

## COMPARATIVE EVALUATION OF WOUND HEALING EFFECTS OF OCIMUM GRATISSIMUM, VERNONIA AMYGDALINE AND ZINGIBER OFFICINALIS EXTRACTS ON INCISION WOUND MODEL IN RATS

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### Abstract

Wound healing effects of *Ocimum gratissimum*, *Vernonia amygdalina* and *Zingiber officinalis* extracts were investigated using incisional wound model in rats. Twenty five (25) male Wistar albino rats, weighing between 100–150 grams were used for the study. They were divided into five groups of five rats each. Group A received the extract of *Zingiber officinalis* whereas groups B and C received the extracts of *Ocimum gratissimum* and *Vernonia amygdalina*, respectively. Group D served as positive control and received hydrogen peroxide and group E served as negative control and received normal saline. Wound healing indices such as wound contraction, re-epithelialization and whooping (fluid exudation) were subjectively and grossly studied. Area of wound contraction was observed to be time dependent regardless of the extract in both groups. In all extracts, wound contraction improved with duration of exposure to extract, being least at day 2 and highest at day 18. Similar results were obtained for both the positive and negative control using hydrogen peroxide and normal saline. The extracts enhanced wound contraction with more percentage area of wound contraction occurring for the group of animals treated with *V. amygdalina* than for the other extracts. Ranging of extracts wound healing effect were noted to be thus *V. amygdalina* > *O. gratissimum* > *Z. officinalis*. Although, animals treated with *V. amygdalina* showed greater healing than other animals of the positive and negative control but it showed no significant difference ( $p < 0.05$ ) when analyzed statistically. Wound epithelialization occurred on the average of two weeks independent of the groups or the extract used. The data from the experiment showed that there was no significant difference ( $p < 0.05$ ) in the mean days of epithelialization for all the five groups. Animals treated with *Vernonia amygdalina* had lesser day of wound epithelialization in comparison to animals treated with other extracts but yet showed no significant difference ( $p < 0.05$ ) when analyzed statistically. Exudation was mostly observed in the untreated wounds. The observation in the course of the experiment shows that whooping occurred in early stage of the wound for some extracts. Animals treated with plant extracts of *Z. officinalis*, *O. Gratissimum*, *V. amygdalina* and even hydrogen peroxide showed significant difference ( $p < 0.05$ ) in exudation when compared to animals treated with normal saline. However, *Vernonia amygdalina* proved more efficacious than any other extract for the treatment of all the phases of wound healing because it has the highest percentage area of wound contraction in almost all the days post-wounding.

**Keywords-** Incisional wound, rats, plant extracts, wound healing

## Introduction

Wounds are physical injuries that result in an opening or breaking of the skin [1]. Wound healing involves various phases. Initially involves acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules, which are later removed to form a scar [2]. Drugs, which influence one phase, may not necessarily influence another [2]. Alterations in any of these steps can lead to delay or inability in dermal wound-healing. Healing of wound starts from the moment of injury and continues for a period of weeks depending on the extent of injury.

Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. In order to establish proper treatment regimen, a number of important local wound factors have to be considered, e.g., appraisal of location, depth, size, neighbouring tissues, organs, necrosis, secretion, colouring and sensibility [3].

Wounds could be incision, burn wound, excision and dead space wound [4]. Incision is a surgical wound caused by cutting open a part of the body with that part remaining intact [5]. Excision is the removal of a particular part of the body which could be tissue or even the skin [5]. A lot of research has been envisaged to develop the better healing agents. Research on drugs which accelerate wound healing is developing and it is a crucial subject in biomedical science. Several plants and herbs have been used experimentally to treat skin disorders including wound healing in traditional medicine [6]. World Health Organization (WHO) has been promoting traditional medicine as a source of less expensive, comprehensive medical care, especially in developing countries [7]. Eight percent of the world's population relies on medicinal plants for their primary health care.

Akinjogunla *et al.* [8] observed that herbal medicine is the oldest form of healthcare known to mankind and over 50% of all modern clinical drugs are of natural origin and natural products play important roles in drug development in the pharmaceutical industry. Several herbs have been suggested to be of good help in wound healing [9]. The objective of the study was to evaluate the wound healing effects of *Zingiber officinalis*, *Vernonia amygdalina* and *Occimum gratissimum* on incisional wound model in Wister albino rats.

## Materials and Methods

### Animals

Male Wister albino rats, weighing between 100 – 150 grams, were used for the study. They were fed with standard feed and allowed to acclimatize for 2 weeks. They were housed in metal cages maintained under standard conditions (12 hour light - dark cycle;  $25 \pm 3$  °C; 35 – 60% humidity). The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was cleared by same before beginning the experiment.

### Description of plant materials

*Zingiber officinalis* (Fig. 1) belongs to the family Zingiberaceae, commonly known as ginger. The part used are rhizomes and it contains curcumin (diferuloyl methane), turmeric oil or turmerol & 1, 7-bis, 6-hepta-diene-3, 5-Dione. Curcumin has potent anti-inflammatory and analgesic activities. Volatile oil isolated from *Z. officinalis* also exhibits antibacterial and potent anti-inflammatory activity. *Zingiber officinalis* also contains protein, fats, vitamins (A, B, C etc) all of which have an important role in wound healing and regeneration. The presence of vitamin A and proteins in turmeric result in the early synthesis of collagen fibers by mimicking fibroblastic activity. Juice of the fresh rhizome is commonly applied to recent wounds, bruises and leech bites. It can also be mixed with ginger oil to prevent skin eruptions [10].

*Vernonia amygdalina* (Fig. 2), commonly called bitter leaf, is a perennial shrub of 2 – 5 m in height that grows throughout tropical Africa. It belongs to the family Asteraceae, has a rough bark with dense black straits, and elliptic leaves that are about 6 mm in length. The leaves are green and have a characteristic odor and bitter taste. In many parts of West Africa, the plant has been domesticated [11]. *Vernonia amygdalina* has been shown to contain significant quantities of lipids, proteins with high essential amino acid score that compare favorably with *Telfairia occidentalis* and *Talinum triangulare*. The plant has also been shown to contain appreciable quantities of ascorbic acid and carotenoids. Calcium, iron, potassium, phosphorous, manganese, copper and cobalt have also been found in significant quantities in *V. amygdalina* [12].

*Ocimum gratissimum* (Fig. 3) is a shrub up to 1.9 m in height with stems that are branched. The leaves measure up to 10 x 5 cm, and are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at base with a coarsely crenate, serrate margin, pubescent and dotted on

both the sides. The leaves show the presence of covering and glandular trichomes. Stomata are rare or absent on the upper surface while they are present on the lower surface [5].

#### **Preparation of herbal extracts**

*Ocimum gratissimum* and *Vernonia amygdalina* plants were prepared by air drying the leaves in room temperature for days. *Zingiber officinalis* plant is prepared by air drying the roots of *Zingiber officinalis* for days. The dried samples were reduced to coarse powder by crushing in a mortar and were finally pulverized into a fine powder in a hammer mill with an in-built sieve. Cold maceration method using 50% methanol as the solvent was used in the extraction. The solvent is prepared by mixing 250ml of methanol (analar grade) with 250ml of the distilled water. 100g of each powdered sample was added to 500ml of the solvent. The solutions were properly mixed by constant shaking at an interval of 3hours for 48hours at room temperature and later filtered using No 1 Whatman filter paper. The filtrates were concentrated (dried) at oven temperature of 37°C for 3 day.

#### **Treatment protocols**

The animals were numbered, weighed and then divided into five groups with five animals in each group. Group A received the extract of *Zingiber officinalis*, Group B received the extract of *Ocimum gratissimum*, Group C received the extract of *Vernonia amygdalina*, Group D served as positive control and received hydrogen peroxide and Group E served as negative control and received normal saline.

#### **Excision wound model**

The rats were first anaesthetized with xylazine (5mg/kg IM) and ketamine (90mg/kg IM). Hairs were shaved off from the dorsal thoracic central region of the rat. A circular area for excision were marked by using marker to make a circular make on the shaved region, and then scapel blade was used to cut off the marked circular skin of the rat. After the excision wound was inflicted by cutting away the skin from a predetermined area; the wound was left undressed. Then the extracts were applied (as stated above) on the injured area. Calculation of the percent reduction in wound area was done.

#### **Assessment of wound healing**

The diameter of the wounds was measured with a

transparent meter rule. The diameter was obtained by measuring horizontally and vertically, and then the mean was taken as the diameter. The area was calculated and recorded as initial wound area which is the wound area for day zero. Treatment of the wound began immediately after the initial measurement. Wounds were measure daily from day 0 to day 21 post surgeries. The area was obtained using the formula:  $A = \pi r^2$ . Where A = Area,  $\pi = 22/7$  and  $r^2 =$  diameter. The rate of wound contraction for each rat was determined by using the formula:  $W_0 - W_1$ . Where  $W_0 =$  initial wound area and  $W_1 =$  wound area of each measuring day. The percentage wound contraction was calculated thus: % Wound contraction = Healed area  $\div$  total area  $\times$  100. Epithelialization time was noted as a number of days required for the first scare of the wound to fall off. Complete wound healing was determined by the total number of days required for the second scare to fall off [14]. Subjective assessment was also considered to determine presence of discharges. Then histopathology of the wound was analyzed to know the relationship in between cells.

#### **Statistical analysis**

Data on percentage of wound contraction mean days and mean days to complete healing was analyzed statistically using one-way analysis of variance (ANOVA). 95% confidence interval was considered that is  $p \leq 0.05$ . F-LSA was employed to ascertain the significant differences of wound healing among the three extracts used.

## **Results**

#### **Wound contraction**

Area of wound contraction is time dependent regardless of the extract. In all extracts, wound contraction improved with duration of exposure to extract, being least at day 2 and highest at day 18. Similar results were obtained for both the positive and negative control using hydrogen peroxide and normal saline respectively (Table- 1). The extract enhanced wound contraction with more percentage area of wound contracted occurring for the group of animals treated with *V. amygdalina* than for the other extracts. Ranging of extract wound healing effect can be thus  $V. amygdalina > O. gratissimum > Z. officinalis$ . More wound contraction was recorded for *V. amygdalina* than the positive and negative controls. Although, animals treated with *V. amygdalina* showed greater healing than other animals of the positive and negative control but it

showed no significant difference ( $p < 0.05$ ) when analyzed statistically.

### **Wound epithelialization**

Wound epithelialization occurs on the average of two weeks independent of the extract used. The data from the experiment showed that there was no significant difference ( $p < 0.05$ ) in the mean days of epithelialization for all extracts and the positive and negative control (Table- 2). Animals treated *Vernonia amygdalina* had lesser day of wound epithelialization in comparison to animals treated with other extracts but yet showed no significant difference ( $p < 0.05$ ) when analyzed statistically.

### **Complete wound healing**

The data from the experiment showed that complete wound healing occurs in the average of two weeks independent of the extract used in wound treatment. From the experiment, data showed that there was no significant difference ( $p < 0.05$ ) in the mean days of complete wound healing for all extracts and the positive and negative control (Table- 3). Animals treated *V. amygdalina* had lesser day of complete wound healing compared to animals treated with other extracts but yet showed no significant difference ( $p < 0.05$ ) when analyzed statistically.

### **Exudation/whooping of wound**

Exudation is unique to untreated wounds (Table 4). The observation in the course of the experiment shows that whooping occurred in early stage of the wound for some extracts. Animals treated with plant extracts of *Z. officinalis*, *O. Gratissimum*, *V. amygdalina* and even hydrogen peroxide showed significant difference ( $p < 0.05$ ) in exudation when compared to animals treated with normal saline.

### **Discussion**

Proper and timely wound healing is a problem faced by all clinicians. In majority of patients normal healing establishes tissue integrity quickly and effectively. However, at times this healing is delayed and the ability to accelerate the wound healing becomes a highly desirable objective [15].

Wound contraction is one of the parameters used in assessing wound healing. It commences approximately a week post surgery [16]. From the research, the data showed that extracts of *V. amygdalina* has higher percentage area of wound contracted than extracts of *Z. officinalis*, *O.*

*gratissimum* and even those of the hydrogen peroxide and normal saline.

*V. amygdalina* shows significant difference ( $p < 0.05$ ) in wound contraction when compared to *Z. Officinalis* and *O. gratissimum* but with hydrogen peroxide and normal saline, it showed no significant difference ( $p < 0.05$ ). It can be said from the result that *O. gratissimum* and *V. amygdalina* is good in treating the inflammatory phase of wound as they showed higher percentage area of contracted in the first four days of post surgery and since different drugs affect various phases of wound. This is in line with work of Velmurugan *et al.* [2], where they used *Gossypium herbaceum* in treating excision and incision wound in rats. Garg [17] recorded that wound epithelialization is another parameter used in evaluation of wound and it occurs when epithelial cell covers the wound bed providing coverage for the new tissue. The data from the research showed that animals treated with *V. amygdalina* have lesser day of wound epithelialization than animals treated with other extracts but still showed no significant difference ( $p < 0.05$ ) when analyzed statistically.

From the research, the mean day of complete wound healing showed that there was no significant difference ( $p < 0.05$ ) when animals treated with *V. amygdalina* were compared to animals treated with *Z. officinalis*, *O. gratissimum*, hydrogen peroxide (positive control) and normal saline (negative control).

Considering the mean days of exudation/whooping, animals treated with normal saline showed significant difference ( $p < 0.05$ ) when compared to animals treated with *Z. officinalis*, *O. gratissimum*, *V. amygdalina* and hydrogen peroxide. This means that exudation is seen in untreated wound than treated wounds. It implies that higher exudation seen in animals treated with normal saline is due to the effect bacteria and other pathogens on the wound. These findings agreed with the work of Kerstein [18]. The positive control (hydrogen peroxide) is good in the treatment of human impetigo conagiosa caused by *Staphylococcus aureus* [19]. From this, we can say since animals treated with *Z. officinalis*, *O. gratissimum*, *V. amygdalina* showed very low day of wound exudation in comparison to those of the normal saline, it is good in the treatment of human *Impetigo conagiosa* caused by *Staphylococcus aureus*.

The increased wound contraction in the treated group may be due to the enhanced activity of fibroblasts in the *Z. officinalis*, *O. gratissimum*, *V.*

*amygdalina* plant extracts and hydrogen peroxide. A significant increase in collagen content due to enhanced migration of fibroblasts and epithelial cells to the wound site was observed during the wound healing process in the treated group. The animals treated with *V. amygdalina* were in the last phase of wound healing (remodeling phase) which shows good healing. The findings in this study can be likened to the work of Devender *et al.* [14], where he used *Plumbago zeylanica* in treating excision model of wound on albino rats.

### Conclusion

In conclusion, this research shows that *Zingiber officinalis*, *Occimum gratissimum* and *Vernonia amygdalina* are good for the treatment of wound and effective in preventing bacteria action. *Vernonia amygdalina* is good for the treatment of all the phases of wound healing because it has the highest percentage area of wound contracted in almost all the days and should be adopted for treatment of wound. The *Zingiber officinalis*, *Occimum gratissimum* and *Vernonia amygdalina* are natural herbs and so are accessible and affordable at all seasons and should be adopted for treatment of wounds as their effectiveness is almost like that of refined medicines for wound healing.

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**Fig. 1:** Picture of *Zingiber officinalis* [10]



**Fig. 2:** Picture of *Vernonia amygdalina* [11]



**Fig. 3:** Diagram of *Ocimum gratissimum* [13]

**Table 1:** Percentage wound contraction of rats treated with varied methanolic plant extracts

Days	Group A ( <i>Z. officinalis</i> )	Group B ( <i>O. gratissimum</i> )	Group C ( <i>V. amygdalina</i> )	Group D (Hydrogen peroxide)	Group E (Normal saline)
2	5.71 ± 0.00 <sup>b</sup>	15.69 ± 0.00 <sup>a</sup>	15.07 ± 0.00 <sup>a</sup>	6.33 ± 1.00 <sup>b</sup>	5.51 ± 0.00 <sup>b</sup>
4	9.39 ± 0.00 <sup>c</sup>	18.61 ± 0.00 <sup>a</sup>	20.22 ± 0.00 <sup>a</sup>	16.03 ± 0.00 <sup>a</sup>	19.07 ± 1.41 <sup>a</sup>
6	20.41 ± 1.41 <sup>b</sup>	23.00 ± 1.41 <sup>b</sup>	27.57 ± 0.00 <sup>a</sup>	23.21 ± 1.41 <sup>b</sup>	29.24 ± 1.41 <sup>a</sup>
8	28.98 ± 1.41 <sup>b</sup>	31.39 ± 0.00 <sup>b</sup>	35.29 ± 1.41 <sup>a</sup>	27.85 ± 1.41 <sup>b</sup>	39.41 ± 1.41 <sup>a</sup>
10	40.00 ± 2.83 <sup>b</sup>	42.20 ± 0.71 <sup>b</sup>	47.43 ± 0.00 <sup>b</sup>	44.73 ± 0.00 <sup>b</sup>	64.41 ± 1.41 <sup>a</sup>
12	52.65 ± 1.41 <sup>c</sup>	61.68 ± 1.41 <sup>b</sup>	72.43 ± 0.00 <sup>a</sup>	59.49 ± 0.00 <sup>b</sup>	77.54 ± 1.41 <sup>a</sup>
14	76.73 ± 0.00 <sup>b</sup>	78.43 ± 0.71 <sup>b</sup>	80.88 ± 0.00 <sup>a</sup>	73.42 ± 1.41 <sup>b</sup>	83.05 ± 1.41 <sup>a</sup>
16	81.63 ± 1.41 <sup>a</sup>	84.31 ± 0.00 <sup>a</sup>	88.24 ± 1.41 <sup>a</sup>	86.92 ± 0.00 <sup>a</sup>	86.86 ± 1.41 <sup>a</sup>
18	86.12 ± 1.41 <sup>b</sup>	87.96 ± 0.01 <sup>b</sup>	95.84 ± 0.37 <sup>a</sup>	94.94 ± 0.00 <sup>a</sup>	93.66 ± 0.03 <sup>a</sup>

Significant difference at  $P < 0.05$  for rats treated with varied extracts. Values are Mean ± SEM from 5 animals in each group. Values with the same superscript are not significant while those of different superscript are significant.

**Table 2:** Wound epithelization of rats treated with varied plant methanolic extracts

Groups	Mean Days
Group A ( <i>Z. officinalis</i> )	10.40 ± 0.00 <sup>b</sup>
Group B ( <i>O. gratissimum</i> )	11.00 ± 1.41 <sup>b</sup>
Group C ( <i>V. amygdalina</i> )	10.00 ± 1.41 <sup>B</sup>
GROUP D (Hydrogen peroxide)	12.80 ± 1.41 <sup>B</sup>
GROUP E (Normal saline)	10.60 ± 1.41 <sup>B</sup>

Significant difference at  $P < 0.05$  for rats treated with varied extracts. Values are Mean ± SEM from 5 animals in each group. Values with the same superscript are not significant while those of different superscript are significant.

**Table 3:** Complete wound healing of rats treated with varied plant methanolic extracts

Groups	Mean Days
Group A ( <i>Z. officinalis</i> )	17.00 ± 1.41 <sup>b</sup>
Group B ( <i>O. gratissimum</i> )	18.40 ± 0.00 <sup>b</sup>
Group C ( <i>V. amygdalina</i> )	16.60 ± 1.41 <sup>b</sup>
GROUP D (Hydrogen peroxide)	17.20 ± 1.41 <sup>b</sup>
GROUP E (Normal saline)	17.40 ± 1.41 <sup>b</sup>

Significant difference at  $P < 0.05$  for rats treated with varied extracts. Values are Mean ± SEM from 5 animals in each group. Values with the same superscript are not significant while those of different superscript are significant.

**Table 4:** Exudation/whooping of wound of rats treated with varied plant methanolic extracts

Groups	Days
Group A ( <i>Z. officinalis</i> )	0.00 ± 0.00 <sup>A</sup>
Group B ( <i>O. gratissimum</i> )	0.20 ± 0.00 <sup>A</sup>
Group C ( <i>V. amygdalina</i> )	0.00 ± 0.00 <sup>A</sup>
Group D (Hydrogen peroxide)	0.20 ± 1.41 <sup>a</sup>
Group E (Normal saline)	3.70 ± 1.41 <sup>B</sup>

Significant difference at  $P < 0.05$  for rats treated with varied extracts. Values are Mean ± SEM from 5 animals in each group. Values with the same superscript are not significant while those of different superscript are significant