INTRODUCTION

Wound healing is an important biological process involving tissue repair and regeneration. A wound is described as ‘a break in the continuity of tissue, from violence or trauma’ and is regarded as healed if there is a restoration of the wounded or inflamed tissue to normal condition (1). 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 (2).

There are four distinct stages involved in wound healing namely – inflammatory stage, debridement stage, proliferation stage and maturation/remodeling stage (2). 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

THE WOUND HEALING EFFECT OF HERBAL OINTMENTS FORMULATED WITH NAPOLEONA IMPERIALIS

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ABSTRACT

The wound healing effect of herbal ointments formulated with *Napoleona imperialis* (NI) embedded in different ointment bases (anionic, cationic and non-ionic) has been evaluated in vivo using the excision wound healing model, on guinea pigs. *Napoleona imperialis* was extracted using methanol and the extract formulated as herbal ointments. The herbal ointments were used to treat wounds inflicted on experimental guinea pigs. The wound healing effects of the formulations were compared to that of a standard antibiotic, Cicatrin®. In all cases, there was a progressive decrease in wound area with time, indicating an efficacy of the formulations in healing the induced wounds. By the 16th day, the ointment containing 100 mg/g of *Napoleona imperialis* in non-ionic emulsifying ointment base showed 100% healing. The wound areas in the animals treated with the standard antibiotic, Cicatrin® showed a 100% healing by the 19th day, indicating that the plant extract, at that given concentration, had a better wound healing property than the standard antibiotic.

Keywords: Wounds, healing, herbal ointments, *Napoleona imperialis*, Cicatrin®
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 (2).

Certain factors that influence wound healing include bacterial infection, nutritional deficiency, drugs, sterility, obesity, movement of wound edges, site of wound, and wasting diseases (3). 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 (4). 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 (5).

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. A study on *Vernonia amygdalina*, a plant used in folklore for wound healing, has for instance, revealed the presence of saponins, alkaloids and tannins (6). The antimicrobial screening of *Landolphia owerrience* among other plants, has shown that the extract inhibits *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger* and the activities were linked to the presence of steroids, tannins and glycosides in the plants (7).

*Napoleona imperialis* is of the family of plants called Lecythidaceae. This family has 15 genera and 325 species. It is a woody plant, several meters high, found mainly in tropical rain forest (8). The pinnate leaves, which are alternatively arranged, are oval in shape with acute apex and reticulate venation. The margin of the leaves is entire and the surface smooth. The leaf is about 12 cm long and 7 cm wide and is used locally as analgesic, tonic, antitussive, antiasthmatic, and wound dressing (9). Preliminary investigation into the antispasmodic properties of the aqueous root bark extract was carried out and the result confirmed its folkloric claim of use in treating asthma (10). In this present study, ointments formulated with the volatile oil from *Napoleona imperialis* (NI) leaves were evaluated for their wound healing activity on guinea pigs using the excision wound healing model.

**MATERIALS AND METHODS**

**Materials**

Shea butter, liquid paraffin and white soft paraffin were obtained from Pharmaceutics Laboratory, University of Nigeria, Nsukka. Other reagents were of analytical grades and include anionic emulsifying wax (Merck, Darmstadt), cetrimide (Merck, Darmstadt), Polyoxylene sorbitan tristearate (Atlas, USA), cetostearyl alcohol, (Merck, Darmstadt), and Cicatrin® powder (Glaxowellcome, Nigeria). Fresh leaves
of *Napoleona imperialis* were purchased from Agbor in Delta State of Nigeria and were identified in the Department of Botany, University of Nigeria, Nsukka where a Voucher specimen was also deposited. The guinea pigs were purchased from Nkwo Ibagwa market in Nsukka.

**Methods**

**Preparation of extract**

The fresh leaves were washed, chopped into tiny fragments and sun-dried. The dried leaves were ground into powder with pestle and mortar. From the pulverized leaves, 20 g was added to a dish and sufficient quantity of methanol added. This was vigorously shaken thrice daily, for 2 days. The mixture was filtered and the filtrate placed in a hot air oven (Gallenkamp, England) maintained at 40°C. After evaporation of the solvent, the resulting extract was placed in a sealed bottle until ready for use.

**Formulation of emulsifying ointments**

Three different emulsifying ointments representing anionic, cationic and non-ionic types respectively, were prepared according to the following formula:

(a) **Anionic emulsifying ointment**

- Emulsifying wax BP – 30%
- Liquid paraffin - 20%
- White soft paraffin - 50%

(b) **Cationic emulsifying ointment**

- Shea butter - 50%
- Cetostearyl alcohol - 45%
- Cetrimide - 5%

(c) **Non-ionic emulsifying ointment**

- Shea butter - 60%
- Cetostearyl alcohol - 36%

Tween 65 EP - 4%

In preparing the anionic emulsifying ointment, the liquid paraffin, white soft paraffin and emulsifying wax were melted together in a beaker and immersed in a thermostated hot water bath. The melted ingredients were stirred until cold. For the cationic and non-ionic emulsifying ointments, the cetostearyl alcohol and shea butter were melted at about 97°C, followed by the addition of cetrimide or Tween 65 as the case may be, and stirring continually till cold.

**Preparation of medicated ointments**

The medicated ointments were prepared according to the following formula:

(a) Anionic

- Anionic emulsifying ointment - 90%
- *Napoleona imperialis* extract – 10%

(b) Cationic

- Cationic emulsifying ointment – 90%
- *Napoleona imperialis* extract – 10%

(c) Non-ionic

- Non-ionic emulsifying ointment – 90%
- *Napoleona imperialis* extract – 10%

The fusion method was employed in the preparation of the medicated ointments. The required quantity of the ointment base was weighed and melted at a temperature of about 70°C in a hot water bath. The designated quantity of the extract (s) were respectively added to the melted base at 40°C and the mix, stirred gently and continuously until a homogenous dispersion is obtained. Each of the exercises was repeated using Cicatrin® powder instead of the NI extracts.

**Animal studies**
About fifteen healthy guinea pigs were employed in the study. The animals were quarantined for a period of three weeks to ensure stabilization before use. Feeds consisted of grower’s mash, grasses and water provided for the animals ad libitum.

(i) Preparation of wound site

The wound site was prepared following the excision wound model (11). The animals were anaesthetized with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades. A circle of diameter 20 mm was marked on each of the two sides of the skin. Circular incisions were then made on the marked areas 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.

(ii) Determination of wound healing rate

Treatment with the ointments started immediately after incision by application on the wound surface, of the ointments, once daily. The two wound sites on each animal were treated similarly. All the ointments were applied topically after dressing the wound with methylated spirit. The wound areas were measured while the animals were under anaesthesia on the 1st, 4th, 7th, 10th, 14th, 16th and 19th day after surgery. The resulting data were analysed statistically using the One-way Analysis of Variance (ANOVA), and the significant means were separated using the Duncan multiple range test. The probability level was 5%.

RESULTS AND DISCUSSION

Table 1 shows the results of the wound healing effects of the various ointment formulations. There was a general decrease in wound area upon application of the ointments and with time. By the 4th day, the animals treated with ointments containing 100 mg/g of *Napoleona imperialis* in anionic emulsifying ointment, all died. There was however a hundred percent (100 %) healing in the animals treated with ointments containing 100 mg/g of NI in cationic emulsifying agent and in the ointment containing Cicatrin powder, by the 19th day after treatment, as the wound sizes reduced to zero.

The ointment containing the negative control had the least rate of wound healing. A drug to be used for effective wound healing should be able to clear the wound by the 19th day after infliction. The death of the animals treated with the ointment containing 100 mg/g of anionic emulsifying could be attributed to contamination or adverse effect of the formulation.

The above result, presented in form of percentage wound size reduction is shown in Figure 1. From the Figure, the ointments prepared with NI in cationic emulsifying ointment base exhibited the highest initial rate of wound healing. It was followed closely by the ointment prepared with non-ionic emulsifying ointment. The control ointment containing neither NI extract nor Cicatrin® had a very low initial...
Table 1 – Effect of ointments containing *Napoleona imperialis* and Cicatrin® on wound sizes

<table>
<thead>
<tr>
<th>Ointment type</th>
<th>Mean Wound size (mm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 4</td>
<td>Day 7</td>
<td>Day 10</td>
<td>Day 14</td>
<td>Day 16</td>
<td>Day 19</td>
</tr>
<tr>
<td>NI (100mg/g) in Anionic Emulsifying ointment</td>
<td>334</td>
<td>264</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NI (100mg/g) in Cationic emulsifying ointment</td>
<td>346</td>
<td>176</td>
<td>110</td>
<td>30</td>
<td>33</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>NI (100 mg/g) in Non-ionic emulsifying ointment</td>
<td>270</td>
<td>214</td>
<td>142</td>
<td>26</td>
<td>16</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Cicatrin Powder</td>
<td>370</td>
<td>245</td>
<td>185</td>
<td>18</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Negative control (without NI)</td>
<td>390</td>
<td>330</td>
<td>305</td>
<td>170</td>
<td>85</td>
<td>45</td>
<td>21</td>
</tr>
</tbody>
</table>
healing rate. By the tenth day however, all the ointment formulations showed almost the same rate of wound healing with the exception of the control ointment which maintained a very slow rate of wound healing even up to the 19th day. The best activity was observed in the ointment containing *Napoleona imperialis* in cationic ointment base. This shows that *Napoleona imperialis* can effectively be employed as a cationic emulsifying ointment in wound healing.

Conclusively, the various ointments prepared with *Napoleona imperialis* exhibited a good wound healing effect comparable to those of Cicatrin®, a standard antibiotic used in wound healing. This finding thus, justifies its use in folkloric medicine for wound healing.

REFERENCES


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