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Wound Healing Potentials of Aqueous Leaf Extract of *Mangifera indica L.* in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OB designed the study, wrote the manuscript and interpreted the data. Authors AOA and LDB participated in experimental design and data interpretation. Authors OOO and GTA carried out haemostatic activities and managed the literature searches. Author JTK carried out wound healing procedures and managed the literature searches and the analysis of data. All authors read and approved the final manuscript.

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ABSTRACT

Aim of the Study: Since scientifically proven investigations on wound healing has not been adequately carried out on *Mangifera indica* leaves, the present work was therefore conducted to evaluate the wound healing potentials of aqueous leaf extract of *Mangifera indica* in Wistar rats.
Study Design: Animal study model of wound healing.
Place and Duration of the Study: Department of Physiology, Faculty of Basic Medical and Health Sciences, College of Health Sciences, Bowen University, Iwo, Nigeria. Between October 2015 to June 2016.
Materials and Methods: Twenty four Wistar rats weighing between 160 -200 g were used. The rat were grouped into 4 groups with 6 rats in each group. Group 1 rats had no wound on them .Group 2 rats were treated topically with Normal Saline .Group 3 rats were treated topically with povidone

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iodine. Group 4 rats were treated topically with aqueous extract of *Mangifera indica*. In all the animals, the wound was induced using a scalpel blade.
The animals after 21 days of treatment were sacrificed and their blood samples, tissue (skin) were collected, processed and examined for haematological and histological analysis.
Results: The results showed that aqueous leaf extract of *Mangifera indica* (*M. indica*) decreased thrombin time, clotting time and bleeding time. However, wound contraction was significantly higher in experimental animals treated with aqueous leaf extract of *M. indica* when compared to control, Normal Saline (NS) and standard povidone iodine. These findings were further confirmed by histological examination of granulation tissue with a lesser number of chronic inflammatory cells, decreased oedema and increased collagenation in the rats treated with *M. indica* than the control.
Conclusion: Therefore, *Mangifera indica* leaves seem to be promising in haemostasis and wound healing.

Keywords: Magnifera indica; aqueous leaf extract; wound healing; Wistar rat.

1. INTRODUCTION

Plants and their extracts have immense potential for the management and treatment of wound. A wound is a trauma to any tissues of the body especially that caused by physical means and with interruption of the continuity or any break in the external or internal surfaces of the body involving a separation of tissue, and caused by external injury or force [1]. Open wounds are particularly prone to infection, especially by bacteria, and also provide an entry point for systemic infections. Infected wounds heal less rapidly and also often result in the formation of unpleasant exudates and toxins that will be produced with concomitant killing of regenerating cells. Consequently, there is a need to stimulate healing and restore the normal functions of the affected part of the body to ease the discomfort and pain associated with wounds, preventing infection, and activating tissue repair processes. Antibacterial and healing compounds in a traditional remedy can induce this occurrence and may be beneficial in treating wounds [2]. Wound healing, or wound repair, is an intricate process in which the skin (or another organ-tissue) repairs itself after injury [3]. In a normal skin, the epidermis (outermost layer) and dermis (inner and deeper layer) exist in a steady-state equilibrium, forming a protective barrier against the external environment. Wounds are unavoidable events of life. It may arise due to any agent that induces stress and injury and their healing has been one of the well-known problems. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and function. Treatment is therefore aimed at minimizing the undesired consequences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Some of these drugs have been screened scientifically for evaluation of their wound healing activity in different pharmacological models, but the potential of many of the traditionally used herbal agents remains unexplored. In few cases, active chemical constituents were identified [4]. Hence, there is dearth of rational pro-healing agents for the wound management programme, which can hasten the healing process. Wound healing can be classified into any of three types – healing by first intention, healing by second intention or healing by third intention, depending on the nature of the edges of the healed wounds.

In wounds healed by the first intention, the edges are smoothly closed that no scar is left. On the other hand, wound healing by second intention involves formation of granulation tissues, which fill up the gaps between the wound edges and is associated with significant loss of tissue, leaving little scars. Wounds healed by third intention, are usually those left open for three to five days until granulation bed falls before they are sutured, generally resulting in extensive scar formation [5].

There are four distinct stages involved in wound healing namely – inflammatory stage, debridement stage, proliferation stage and maturation/remodelling stage [5]. When an injury occurs, the vascular integrity of the injured area is disrupted leading to extravasations of blood into the surrounding tissue or plasma when the damage is minor. The inflammatory stage is directed at preventing further loss of blood by platelet adhesion/accumulation at the site leading to coagulation that results to the formation of thrombus. The debridement stage occurs from the third to the sixth day after injury and involves

the appearance of neutrophils to clear contaminating organisms. The proliferation or repair stage is characterized by endothelial budding in the nearby blood vessels forming new capillaries that penetrate and nourish the injured tissue. The maturation stage commences from the tenth day to several months depending on wound severity during which the number of capillaries decreases and wound changes from pink to white [5].

Certain factors that influence wound healing include bacterial infection, nutritional deficiency, drugs, sterility, obesity, movement of wound edges, site of wound, and wasting diseases [6]. Several drug classes have been used in the management of wounds. Among these are the antibiotics. Penicillin and streptomycin have been widely employed in combating post-operative infections in man and animals [7]. The antibiotics are chosen based on their ability to destroy or inhibit the growth of pathogenic organisms, while the tissue is left unharmed. Antibiotics used should be applied to achieve maximum concentration in the tissue as quickly as possible and continued until 48 h after disappearance of symptoms unless signs of toxicity are shown [8].

The wound healing activities of plants have since been explored in folklore. The significant successes recorded have led to investigation into medicinal plants with a view to confirming these acclaimed properties. Records have it that different parts of plants used for wound healing contain some active principles or components that are antimicrobial and nutritive in function. These natural agents induce healing and regeneration of the tissue by multiple mechanisms, however, *Mangifera indica* has been reported by various researchers to possess antimicrobial, antifungal, astringenic and haemostatic activities and as a result this research was conducted to evaluate the wound healing potentials of aqueous leaf extract of *Mangifera indica* in wistar rats model.

2. METHODOLOGY

2.1 Plant Material

Mangifera indica young leaves were collected from different area of BOWEN University, Iwo Osun State. The leaves were identified and authenticated by Dr. Ayanbamiji of the Department of Biological Sciences BOWEN University, Iwo Osun State, Nigeria with voucher number BUH 090. The leaves were clean; Shade dried and was crushed to moderately coarse powder. The powder was stored in an air tight container for subsequent use. 500 ml of boiled distilled water was added to 500g of the powdered sample in a 1 litre of round bottom flask, stirred with a glass rod, covered, shaken continuously for 6 h using a mechanical shaker. Then it is allowed to stand for another 18 h at room temperature. The extract was then filtered using Whitman filter paper No. 91 (18.5 cm). The filtrate was concentrated on a water bath at a controlled temperature. Then the concentrated extract of *M. indica* was completely evaporated in a lyophilizer (MAC) to get in the dry powder form [9].

2.2 Care and Management of Animals

Twenty-four (24) albino Wistar rats weighing 160-200g were purchased from the animal house of the college of health sciences, Bowen University, Iwo, Osun State, Nigeria. The rats were kept in wooden cages and maintained at normal and standard laboratory condition of (28 ± 2°c), relative humidity (46 ± 6%) with 12-hour light-dark cycle and adequate ventilation in the animal house of the Physiology Department, Faculty of Basic Medical and Health Sciences, Bowen University, Iwo, Osun State, Nigeria. All the animals were fed with commercially formulated rat feed and water ad libitum. After randomization into various groups, the rats were acclimatized for a period of 2 weeks before the initiation of the experiment. Their cages were cleaned of waste daily. All procedures involving the use of animals in this study complied with the guiding principles for research involving animals as recommended by the declaration of Helsinki and the Guiding principles in the care and use of animals [10].

2.3 Experimental Design

Twenty-four rats (24) randomly divided into 4 groups of 6 rats each were used during the experiment.

Group 1: The animals in this group had no wound on their skins.

Group 2: The animals in this group had their wounds treated topically with normal saline infusion and served as negative control.

Group 3: The animals in this group had their wounds treated topically with providine iodine solution applied once daily and served as standard group.

Group 4: The animals in this group had their wound treated topically with 40% w/w aqueous leaf extract of *M. indica* once daily.

2.4 Wound Infliction and Treatments

An excision wound model was used to evaluate the wound healing potentials of the plant extracts as described by Morton and Malone, 1972 [11]. The animals were treated humanely during the inducement of the experimental wound. The anticipated area was shaved, marked with a permanent marker and disinfected with 80% alcohol. New surgical blades were used for each of the animals to completely excise the tissue of the skin to its full thickness under a light anaesthesia (chloroform). An area of tissue of 2 cm by 2 cm was excised from the depilated side about 5 mm from the dorsal back of the rats. After achieving complete haemostasis by blotting of the wound with a cotton wool soaked in ethanol, the animals were placed singly in an individual cages.

2.5 Blood Collection

The blood samples were collected by cardiac puncture from the heart of the rats for haematological analysis of the thrombin and prothrombin time after the last day of treatment of the animals with extract. Also the blood samples for clotting and bleeding time was done using the tail vein of the rats.

2.6 Sample Analysis

2.6.1 Determination of bleeding time

This was determined using a modified Duke method [12]. A skin puncture was made quickly using disposable lancet and the stopwatch was started as soon as bleeding started. The puncture was dabbed with filter paper every 15 s until the paper no longer stained red with blood. Bleeding time was then taken as the time when the blood stopped flowing from the puncture.

2.6.2 Determination of Thrombin time

After separating the plasma from the whole blood by centrifugation, bovine thrombin is added to the sample of plasma. Clot formation is detected optically or mechanically by a coagulation instrument. The time between the addition of the thrombin and the clot formation is recorded as the thrombin clotting time.

2.6.3 Determination of prothrombin time

Blood was collected into sample vials containing 3.2% sodium citrate (as specified in the prothrombin time (PT). test kit used). in the ratio 1:9 with the blood sample. The blood was then centrifuged at 1000 g for 15 min to obtain platelet poor plasma. Thromboplastin PT-S was placed in a water bath at 37°C; and 0.1 ml of test plasma was also put into a test tube and placed in the water bath to prewarm to 37°C. A 0.2 ml of warmed thromboplastin PT-S was then forcibly added to the test plasma and the stopwatch was started. The tube was tilted repeated until a clot was formed and the time taken for clot to form was noted. This was repeated for all the blood samples (five in each group). Precaution was taken to perform test within 3 h of blood collection since the labile factor deteriorates quickly at room temperature.

2.6.4 Determination of clotting time

Blood was taken directly from the heart to avoid contamination with tissue thromboplastin (0.8 ml from each rat). A 0.2 ml of blood was then delivered into four glass test tubes that had previously been warmed and maintained at 37°C and the tubes immediately placed in a 37°C water bath to mimic the temperature of the internal environment. The stopwatch was started immediately the blood was delivered into the glass test tubes and the tubes were continually tilted at 40 s intervals (until blood in them stopped flowing when tilted at an angle of 90°)., starting with the first, to see and note the time when the blood clotted. The clotting time was taken as the average of the times blood clotted in the four tubes.

2.7 Histological Analysis

Skin tissues were collected and transferred to 10% neutral buffered formalin (NBF). for 24 h at 4°C. The formalin-fixed tissues were dehydrated through grades of alcohol and cleared in xylene, and then embedded in paraffin wax (58–60°C m.p.). 5 to 7 µm sections were deparaffinized and stained with hematoxylin, and then counterstained with Eosin [13].

2.8 Statistical Analysis

Results were expressed as group Mean ± SEM (Standard Error of Mean). Data were analysed using appropriate statistical methods and program of Microsoft excel, Graph pad prism and SPSS version16. Comparison between groups

and the significant difference between the control and treated groups were analysed using one way analysis of variance (ANOVA) and the values of $p \leq 0.05$ were considered statistically significant.

3. RESULTS

Efefect of *M. indica* on haemostatic indices.

3.1 Bleeding Time

The extract decreased bleeding time in rats of the experimental group in comparison with the control group. The mean bleeding time in control group was 6.33±0.33 mins while 5.39 ± 0.49 mins, 5.39 ± 0.31mins and 4.35 ± 0.13 mins were mean values obtained for normal saline, Iodine and *M. indica* group respectively. The analysis showed that the decrease was significant in the extract group at $p < 0.05$ but not significant in normal saline and Iodine group when compared independently with the control.

3.2 Clotting Time

There was a decrease in clotting time of *M. indica* aqueous extract group when compared to control group as shown in Table 1. The mean value of control was 6.76 ±0.08 mins while 6.08 ±0.49 mins, 6.35 ±0.51 mins and 4.66 ±0.74* mins were mean values obtained for normal saline, Iodine and *M. indica* group respectively. The analysis showed that this decrease was statistically significant ($P<0.05$).

3.3 Prothrombin Time

The mean value of prothrombin time was 13.67 ±0.81 mins in control group, 11.89 ±0.12 mins (normal saline group), 11.24 ±0.89 mins (Iodine group). and 15.92 ±0.78 mins (*M. indica* group) as shown in Table 1. There were significant decreases ($p < 0.05$) in prothrombin time in the normal saline and Iodine groups compared with the control group but there was no significant decrease ($p > 0.05$) in prothrombin time in the *M. indica* extract groups compared with the control group.

3.4 Thrombin Time

The mean value of thrombin time was 15.92 ±0.78 mins in control group, 19.17±0.75 mins (normal saline group), 18.00±0.57 mins (Iodine group). and 16.74 ±0.09* mins (group III) as shown in Table 1. There was a significant decrease ($p < 0.05$) in prothrombin time in the *M. indica* experimental groups compared with the control group.

3.5 Effect of *M. indica* Aqueous Extract on Wound Healing

Rats treated with *M. indica* aqueous extract, were observed to show significant ($P < 0.05$) increase in wound healing activity compare with those treated with normal saline and a significant ($P < 0.01$) when compared with those treated with povidone iodine. There were no significant differences between wounds treated with Povidone Iodine and normal saline.

Wound contraction was expressed as percentage reduction of original wound size.

$$\% \text{ Wound Contraction} = \text{Healed Area} \div \text{Total Area} \times 100$$

On day 14 of this research work the animals treated with *M. indica* aqueous extract showed wound contraction of 94%, while those treated with povidone iodine and normal saline showed wound contraction of 75% and 84% respectively. The lower percentage wound contraction observed with the other experimental rats reflect the wound delay in the group.

Table 1. Effect of *M. indica* aqueous extract on haemostasis

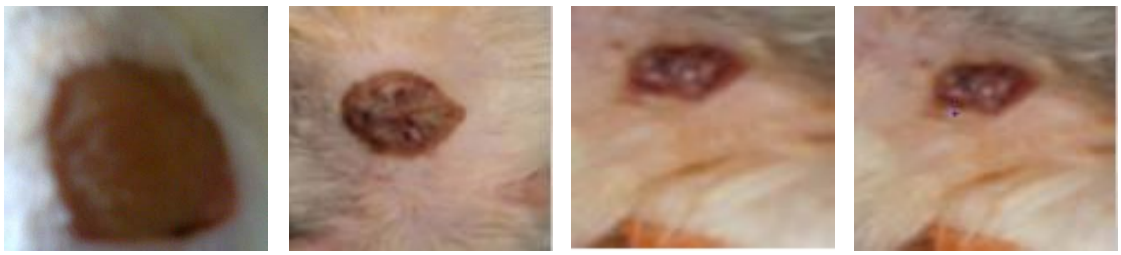
Group	Treatment	Prothrobin time	Thrombin time	Clotting time	Bleeding time
1	No wound	13.67 ±0.81	19.54 ±0.06	6.76 ±0.08	6.33 ±0.33
2	Normal saline	11.89 ±0.12*	19.17±0.75	6.08 ±0.49	5.39 ± 0.49
3	Iodine	11.24 ±0.89*	18.00 ±0.57	6.35 ±0.51	5.39 ± 0.31
4	*Mangifera indica*	15.92 ±0.78	16.74±0.09*	4.66±0.74*	4.35± 0.13*

*Prothrombin Time (P.T), Thrombin Time (T.T), Clotting and Bleeding Time comparison among the Experimental rats.Values were expressed as mean±S.E.M. *p < 0.05, (n=5)*

Table 2. Shows *M. indica* wound healing activity in experimental rats using excision wound model

Post wounding day	Wound area (mm2).		
	N.S	P.I	M.I
0	400.00 ±00	400.00 ± 00	400.00 ± 00
3	375.77 ± 5.04	380.05 ± 5.76	296.17 ± 2.23*
5	368.25 ± 7.08	364.62 ± 9.04	271.15 ± 2.22*
10	71.73 ± 1.11	124.45 ± 2.05	99.63 ± 1.35
14	64.38 ± 9.71	101.22 ± 4.39	30.76 ± 2.48**
16	26.46 ± 9.33	34.96 ± 7.64	8.34 ± 1.32*
19	5.61 ± 1.66	11.15 ± 7.54	3.69 ± 0.90

N.S- normal saline, P.I- Povidone Iodine, M.I – Mangifera indica
*Values are presented as mean ± S.E.M; n = 5; *P<0.05 vs control and **P<0.01 vs. Iodine*



Day 1 Day 7 Day 14 Day 19

Fig 1. Excision wound healing activity of standard povidone iodine group

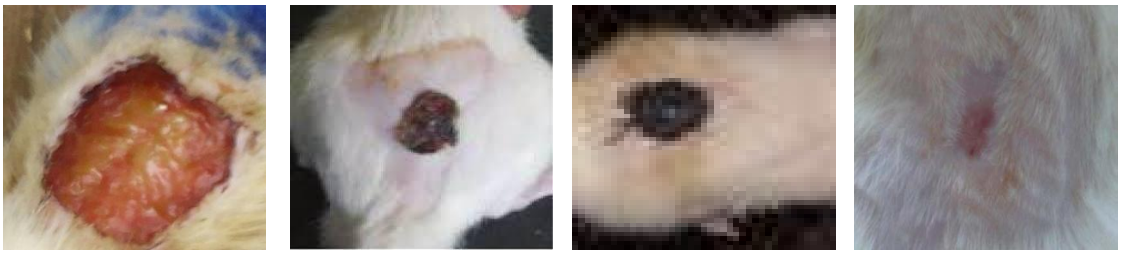


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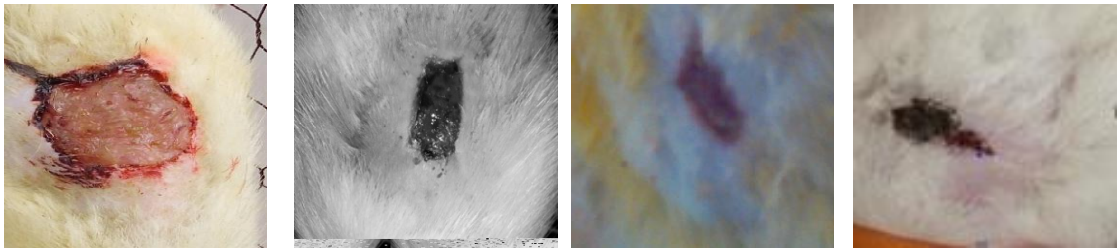
Fig. 2. Excision wound healing activity of *M. indica* aqueous extract group



Day 1 Day 7 Day 14 Day 19

Fig. 3. Excision wound healing activity of control normal saline group

3.6 Histological Analysis

Histologically, wounds treated with *M. indica* aqueous extracts showed markedly less scar at wound enclosure and granulation tissue contained markedly increased collagen fibres, fibroblasts and proliferating blood capillaries, and absence of inflammatory cells, while

wounds treated with other experimental groups which showed less collagen fibre, fibroblasts and blood capillaries, and more inflammatory cells (Fig. 4).

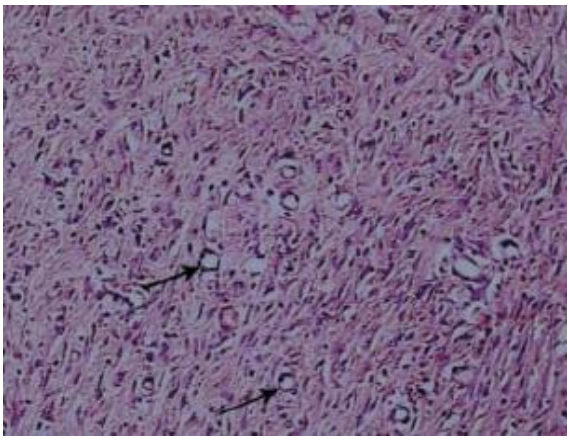


Fig. 4. The *M. indica* treated lesion on day 10 post-injury. Numerous blood vessels of different sizes are seen in this section (arrows). The collagen fibres show a primitive organized pattern and the tissue alignment is greater as compared to untreated lesions (H&E ×100)

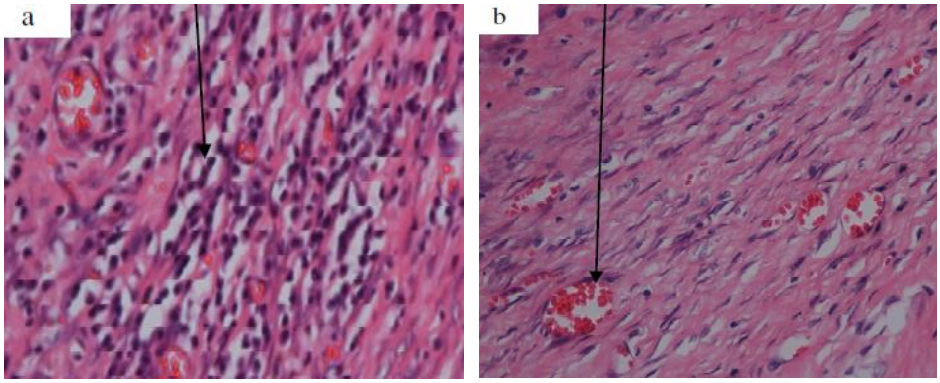


Fig. 5. Histological section of wound healed on day 13 post wound day in (a) normal saline - treated group. Granulation tissue contains comparatively less collagen, fibroblast and blood capillaries, and more inflammatory cells (b) *M. indica*-treated group. Granulation tissue contains comparatively more collagen, fibroblast and blood capillaries, and less inflammatory cells (H& E stain x40)

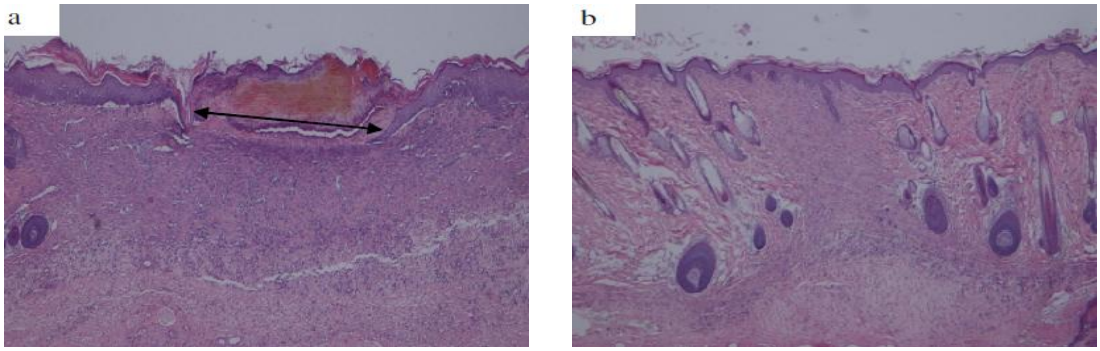


Fig. 6. Histological section of healed wound in (a) Povidone iodine-treated group showing incomplete wound closure (no epidermis, arrow), (b) *M. indica*-treated group showing complete wound healing closure, (H& E stain x40) on day 20

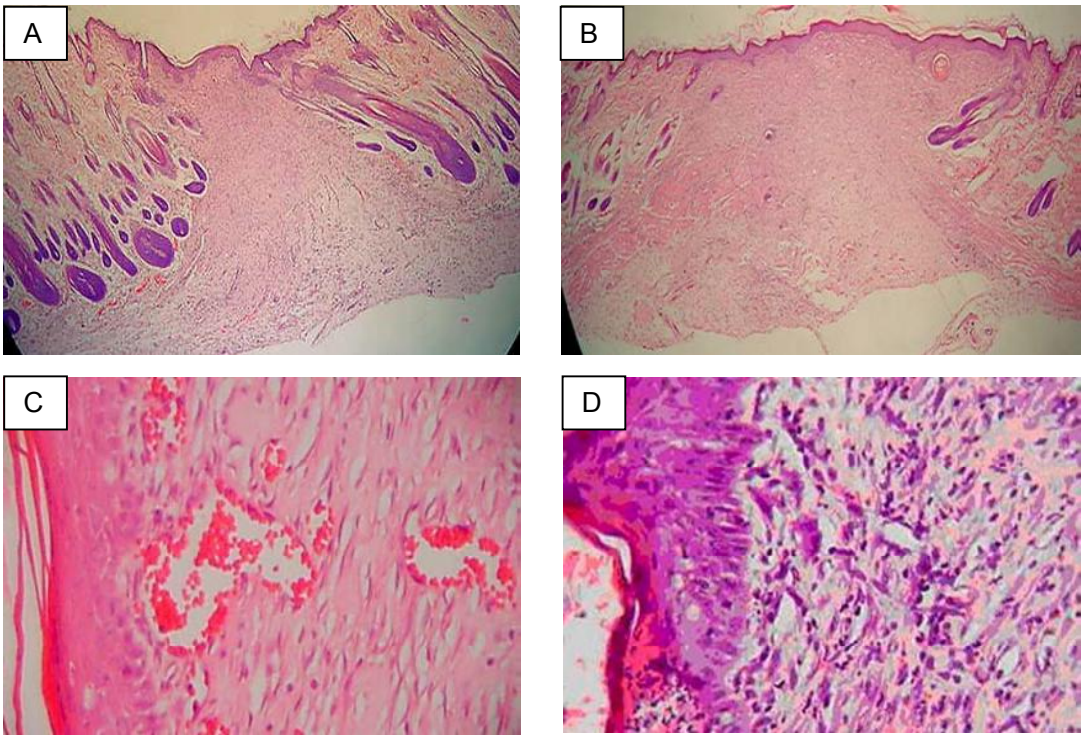


Fig. 7. Histological sections of healed wounds treated with 40%. *M. indica* aqueous showing narrow scar at the wound closure (A), treated with Povidone Iodine revealed wide scar at the wound closure (B), treated with placebo containing 40%. *M. indica* aqueous extract showing granulation tissue with more collagen, fibroblast and blood capillaries as well as absence of inflammatory cells (C), and after treatment with Normal saline where granulation tissue contains less collagen, fibroblast, and blood capillaries as well as more inflammatory cells (D) (H & E stains ×80)

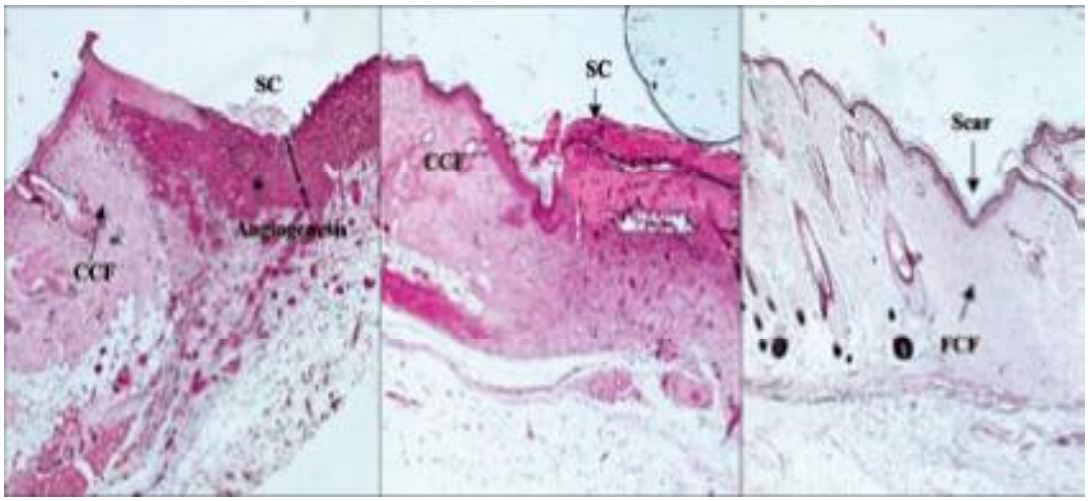


Fig. 8. Photomicrograph of the control wounded skin tissue stained with standard haematoxylin and eosin, (H&E×40)

Note that poor visible of fine and coarse collagen fibre and its arrangement throughout the wound healing processes. CCF: Coarse Collagen fibres, FCF: Fine Collagen fibres, HF: Hair Follicles, SC: Scab

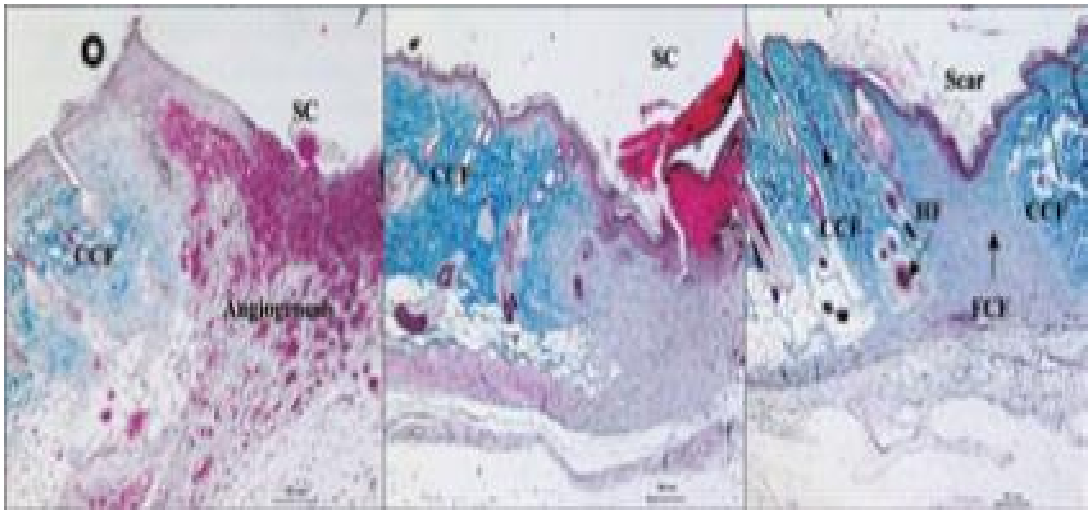


Fig. 9. Photomicrograph of the control wounded skin tissue stained with modified Masson's trichrome staining, (H& E×40)

Note that clear visible and differentiation of fine and coarse collagen fibre and its arrangement, scab formation and angiogenesis throughout the wound healing processes. CCF: Coarse Collagen Fibres, FCF: Fine Collagen fibres, HF: Hair Follicles, SC: Scab

4. DISCUSSION

This study was carried out to determine the effect of aqueous extract of *M. indica* on wound healing in normal rats with interest on haemostasis, wound closure, collagenation, angiogenesis and re-epithelisation.

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. In the maturational phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue [14].

In this present study, topical application of *M. indica* aqueous extract significantly accelerated the rate of haemostatic ability, wound healing, collagen, fibroblasts, and blood capillaries were contained in granulation tissue with little or no inflammatory cells. Haemostatic ability was exhibited by decreasing bleeding, thrombin and clotting time which measures blood coagulation and this effect was as result of bioactive component such as tannins which have been implicated in the haemostatic activity of plants where they arrest bleeding from damaged or injured vessels to form vascular plugs [15].

The accelerated wound healing effects seen in this study may be due to regulation of collagen expression [16] and increase in tensile strength of the wounds [17]. Similarly, enhanced healing activity has been attributed to increased collagen formation and angiogenesis [18,19]. Collagen played a central role in the healing of wounds and it is a principal component of connective tissue and provides a structural framework for the regenerating tissue [20]. Angiogenesis in granulation tissues improves circulation to the wound site thus providing oxygen and nutrients essential for the healing process [21] that include re-epithelisation, stimulate epithelial cell proliferation and angiogenesis are important for wound healing process [22].

Histological analysis of the treated healed wound group contained a large amount of fibroblast proliferation, collagen synthesis, and neovascularization, which resulted in an increased wound tensile strength and accelerated healing of wound. There were no significant differences between wounds treated with Povidone Iodine and normal saline, although normal saline showed much effectiveness in terms of accelerated rate of wound healing process. There is extensive evidence to support the use of povidone iodine in wound healing, but its use is not without controversy due to perceived issues with toxicity, systemic absorption and delayed healing. It has been suggested that iodine has a negative impact on

cells involved in the wound healing process and because of this its safety and efficacy have been questioned whereas Physiological saline is a widely recommended irrigating and wound dressing solution, as it is known to be compatible with human tissue [23,24,25,26,27]. Normal saline causes no damage to new tissue and does not affect the functions of fibroblast and keratinocytes in healing wounds. Its effectiveness in preventing infection has not been ascertained [24]. Although the capacity of the skin to heal itself is large, restrictions on this ability include dryness of the wound surface and infections [28]. Other workers have documented this effect of normal saline on healing wounds but the mechanism of this effect is presently not known [28,29]. Probably the ability of normal saline in keeping the wound surface moist and not affecting the healing process, as it is a physiological solution may be the main reason.

The extract of *M. indica* possess significant antioxidant activity. It is likely that the antioxidant property of *M. indica* extract could be linked to its wound healing acceleration. Topical applications of compounds with antioxidant properties significantly improve wound healing and protect tissues from oxidative damage [30]. The tannins are known to possess antioxidant activity. It could be conceivable that the *M. indica* extract exert their wound healing activity through the tannins, magniferin and flavonoids since they are reported to improve wound healing and protect tissues from oxidative damage. In addition, wound healing activities of aqueous leaf extract of *M. indica* might also be connected to its anti-microbial and anti-inflammatory properties which helped to hasten wound contraction.

5. CONCLUSION

Therefore, from these findings it could be concluded that topical application of *M. indica* extract to an open wound has beneficial effects on haemostatic activity, wound contraction and wound healing and may be a promising medication for wound treatment.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved to be conducted by College of Health Sciences Ethical Committee, Bowen University, Iwo, Osun State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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