



Restorative Effect of Methanol Leaf Extract from *Vernonia amygdalina* on Dermal Wounds Modeled with Albino Rats

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ABSTRACT

The wound healing activity of the leaf of *Vernonia amygdalina* (Asteraceae) was evaluated using contraction of excision wounds on rat skin. The work investigated the healing process on excision wounds on the skin of rats treated with two concentration levels of methanol leaf extract (2.5 and 5 %), the expressed natural leaf extract of *Vernonia amygdalina* and their effects compared with standard antibiotic (neomycin) and the untreated wounds by monitoring the wound healing process for three weeks. Results showed that the extracts increased the rate of wound contraction better than the untreated cases and the effect of the methanol leaf extracts were comparable to the standard antibiotic. Therefore, methanol leaf extracts of *Vernonia amygdalina* have values in management of superficial skin wounds.

KEY WORDS: *Vernonia amygdalina*, excisional wound, topical, closure rate, toxicity

INTRODUCTION

A wound is a disruption of normal anatomic structure and function of skin [1] and living tissue that can be caused by physical, chemical, microbiological or immunological injury [2]. A wound may be chronic (pressure, vascular, diabetic ulcer etc) or acute (those caused by surgery, trauma, burns etc) [3]. Normal wound healing response begins as soon as the tissue is injured. From ancient times, for effective healing of wound, a suitable material had to be used to cover the wound in order to prevent any infection. For an effective design of a functional wound bandage, characteristics of the wound type, wound healing time, physical, mechanical and chemical properties of the bandage must be taken into consideration. Ultimately, the main purpose is to achieve the highest rate of healing and the best aesthetic repair of the wound [4]. There are numerous reports describing the various biological and physiological stages of the healing process of a wound summarized into five consecutive cascades of events of haemostasis, inflammation, migration, proliferation and maturation [5, 6]. The healing process involves a series of continuous phase by mechanical and chemical injuries; tissue release of some factors at the wounding sites; and can also be based on three types of principle/intensifying

stages- healing by first intention, healing by second intention or healing by third intention [7]. Clinical studies have demonstrated that a measure of the tissue microbial load in a wound can predict delayed healing or infection [8, 9].

V. amygdalina has been found to possess a wound healing effect as the isolates vernolide and vernodalol have shown activity against gram-positive (*B. cereus*, *S. epidermidis*, *S. aureus*, *Micrococcus kristinae* and *S. pyogenes*) and gram-negative (*Salmonella pooni*), justifying the use in wound treatment [10]. The presence of flavonoids, alkaloids, terpenes, saponins are also essential in wound healing process [11, 7] while other biological active constituents are steroids, coumarins, phenolic acids, lignans, xanthenes and anthraquinone [12], edotides [13] and sesquiterpenes [14]. The objective of the present study was to use graded dose of methanol leaf extract of *V. amygdalina* and evaluate its wound healing activity in rats.

MATERIALS AND METHODS

Materials

Fresh leaves of *Vernonia amygdalina* were collected in the month of September 2011 from Crop Science farm, University of Nigeria Nsukka,



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methanol (Sigma-Aldrich, Germany), Ketamine® (Laborate Pharmaceutical, India), neomycin® (Drugfield, Lagos, Nigeria), methylated spirit, gentian violet (Kenol Pharm Ltd, Nsukka, Nigeria), distilled water (Lion Water, Nigeria), Albino rats obtained from animal house, Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. All other chemicals and reagents were of analytical grade and were used without further purification.

Methods

Preparation of crude extract

Fresh leaves of *V. amygdalina* weighing 1 kg were washed, sun-dried and milled with a hammer mill. After the milling, the powder obtained weighing 342 g was extracted with 500 ml of methanol in a Soxhlet extractor for 4 h. The methanol extract was poured into flat trays and allowed to concentrate under fan. The quantity of yield of the extract was calculated using the formula:

$$\frac{\text{percentage yield of extract} - \text{Eqn 1}}{\frac{\text{weight of milled leaf}}{\text{weight of extract}} \times 100}$$

Phytochemical screening of the extract

Phytochemical screened was carried out on the methanol leaf extract for the following components; carbohydrates, cardiac and cyanogenic glycosides, tannins, saponin, anthraquinones, flavonoids and alkaloids.

Experimentally induced excision wounds

The animal experimental protocols were in accordance with the guidelines for conducting animal experiments stipulated by our Institution's Animal Ethics Committee and in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC) [15]. The initial procedure was according to previous author [16]. Twenty albino rats weighing 230-250 g were used for the study. Briefly, the rats were divided into five groups (1, 2, 3, 4 and 5) of four rats per group. They were fed for one week and allowed to acclimatize to laboratory condition. The animals were anesthetized with 0.09 ml of ketamine® i.m injection (30 mg/kg, 100 mg/ml) and 0.01 ml of xylazil i.m. injection (3 mg/kg, 100 mg/ml) prior to creation of the wounds. The wound site was prepared following the excision wound model [17], the hairs on the skin of the back of the albino rats were shaved with sterile razor blade, disinfected with 70 % alcohol and injected with 1 ml of subcutaneous lignocaine HCl (2 %, 100 mg/5 ml). A circular diameter of 20 mm was marked on the

dorsum of the skin of each rat and circular excision made on the marked areas of the skin [18].

Evaluation of the wound healing effect of the extracts

Treatment on the excision was done after every three days with groups 1, 2, 3, 4 and 5 receiving 2.5 g% of methanol extract of *V. amygdalina*, 5.0 g% of methanol extract of *V. amygdalina*, natural squeezed fresh leaf extract of *V. amygdalina*, neomycin powder and no treatment respectively. In each case all the applications were made topically after dressing with methylated spirit and the wound area measured once every 3 days.

Macroscopic evaluation of the wounds

During the 24 days of the experiment, wound closure was macroscopically recorded with photography. All wounds were imaged using a high resolution Lebeca camera (PanWest, China) with a built in image sensor (8,000 pixels).

Determination of wound closure rate

The wound closure rate was calculated for each batch using the formula:

$$\text{wound closure rate} = \frac{L_o - L_f}{L_o} \times 100\% \quad \text{Eqn 1}$$

Where L_o is the lengths of the originally created wound; L_f the length of the wound for a specified time interval.

Toxicity studies of the wound

This was done by checking the following parameters on the rats: dryness of wound area, wound odor, exudation, wound contraction, effect of food intake, effect of water intake, itching and physical state.

Statistical analysis

All values were reported as mean \pm S.E.M. Statistical significance of differences among groups were assessed using one-way ANOVA. Value of $p < 0.05$ was considered significant.

RESULT AND DISCUSSION

Yield of extract

The extraction process afforded 40 g (11.69 %) of the methanol extract.

Evaluation of wound healing

Wound healing as a process consists of integrated cellular and biochemical events leading to re establishment of structural and functional integrity with regain of strength of injured tissue. The phenomenon of wound healing can be defined as the body's natural process of regenerating dermal and epithelial tissues. When a wound is inflicted, a set of intricate biochemical events occur in a

programmed cascade to repair the damage [19, 20]. Topical application of the extracts caused a

significant concentration related reduction in wound diameter over time (Table 1).

Table 1: Effects of treatment (wound closure in cm) with exposure time

| Groups | Exposure time (days) | | | | | | | | | |
|--------|----------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--|
| | 1(cm) | 3(cm) | 6(cm) | 9(cm) | 12(cm) | 15 cm) | 18(cm) | 21(cm) | 24(cm) | |
| 1 | 2.15 ± 0.03 | 1.93 ± 0.03 | 1.70 ± 0.07 | 1.70 ± 0.06 | 1.30 ± 0.1 | 0.97 ± 0.22 | 0.43 ± 0.09 | 0.15 ± 0.00 | 0.00 | |
| 2 | 2.00 ± 0.57 | 1.88 ± 0.04 | 1.77 ± 0.03 | 1.63 ± 0.03 | 1.27 ± 0.07 | 0.57 ± 0.07 | 0.20 ± 0.06 | 0.00 | 0.00 | |
| 3 | 2.10 ± 0.06 | 1.95 ± 0.03 | 1.77 ± 0.03 | 1.30 ± 0.15 | 0.70 ± 0.15 | 0.30 ± 0.12 | 0.00 | 0.00 | 0.00 | |
| 4 | 2.00 ± 0.06 | 1.95 ± 0.03 | 1.80 ± 0.06 | 1.63 ± 0.07 | 1.50 ± 0.06 | 0.90 ± 0.15 | 0.43 ± 0.13 | 0.15 ± 0.00 | 0.00 | |
| 5 | 2.00 ± 0.12 | 1.98 ± 0.01 | 1.92 ± 0.28 | 1.85 ± 0.03 | 1.63 ± 0.03 | 1.30 ± 0.10 | 0.90 ± 0.15 | 0.47 ± 0.12 | 0.23 ± 0.08 | |

Since flavonoids are established as possessing antioxidant activity [21-26]. It can be speculated that the antioxidant properties of *V. amygdalina* can be attributed to the presence of these flavonoids as reported by Igile *et al.* [27] which can traverse the blood brain barrier [28]. Owoeye *et al.* reported [29] the neuroprotection of the cerebellum by the methanol leaf extract of *V. amygdalina* leaves on the gamma-irradiated brain of Wistar rats which is in conformity with the properties of the plant.

Mechanisms of wound healing may be attributed to stimulation of the production of antioxidants in wound site and which provides a favorable environment for tissue healing and these antioxidants may play a significant role in the wound

healing process and may be important contributory factor in the wound healing property [30]; antioxidants also improve wound healing and protect tissues from oxidative damage [31]. Flavonoids can scavenge for the reactive oxygen species (super-oxide anions) and free radicals produced by ethanol and these reactive intermediates are potentially implicated in delayed wound healing [32], thus the higher the flavonoids content, the stronger the antioxidant activity [33].

Percentage wound closure rate

To elucidate the role of *A. amygdalina* in wound contraction, the wound sites were evaluated by visual examination and measurement of wound photographs.

Table 2: Percentage wound closure on the mice for the period of study

| Time (days) | Group 1 (%) | Group 2 (%) | Group 3 (%) | Group 4 (%) | Group 5 (%) |
|-------------|-------------|-------------|-------------|-------------|-------------|
| 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 3.00 | 3.50 | 6.00 | 2.50 | 2.50 | 1.00 |
| 6.00 | 15.50 | 11.50 | 11.50 | 10.00 | 4.00 |
| 9.00 | 15.00 | 18.50 | 35.00 | 18.50 | 7.50 |
| 12.00 | 35.00 | 36.50 | 65.00 | 25.00 | 18.50 |
| 15.00 | 51.50 | 71.50 | 85.00 | 55.00 | 35.00 |
| 18.00 | 78.50 | 90.00 | 100.00 | 78.50 | 55.00 |
| 21.00 | 92.50 | 100.00 | 100.00 | 92.50 | 76.50 |
| 24.00 | 100.00 | 100.00 | 100.00 | 100.00 | 88.50 |

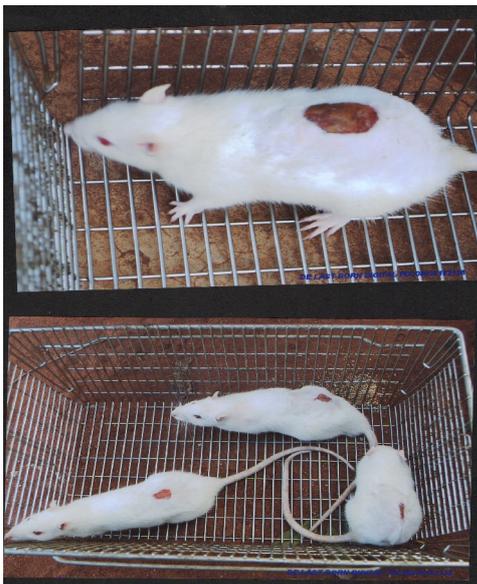
Group 3 containing the squeezed leaf extract healed fastest at the 18th day, followed by group 2 containing 5 % of the methanol extract which healed on the 21st day while group 1 containing 2.5 % of methanol and group 4 containing the neomycin healed on the 24th day. As seen from group 5 in Tables 1 and 2, it is accepted that wound repair is an immune-mediated physiologic mechanism;

wound healing, or wound repair, is an intricate process in which the skin repairs itself after injury [34]. The wound margins were easily recognized by an abrupt interruption in the epithelial and dermal continuity. Different patterns of wound contraction ranging from moderate to incomplete wound

contraction were observed among different groups at day 18 post exposure (Table 1). A significant reduction ($p < 0.05$) in wound contraction of all treated groups was observed at day 18 post

exposure (Table 2). At day 21 post-wounding, a highly significant reduction ($p < 0.01$) in wound contraction was observed among all treated groups with a maximum reduction ($p < 0.001$) in group 3. A highly significant reduction ($p < 0.001$) and completely contracted wounds were prominent in groups 2 and 3 at day 21 post-wounding. Moreover, a non-significant ($p > 0.005$) difference was observed among different *A. amygdalina* treated groups. This implies that the wound may eventually heal though taking longer time than the drug treated groups only if no infection invaded the wound area.

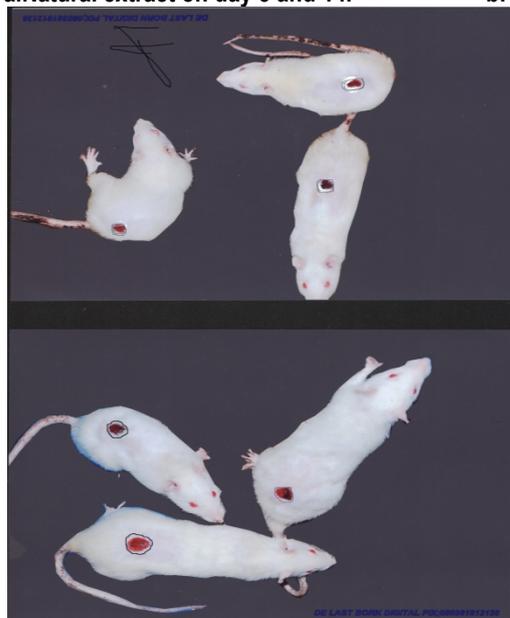
In the present study, rats treated with different doses of *V. amygdalina* had larger wound margins at different days post-wounding when compared with the control groups except the 2.5 % extract that has the same wound closure rate with the positive control (group 4). Continuous application of *A. amygdalina* at wound sites caused progressive acceleration in wound closure ranging from a significant ($p < 0.05$) to highly significant ($p < 0.001$) closure at day 18-21 postwounding; (Table 2), indicating that continuous application of *V. amygdalina* has positive effect on wound closure.



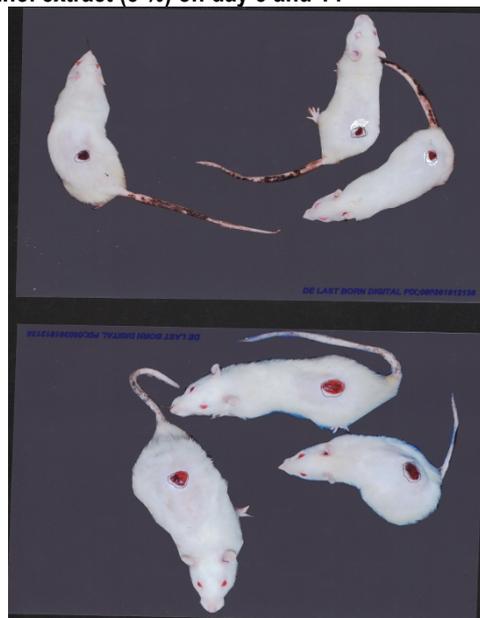
a. Natural extract on day 3 and 14.



b. Methanol extract (5 %) on day 3 and 14



c. Methanol extract (2.5 %) and neomycin



d. negative control day 3 and 14

Fig.1: Effect of *V. amygdalina* on wound healing

The Figs.1a-1d showed a representation of the gradual decrease in wound size as the time prolongs. Group 4 containing the natural squeezed fresh leaf extract of *V. amygdalina* had the greatest decrease in wound while the least healed was the group 5 which had no treatment.

Table 3: Wound evaluation study

| | Group 1 (%) | Group 2 (%) | Group 3 (%) | Group 4 (%) | Group 5 (%) |
|------------------------|-------------|-------------|-------------|-------------|-------------|
| Dryness of wound area | ++ | +++ | +++ | ++ | + |
| Wound odour | - | - | - | - | + |
| Exudation | - | - | - | + | ++ |
| Wound contraction | ++ | +++ | +++ | ++ | + |
| Effect of food intake | + | ++ | ++ | + | + |
| Effect of water intake | + | ++ | ++ | + | + |
| Itching | ++ | + | + | ++ | +++ |
| Physical state | ++ | ++ | +++ | ++ | ++ |

CONCLUSIONS

A number of chemical compounds including edotides, and sesquiterpene lactones have been isolated from the leaf of *V. amygdalina*. These compounds elicit remarkable antioxidant: in rodent models and it is this antioxidant property that plays a significant role in suppressing the proliferation of excision wounds thereby preventing invasion of pathogenic micro-organism.

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