Evaluation of the wound healing activity of ethanol extract of Terminalia avicennioides root bark on two wound models in Rats

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Abstract: The wound healing activity of ethanol extract of Terminalia avicennioides root bark was evaluated in two wound models (excision and incision) in Rattus norvegicus using standard procedures. The present study was aimed to evaluate the scientific validity of the use of Terminalia avicennioides root bark by folklore traditions in Nigeria for treatment of cuts, wounds and burns. Wound area and period of healing were used to evaluate the effect on wound healing. The effects produced in terms of wound contracting ability, wound closure, decrease in surface area of wound, tissue regeneration at the wound site and reduced days of healing were significant (p < 0.05) in ethanol extract of Terminalia avicennioides root bark treated rats. The wound area in 8 days and 16 days post wound for standard drug and tested extract were 260.5mm² (8d), 4mm² (16d) and 252.5mm² (8d), 20mm² (16d) respectively. Both excision and incision wound area completely closed on day 18 for penicillin treated ointment and on day 19 for tested extract treated wound. However, as at the end of experiment the wound area for both excision and incision wounds in simple ointment treated group had not completely closed and were 17.5mm² and 10mm² for excision and incision wounds respectively. The present study thus provides a scientific rationale for the traditional use of this plant in the management of wounds.

Keywords: Wound healing activity; Terminalia avicennioides; Rattus norvegicus; Doko Nigeria.

Introduction

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 wound site or inflamed tissue to normal condition (Adikwu and Ikejiuba 2005). The treatment of wounds is multidisciplinary. The treatment of wounds is essentially the same in most cases (Nguyen et al. 2009). Minor wounds like bruises will heal on their own in no time. The skin discoloration usually disappears in 1-2 weeks. Wound healing is an intricate process in which skin or another organ repair itself after injury (Nguyen et al. 2009). Factors affecting wound healing are nutritional status, dehydration, immune responses, inadequate blood supply to the wound site, age and weight. Wounds are inescapable events in life. Wounds may arise due to physical, chemical or microbial agents. Topical antimicrobial therapy is one of the most important methods of wound care (Meenakshi et al. 2006).

The goal of topical antimicrobial therapy in wound care is to control microbial colonization and subsequent proliferation thus promoting the healing of wounds. Many plant materials are used in the traditional medicine to treat wounds in rural area (Burkill 1985). Medicinal proper-
ties of plants are normally dependent on the presence of certain phytochemical principles such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins and polyphenols which are the bioactive bases responsible for the antimicrobial property (Ebana et al. 1993). Medicinal plants contain pharmacologically active principles which over the years have been exploited in traditional medical practice for the treatment of various ailments (Adebanjo et al. 1983). This could be attributed to its affordability, accessibility and uneven distribution of health personnel between rural and urban areas.

*Terminalia avicennioides* Guill & Perr belongs to the Combretaceae family and it is commonly found in the savannah region in West Africa (Burkill 1985). It is a tree with yellowish brown, hard and durable wood. Its roots are used as chewing sticks in Ibadan area of Nigeria and have been claimed to cure dental caries and skin infections (Lewis and Elvin-Lewis 1977); the root-bark decoction is used in Ivory Coast for draught and enema for severe jaundice (Kerharo and Bouquet 1950); in Senegal for sores and ulcers (Kerharo and Adam 1963); in Nigeria for gastrointestinal disorders (Abdullahi et al. 2001); as well as for syphilis by the Jukuns (Irvine 1961). The seeds, fruits, roots, and rind are used in the treatment of bloody sputum and cough in humans by the Nupes in North Central Nigeria (Mann 2007).

Therefore, the aim of this research work is to evaluate the wound healing activity of ethanol extract of *Terminalia avicennioides* root bark in both excision and incision wounds in *Rattus norvegicus* and also to evaluate the scientific validity of the use of *Terminalia avicennioides* root bark by folklore traditions in Nigeria for treatment of cuts, wounds and burns.

**Materials and Methods**

**Collection of Plant Material**

2kg of fresh root bark of *Terminalia avicennioides* Guill & Perr (Nupe: Kpace) were obtained from their natural habitats in a farm land at Doko, Lavun Local Government, Niger State, Nigeria in April 2010 and identified by Mallam Ibrahim Muazzami of the Department of Medicinal Plant Research and Traditional Medicine of National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria where a voucher specimen (NIPRDH 5735) was deposited at the herbarium unit.

**Experimental**

Plant material (*Terminalia avicennioides*) was prepared using the method described by Ajiboso and Adejumo (2003) by washing plant material with portable water after which it was air-dried and ground into fine particle size which was passed through 75µm mesh size sieve range. 1000g of powdered sample was weighted into 70% ethanol (2.7L) and then percolated for 48 hours. The ethanol extract obtained after filtering through a millipore filter into conical flasks was concentrated and evaporated to dryness using water bath. The solid mass obtained was used to evaluate wound healing property of the plant extract.

**Animal**

12 eight weeks old healthy *Rattus norvegicus* rats of both sex with mean weight of 112.0±2.0 obtained from Ahmadu Bello University, Zaria, Nigeria were used to evaluate wound healing property of ethanol extract of *Terminalia avicennioides* root bark. The rats were kept in rat cages in Animal Farm of Department of Science Laboratory Technology, The Federal Polytechnic Bida, Niger State. The rats were grouped into three different groups of four rats in each group. They were maintained in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (DHHS, NIH Publication No. 85–23, 1985). The rats were acclimatized for two weeks under day/night condition, the rats were fed ad libitum and were exposed to rat chow and clean water throughout the experimental period.

**Wound Models**

**Excision wound model**

The dorsal skin of the mice was shaved. The mice were divided into three groups of four animals each. The animals were depilated on the...
paravertebral area prior to wound creation and predetermined area of 7mm×7mm skin in its full thickness was excised under ether anaesthesia (Suguna et al. 1996). Group I – III were treated with simple ointment base (Reference), penicillin ointment (Control) and ethanol extract of *Terminalia avicennioides* root bark (Tested) respectively, once daily for 22 days. Wounds were left undressed to the open environment and the animals were kept individually in separate cages.

**Incision wound model**

The animals were anaesthetised and paravertebral incision of 6cm long was made on either side of the vertebral column of the rat. Care was taken to make the incision at least 1cm lateral to vertebral column. The wounds were covered with interrupted sutures of 1cm apart. No ligature was used for stitching. After the incision was made, the parted skin was kept together and stitched with green silk at 1cm intervals, surgical thread (size 1.5meters) and a curved needle was used for stitching. The wound was left undressed.

**Measurement of wound area**

The progressive changes in wound area were measured in mm at every 3 days interval. Progressive decrease in the wound size was monitored periodically.

**Drugs administration**

Simple ointment base B.P (Control), 0.2% w/w penicillin ointment (Standard drug) and 5% w/w ethanol extract of *Terminalia avicennioides* root bark (Tested extract) were applied to group I, II and III respectively. These rats were treated according to group once daily till the wound was completely healed. When the wounds were completely healed in the case of incision, the sutures were removed on the 8th day after surgery. The animals were lagged separately according to groups.

**Statistical Analysis**

The data obtained as wound contraction area (mm²) was statistically analysed using paired t-test for test of significance at 5% confidence level.

**Results and Discussion**

Evaluation of the wound healing activity of ethanol extract of *Terminalia avicennioides* root bark on two wound models (excision and incision) in rats was carried out in this present investigation. At p<0.05, there was no significant difference in the performance in term of wound contraction and healing between standard drug (Penicillin ointment) and ethanol extract of *Terminalia avicennioides* root bark) but healing activities of penicillin and tested extract significantly differ from simple ointment healing activity as obtained from paired t-test for significance at 5% confidence level (Tables 1 and 2). As shown in Figure 1, mean values of wound area (mm²) of excision wound revealed that the performance of tested extract (ethanol extract of *Terminalia avicennioides* root bark) in term of healing (wound contraction) and healing days compared favourably with standard drug- penicillin ointment.

**Table 1**: Paired t-test of excision wound for significance at 5% confidence level.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Means</th>
<th>T-calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO-SO</td>
<td>1</td>
<td>PO= 123.2</td>
<td>12.71</td>
</tr>
<tr>
<td>PO-EE</td>
<td></td>
<td>SO= 291</td>
<td>0.047</td>
</tr>
<tr>
<td>EE-SO</td>
<td></td>
<td>EE= 132.2</td>
<td>13.21</td>
</tr>
</tbody>
</table>

Decision rule

If t_calculated < 0.05, accept H₀

If t_calculated > 0.05, reject H₀ and accept H₁

H₀ = There is no significant difference

H₁ = There is significant difference

**Table 2**: Paired t-test of incision wound for significance at 5% confidence level.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>Means</th>
<th>T-calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO-SO</td>
<td>1</td>
<td>PO= 122.3</td>
<td>10.15</td>
</tr>
<tr>
<td>PO-EE</td>
<td></td>
<td>SO= 164.1</td>
<td>0.025</td>
</tr>
<tr>
<td>EE-SO</td>
<td></td>
<td>EE= 117.2</td>
<td>9.73</td>
</tr>
</tbody>
</table>
Figure 1: Mean values of wound area (mm$^2$) of excision wound treated with simple ointment (SO), penicillin ointment (PO) and ethanol extract of *Terminalia avicennioides* root bark (Simple ointment= Green; Penicillin ointment= Dotted white; Extract= Grey).

Figure 2 shows mean values of wound area (mm$^2$) of incision wound treated with simple ointment (SO), penicillin ointment (PO) and ethanol extract of *Terminalia avicennioides* root bark. Ethanol extract of *Terminalia avicennioides* root bark healed the incision wound better than other ointments throughout the experimental period (22 days). The standard drug (penicillin ointment) also showed significant healing property but not as effective as ethanol extract of *Terminalia avicennioides* root bark on incision wound. However, the performance of simple ointment as wound healing agent was of no statistical significance when compared to healing activity of other ointment and tested extract.
The results showed accelerated wound healing and repair by ethanol extract of *Terminalia avicennioides* root bark, this was shown by the full thickness coverage of the wound area by an organized epidermis and reduced healing days when compared with standard drug- penicillin. The wound healing property of the plant could be explained on the basis of anti-inflammatory effects of the plant that are well established in the folklore traditions in Nigeria for treatment of cuts, wounds and burns. Study on animal models in both excision and incision wounds showed enhanced rate of wound contraction and drastic reduction in healing time in group treated with ethanol extract of *Terminalia avicennioides* root bark than control, which might be due to enhanced epithelisation and complete acute wound healing phases. Acute (normal) wound healing phases include haemostasis, early inflammatory phase, late inflammatory phase, proliferative phase (fibroblast migration, collagen synthesis, angiogenesis, granulation tissue formation and epithelialisation) and remodelling phase.

Complete marked dryness of wound margins with tissue regeneration was observed in the groups treated with ethanol extract of *Terminalia avicennioides* root bark and standard drug- penicillin ointment after 20 days of post wound. During cell injury, there is a mediation of disturbance in cellular and humoral responses (Ajiboso 2004), generation of free radicals such as reactive oxygen specie, hydroxyl and peroxyl ions is usually high during this disturbance. The body reacts to these prooxidants by releasing antioxidants such as ascorbic acid, tocopherol, reduced glutathione, superoxide dismutase, catalase and glutathione peroxidase in form of protective and compensatory mechanisms. However, if there is an imbalance in the levels of prooxidants and antioxidants, where the prooxidants are more than antioxidants, the cell will experience oxidative damage resulting from oxidative stress (Ajiboso 2009). Increased cellular proliferation of both excision and incision wounds may be attributed to adequate amount of antioxidants in both the animals and plant extract. This might have significantly contributed to healing process of early dermal and epidermal regeneration in treated rats.

A number of secondary metabolites/active compounds isolated from plants have been demonstrated in animal models (*in vivo*) as active principles responsible for facilitating healing of wounds. Some of the most important ones include tannins from *Terminalia arjuna*, (Chaudhari and Mengi 2006), oleanolic acid from *Anredra diffusa* (Letts et al 2006) polysaccharides from *Opuntia ficus-indica* (Trombetta et al 2006), Tannins and anthraquinones have been earlier documented by Mann et al. (2007) as the major phytoconstituent present in this plant which may be responsible for wound healing action. The ethanol extract of *Terminalia avicennioides* root bark possesses wound healing action by improving regeneration and organization of the new tissue due to the presence of tannins.

**Conclusion**

Penicillin ointment and the extract both showed wound healing activity in rats. This study demonstrates the wound healing activity of ethanol extract of root bark of the *Terminalia avicennioides* and found it to be effective in the functional recovery of the healing of both excision and incision wounds.

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**References**


