Research Article

Natural Healing Compound for the Treatment of Excision and Incision Wound in Rats Model

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ABSTRACT

The aim of the study was to evaluate the wound healing properties of ethanolic extract of *Moringa olifera* leaves. The ethanol extract of *Moringa olifera* leaves was prepared and evaluated for its wound healing potentials in Wistar rats using excision and incision wound models. The breaking strength and the hydroxyproline content of the wounds were also studied. The results indicated that the rate of wound healing in groups treated with *Moringa olifera leaves* extract ranged from 32.2 ± 0.0 to 100.0 ± 1.1 % at 4 and 15 days respectively, while the groups that received nitrofurazone ointment exhibited 11.0 ± 1.0 to 77.2 ± 2.0 % wound healing at 4 and 15 days respectively. Batches A, B and C treated with varying concentrations of the leaves extract exhibited greater breaking strength and higher contents of hydroxyproline significantly different from the reference and the control groups ($p < 0.05$). Therefore, *Moringa olifera* leaves extract exhibited faster wound healing rates than nitrofurazone ointment used as the reference drug.

Keywords: *Moringa olifera*, wound healing, hydroxyproline, excision wound, incision wound.

INTRODUCTION

Moringa oleifera Linn. (Moringaceae) is native to the Indian sub-continent and naturalized in tropical and sub-tropical areas around the world and has been an ingredient of Indian diet since centuries [1]. This rapidly growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics. The leaves of the plant have also been reported for its anti-tumor, hypotensive, antioxidant, radio-protective, anti-inflammatory and diuretic properties. The aqueous extract was studied and it was found that there was significant increase in wound closure rate, skin-breaking strength, granuloma breaking strength, hydroxyproline content, granuloma dry weight and decrease in scar area was observed[1-3].

Moringa oleifera has antibiotic, antitryponosomae, hypotensive, hypoglycemic and anti-inflammatory activities[4]. The antibiotic properties of *Moringa oleifera* has been attributed to some of its phytochemcials such as benzyl isothiocyarate and other isothiocynates. These compounds had antibiotic activity against a wide range of bacteria and fungi [4].

Specific phytochemicals of *Moringa* preparations that have been reported to have hypotensive, anticancer, and antibacterial activity include 4- (4'-O-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocy- anate, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocy-anate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate[2, 5-7]. While these compounds are relatively unique to the *Moringa* family, it is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including β-carotene or pro-vitamin A) [2]. Several antibiotic drugs have been used in the treatment of various types of wound, and many have also been formulated such as ointments and wound dressings used in the treatment of severe skin wounds or ulcers including bedsores and burn wounds [8-9]. Wounds recovery processes have been subdivided into several diseases stages. In bedsores and burn wounds, the wound stages are often divided into an infectious period, necrosis and agglutination period, proliferation period and epidermis formation period[10].

Generally, formulations are selected based on the disease stage and the causes of the wound. There are increasing resistance to some of the orthodox antibiotics used in wound healing, which may be due to the high level of bacteria resistance, poor drug combination and inactivation of the antibiotic by some enzyme producing bacteria[10]. Plants and plant products present some hope to scientists, serving as an alternative avenue to discovery from the current mainstream approach of attempting to find solution to diseases that have proved very resistance to orthodox drugs for specific health problems [11-13].

The wound healing activities of plants have since been explored in folklore[1]. Many herbal plants have very important roles in the process of wound healing. Plants are potent healers because they promote the repair mechanisms in the natural way. Extensive research has been carried out in the area of wound healing management through medicinal plants. Herbal medicines in wound management involve disinfection, debridement

and providing a moist environment to encourage the establishment of the suitable environment for natural healing process [1, 14]. The aim of the study is to confirm the wound healing properties of *Moringa olifera* leaves extract and to give insight for the standardization for possible use in modern medicine.

MATERIALS AND METHODS

Moringa olifera leaves were collected from the Ibagwa forest in Nsukka, Enugu State, Nigeria in the month of February, 2012. The plant material was authenticated by Mr. A.O. Ozioko, a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka. The voucher specimen of the plant studied was deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. *Moringa olifera* leaves extract was obtained from a batch processed in our laboratory. Ethanol (BDH, England), distilled water (Lion water, Nsukka, Nigeria). All other reagents and solvents were analytical grade and were used as supplied.

Extraction of the *Moringa olifera*

Moringa olifera leaves were washed with distilled water and shade dried for 4 to7 days and was then finely powdered using electrical blender. About 300 g of powder was subjected to soxhlet extraction with 75 % ethanol (2.5 liters) in a conical flask for about 48 h. The extract was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1) and concentrated in vacuum under reduced pressure using a rotary flash evaporator (Sigma – Aldrich, USA) and dried in a desiccator over fused calcium chloride. The semisolid extract (17.4 % yields) was packed into a close tight container and used for further studies.

Animal protocol

Wistar albino rats of either sex weighing 200-220 g were procured from the Biochemistry Department, University of Nigeria, Nsukka and were maintained at standard housing conditions with 12 h light. The animals were fed with a commercial diet (Feeds BC, Nsukka, Nigeria) and water during the experiment. All animal experimental protocol was in accordance with the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka and in accordance with the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Preparation of the extract for wound healing activity

Three different concentrations of the extract were prepared by dissolving a known weight of the methanolic extract in a known volume of purified distilled water to obtained 2.5, 5.0 and 10 % w/v of the crude extract.

Wound healing evaluation

The excision and incision wound models were used to evaluate the effectiveness of *Moringa olifera* extract on wound-healing activity.

Excision wound

The wound site was prepared following the excision wound model[15]. The animals were anaesthetized with ketamin® (Rotex Ltd – Indian), at a dose of 1ml/kg body weight, a clean sterile surgical blade of size (No. 20) was used to shave the hairs on the skin of both thighs of the rat immediately the effect of the drug was noticed. A circular diameter (20 mm) was gently marked on the shaved portion of the thighs. Circular excisions were then made on the marked area of the skin surface and the skin carefully dissected out.

The area was measured immediately by tracing out the wound area using a transparent tracing paper and the squares counted. The animals were divided into five groups of five animals each. Batches A, B and C were treated 2.5, 5.0 and 10.0 % *Moringa olifera* extract respectively. The control group received normal saline, while the reference group was treated with nitrofurazone ointment. The extract was topically applied once daily from the day of the operation until complete epithelization. The wounds were traced every three days for six times and thereafter monitored until healing was complete. The percentage of wound closure was calculated [9].

Incision wound model

Rats were anesthetized and two par vertebral long incisions made through the skin of about 5.0 cm from the midline on each side of the back using sterile scalpel surgical blade size 20. The animals were treated as discussed in the excision study.

The open skins were carefully stitched with a black silk-0 on alternate positions to ensure a good closure with minimal fluid loss. Batch Batches A, B and C were treated 2.5, 5.0 and 10.0 % *Moringa olifera* extract respectively. The control group received normal saline, while the reference group was treated with nitrofurazone ointment. The sutures were removed at day 8 post surgery and the tensile strength of the healed skin was assessed at the 10th day as described in the previous method [16].

Determination of hydroxyproline content

Hydroxyproline is an amino acid present in collagen fibers of granulation tissues. Its estimation helps clinically to understand progress rate at which the healing process is going on in connective tissues of the wound. The hydroxyproline contents were determined by calorimetry from standard curves [17].

Statistical analysis

Statistical analysis was carried out using SPSS version 14.0 (SPSS Inc. Chicago, IL.USA). All values are expressed as mean ± SD. Data were analysed by one-way analysis of variance (ANOVA). Differences between means were assessed by a two-tailed student's T-test. $P < 0.05$ was considered statistically significant.

RESULTS

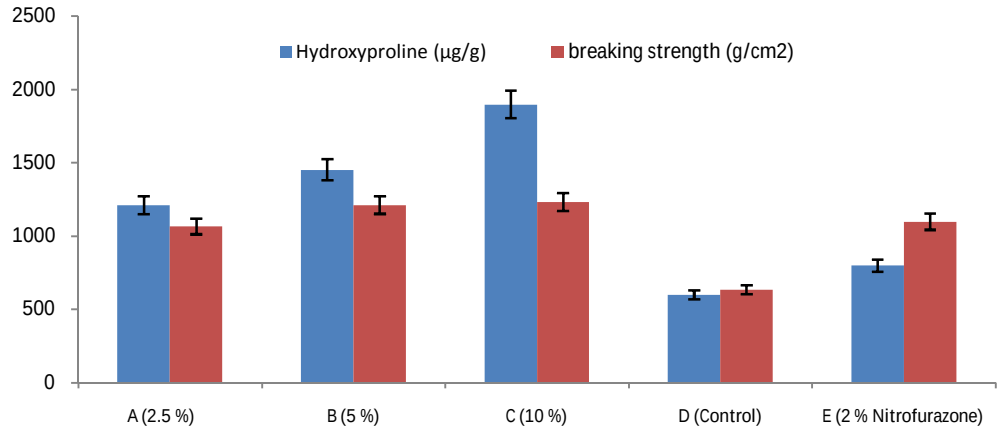
The results of incision and excision wound healing properties of ethanolic extract of *Moringa olifera* leaves is shown in Table 1, it was observed that the leaves extract exhibited higher percentage wound healing properties than the reference drug (nitrofurazone ointment) ($p < 0.05$). Batch B (5 % extract) showed higher wound healing properties than batches A and C treated with 2.5 % and 10 % from 8 days to 15 days as shown in Table 1. Batches B and C treated with 5 and 10 % of the extract showed complete epithelization at 15 days, while batch A treated with 2.5 % of extract showed complete epithelization at 20 days. All the groups treated with ethanolic extract of *Moringa olifera* leaves exhibited faster epithelization period which varied significantly from the control and the reference groups ($p < 0.05$) and were not absolutely dose dependent. It could be observed that as the concentration of the extract was increased to 10 % there was no corresponding increased in the activity of the formulation, this could be attributed to the amount of the extract that was adhered to the wound surfaces, which invariably showing that increasing the concentration above 5 % have no impact on the action of the formulation. This is a suggestive that the best wound healing could be pegged at 5.0 % of crude extract.

The breaking strength and hydroxyproline content of wound are shown in Fig. 1. From the results, the control which did not receive any treatment exhibited a low breaking strength significantly different from the test and the reference groups ($p < 0.05$). Also, batch C (10 %) showed higher breaking strength values significantly different from other batches ($p < 0.05$) as shown in Fig. 1. The hydroxyproline content of wounds shown in Fig. 1 also showed that batches A, B and C treated with varying concentrations of the ethanolic extract of *Moringa olifera* exhibited higher contents of hydroxyproline significantly different from the reference and the control groups ($p < 0.05$).

Table 1: Percentage wound healing of ethanolic extract of *Moringa olifera* leaves

Treatment duration (Day)	1	4	8	12	15	18	20
Groups	Wound healing rate (%)						
A (2.5 %)	0.0	27.5 ± 2.1*	53.4 ± 0.0*	74.2 ± 1.0*	94.1 ± 2.0*	100.0 ± 0.0*	-
B (5.0 %)	0.0	32.2 ± 0.0*	65.3 ± 4.0*	83.8 ± 2.3*	100.0 ± 1.1*	-	-
C (10.0 %)	0.0	36.5 ± 1.0*	47.2 ± 2.1*	79.3 ± 5.0*	100.0 ± 0.0*	-	-
D (Control)	0.0	8.1 ±1.0	8.0 ± 1.0	23.1 ± 0.3	29.1 ± 1.0	32.1 ± 0.2	37.2 ± 2.0
E (Nitro)	0.0	11.0 ± 1.0	23.1 ± 1.1	49.1 ± 0.1	77.2 ± 2.0	86.0 ± 10	100.0 ± 1.0

Values shown are mean ± standard deviation; A, B, and C were treated with Moringa olifera leaves extract, D= Control, while E was treated with nitrofurazone ointment, *$p < 0.05$ was considered statistically significantly compared to control.



A, B, and C were treated with *Moringa olifera* leaves extract, D= Control, while E was treated with nitrofurazone ointment.

Figure 1: Breaking strength and hydroxyproline content of wounds.

DISCUSSIONS

Wound healing involves a highly dynamic integrated series of cellular, physiological and biochemical processes, which occur in living organism [17]. Repair through regeneration is very common in unicellular and the lower metazoan animal groups while it is highly restricted in the higher animals [18]. Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent to each other [9]. Though healing process takes place by itself and need not require much help, but various risk factors such as discomfort, inability to heal by primary intention due to infection and delay in healing has brought attention to

promote this process [19]. Medically, any substances either plant or synthetic origin that must be used as healing agent or to balance the loss tissue on the infliction area must be able to do so within the first 16 days of the wound [20].

A wound is a generally defined as a disruption in the normal anatomical structure and function of living tissue that resulted by physical [21], chemical, microbiological or immunological injury [20].

Wound healing is essentially a survival mechanism and represents an attempt to maintain normal anatomical structure and function which can take place without any external application, this depend on the integrity of the immune system and the state of the physiological wellbeing of an individual [22]. When healing takes place in a direction away from its normal course, it may result in non-healing, under-healing or over healing [19]. In such case there is need for helper agent, because of the likely secondary infection that may set in as a result of microbial inversion into the open wound.

The results of wound healing rate showed that the groups treated with ethanolic extract of *Moringa olifera* leaves exhibited faster wound healing rate than the group treated with nitrofurazone ointment. Therefore, *Moringa olifera* leaves extract posses greater wound healing properties than nitrofurazone ointments. Also the content of hydroxyproline calculated in animal groups treated with ethanolic extract of *Moringa olifera* leaves were higher than those treated with nitrofurazone ointment, hence, indicating that wound healing processes were fasters in these groups. During the healing of wound, collagen is synthesized which is one of the constituents of growing cell. Constituents of hydroxyproline are a measure of concentration of collagen. Higher concentration of hydroxyproline indicates faster rate of wound healing [19]. Also groups treated with *Moringa olifera* leaves extract showed higher breaking strength than the control and the reference groups indicating higher wound healing effect in these groups. The higher wound healing properties seen in groups treated with *Moringa olifera* leaves extract may be due to the antimicrobial properties of this plant. The antibiotic properties of *Moringa oleifera* has been attributed to some of its phytochemicals such as benzyl isothiocyarate and other isothiocynates. These compounds had antibiotic activity against a wide range of bacteria and fungi [4].

CONCLUSION

Moringa olifera leaves extract exhibited faster wound healing rates than nitrofurazone ointment used as the reference drug. Wounds treated with ethanolic extract of *Moringa olifera* leaves showed higher breaking strength, higher hydroxyproline content and faster rate of epithelization or wound contraction than those treated with nitrofurazone ointment. And the concentration that offered best healing was 5.0 % concentration of the extract and as such is recommended for further verification using different delivery method for the same wound healing.

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