph of heart for group 4 animals treated with 10 % v/v *M. indica* hexane extract ointment (H&E x400)

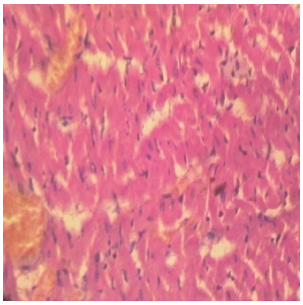


Fig 5.1e Photomicrograph of heart for group 5 animals treated with 10 % v/v *M. indica* methanol extract ointment (H&Ex400)

Figure 5.1: Photomicrograph of heart for all test groups

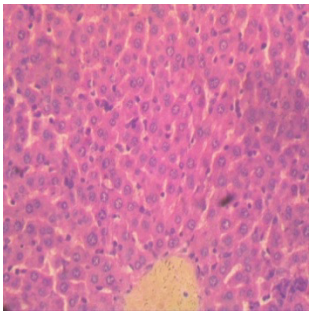


Fig 5.2a: Photomicrograph of liver for group1 animals treated with control base ointment (H&E x400)

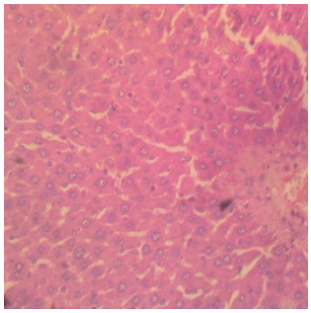


Fig 5.2b: Photomicrograph of liver for group 2 animals treated with 5 % v/v *M. indica* hexane extract ointment (H&E x400)

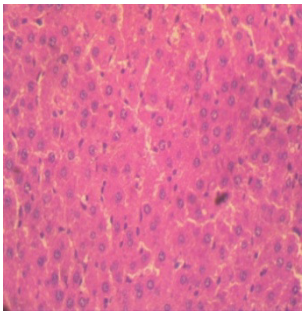


Fig5.2c: Photomicrograph of liver for group 3 animals treated with 5 % v/v *M. indica* methanol extract ointment (H&Ex400)

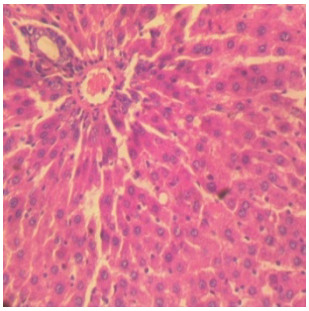


Fig 5.2d: Photomicrograph of liver for group 4 animals treated with 10 % v/v *M. indica* hexane extract ointment (H&E x400)

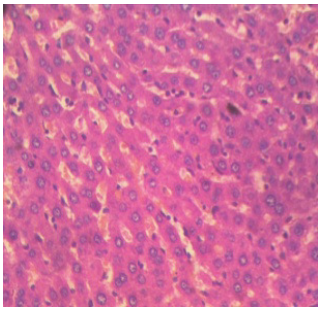


Fig 5.2e Photomicrograph of liver for group 5 animals treated with 10 % v/v *M. indica* methanol extract ointment (H&Ex400)

Figure 5.2: Photomicrograph of liver for all test groups

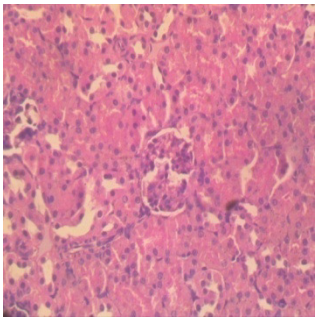


Fig 5.3a: Photomicrograph of kidney for group1 animals treated with control base ointment (H&E x400)

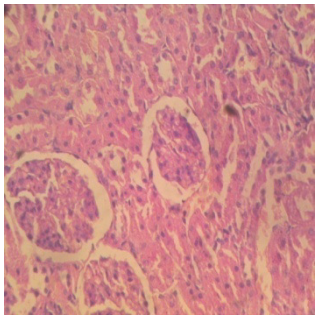


Fig 5.3b: Photomicrograph of kidney for group 2 animals treated with 5 % v/v *M. indica* hexane extract ointment (H&E x400)

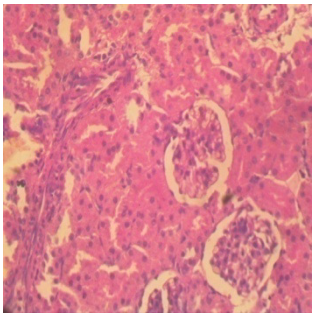


Fig5.3c: Photomicrograph of kidney for group 3 animals treated with 5 % v/v *M. indica* methanol extract ointment (H&Ex400)

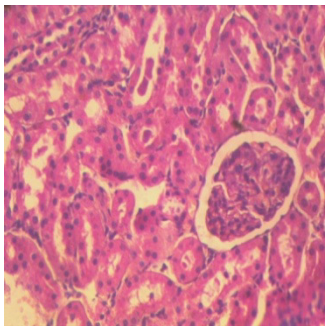


Fig 5.3d: Photomicrograph of kidney for group 4 animals treated with 10 % v/v *M. indica* hexane extract ointment (H&E x400)

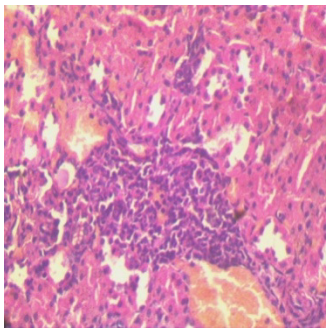


Fig 5.3e Photomicrograph of kidney for group 5 animals treated with 10 % v/v *M. indica* methanol extract ointment (H&Ex400)

Figure 5.3: Photomicrograph of kidney for all test groups

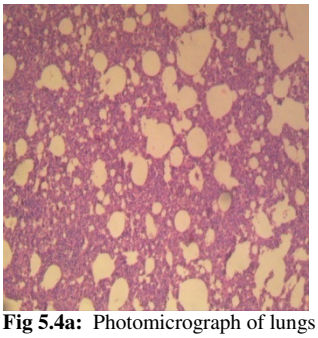


Fig 5.4a: Photomicrograph of lungs for group1 animals treated with control base ointment (H&E x100)

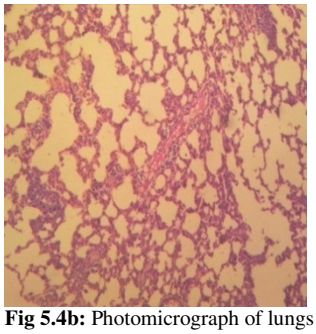


Fig 5.4b: Photomicrograph of lungs for group 2 animals treated with 5 % v/v *M. indica* hexane extract ointment (H&E x100)

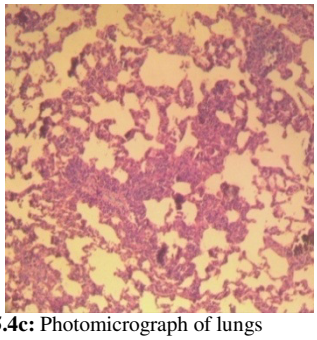


Fig5.4c: Photomicrograph of lungs for group 3 animals treated with 5 % v/v *M. indica* methanol extract ointment (H&Ex100)

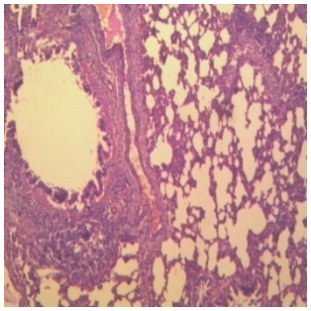


Fig 5.4d: Photomicrograph of lungs for group 4 animals treated with 10 % v/v *M. indica* hexane extract ointment (H&Ex100)

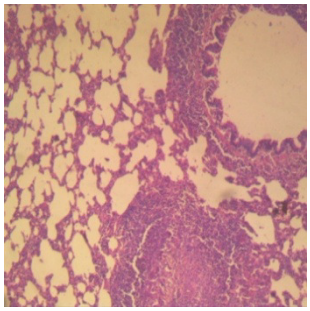


Fig 5.4e Photomicrograph of lungs for group 5 animals treated with 10 % v/v *M. indica* methanol extract (H&E x100)

Figure 5.4: Photomicrograph of lungs for all test groups

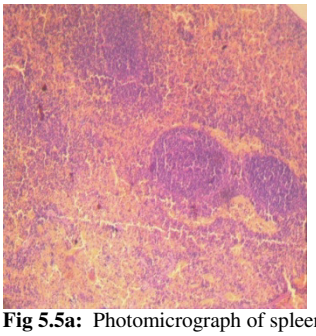


Fig 5.5a: Photomicrograph of spleen for group1 animals treated with base ointment (control) (H&E x100)

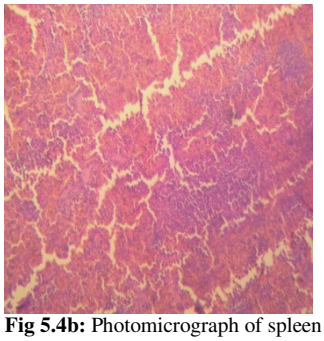


Fig 5.4b: Photomicrograph of spleen for group 2 animals treated with 5 % v/v *M. indica* hexane extract ointment (H&E x100)

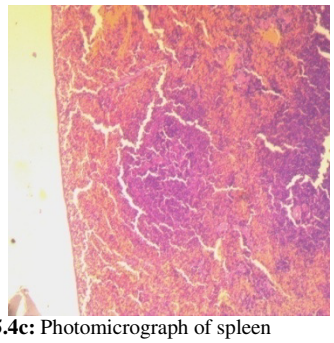


Fig5.4c: Photomicrograph of spleen for group 3 animals treated with 5 % v/v *M. indica* methanol extract ointment (H&Ex100)

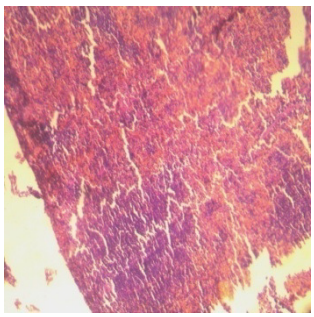


Fig 5.5d: Photomicrograph of spleen for group 4 animals treated with 10 % v/v *M. indica* hexane extract ointment (H&E x100)

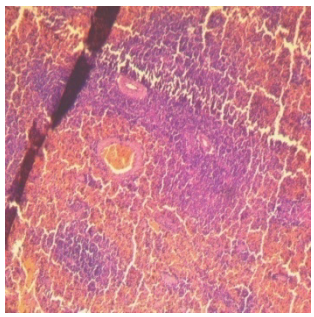


Fig 5.5e Photomicrograph of spleen for group 5 animals treated with 10 % v/v *M. indica* methanol extract ointment (H&Ex100)

Figure 5.5: Photomicrograph of spleen for all test groups