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Preliminary Study on the Wound Healing Activity of Ethanolic Extract of *Vitellaria Paradoxa* C. F. Gaertn. in Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *Vitellaria paradoxa*, an African indigenous plant is well-known for its several medicinal applications in Nigeria, especially in skin diseases.
Objectives: The present study was aimed at evaluating the scientific claim of the use of *Vitellaria paradoxa* stem bark by traditional medical practitioners in Nigeria for treatment of wounds.
Materials and Methods: Powdered stem bark sample of *V. paradoxa* was screened for various classes of secondary metabolites using standard procedure. The wound healing activity of ethanol extract of the stem bark was evaluated using incision wound model in rats. Fifteen rats were divided into three groups of five rats each. Group 1 served as negative control and received normal saline. Group 2 served as positive control and received Mupirocin ointment. Group 3, the treatment group received crude ethanol extract of *Vitellaria paradoxa* stem bark. The gradual changes in wound size were measured in cm every three days.
Results: The stem bark of *V. paradoxa* showed the presence of: tannins, saponins, alkaloids, flavonoids, steroids and cardiac glycosides in varying amounts. The wound size reduced from 5.0±0.0 cm on day 0 to 1.5±0.09 on day 3 and 1.2±0.09 on day 6. The reduction of 0.4±0.05 (92%) in wound area on day 9 in the ethanol stem bark extract was more than the reduction of 0.5±0.05 (90.8) observed in Mupirocin, the positive control group.
Conclusions: The study therefore provides a scientific justification for the traditional use of this plant in the management of wounds.

Keywords: *Vitellaria paradoxa*, Wound healing, Incision wound model, Ethanol extract.

INTRODUCTION

Wounds are physical injuries that result in an opening or break of the skin that cause disturbance in the normal skin anatomy and function (Strodbeck, 2001). Wounds are major causes of physical disabilities (Nagori and Solanki, 2011). A wound may be caused as a result of a fall, a surgical procedure, an infectious disease or an underlying pathological condition. There are many different types of wounds ranging from mild to severe to potentially fatal. Contusions, small incisions, and abrasions tend to be non-threatening, though some may pose the risk of infection. Deep punctures, avulsions, and amputations, however, may be life threatening. In most cases, the risks posed by all types of wounds differ in severity based on the instrument causing them, the ease of blood flow, and the cleanness of the edges of the damaged skin.
The normal skin, the epidermis (outermost layer) and dermis (inner or deeper layer) exist in steady-state equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, the normal (physiologic) process of wound healing is immediately set in motion. The wound healing process involves continuous cell – cell interaction and cell matrix interactions in phases which include: Inflammation, wound contraction, re epithelialization tissue, re-modeling, and formation of granulation tissue with angiogenesis. However, this process may be delayed by several factors including bacterial infection, necrotic tissue, and interference with blood supply, lymphatic blockage and diabetes mellitus (Chitra *et al*., 1998).
The wound healing activities of plants have been explored in different parts of the world. Extensive research has been carried out in wound healing management through medicinal plants (Kumari et al., 2010; Shivhare et al., 2010; Agra et al., 2013; Gupta et al., 2014). The plant selected for the present study; *Vitellaria paradoxa* (formerly known as *Butyrospermum parkii*), belonging to

the family Sapotaceae, is a well-known medicinal plant in traditional medicine practice. It is popularly known as Shea butter tree. It is the only species in the genus *Vitellaria* (Byakagba et al., 2011) and is indigenous to Africa. *Vitellaria paradoxa* is a medium-sized tree, up to 25 m high; much branched, dense, spreading, round to hemispherical crown. The bole is 3-4 m in mature trees. Medicinal application of Shea butter includes; its ability to protect against sunburn, hence it is a useful ingredient in sun-protection or post-sun-exposure products. It also encourages wound healing and soothes skin irritation. Different parts of the plant including leaves, roots, seeds, fruit and stem bark have been used in the treatment of enteric infections such as diarrhea, dysentery, helminthes and other gastrointestinal tract infections, skin diseases and wound infections (Soladoye *et al*., 1989). The fruit pulp can be eaten fresh when ripe (Lovett *et al.,* 2000). Shea butter is stable and permits the fast release of medicaments; it can therefore be used as a base for suppositories and ointments (Bayalal et al., 2009).

In some parts of Africa it is used to prepare meals as cooking oil and the leaves of the tree are also consumed as a vegetable. In the neighborhoods of Northern Benin it is used as the main edible oil. The estimated usage on a daily basis by individual in the area is estimated at 26.3 grams per person (Honfo *et al.*, 2012). Shea butter is also used as cocoa butter additive in manufacturing chocolate (Honfo, 2012). Due to its importance in rural farmers' economies, the Shea tree is a species with great value for domestication (Franzel *et al.,* 1996; Bounkoungou *et al.* 1998). The present was designed to evaluate the wound healing activity of ethanol extract of *Vitellaria paradoxa* stem bark in incision model.

MATERIALS AND METHODS

Collection and preparation of Plant material

Vitellaria paradoxa stem bark was collected at Papa area, Iseyin, Oyo state, Nigeria in October, 2013 and identified by Mr Afilaka of Forest Herbarium, Ibadan (FHI), Nigeria where a voucher specimen (FHI 109816) was deposited. Samples were air-dried at room temperature under shade for three weeks. The dried sample was ground into powder using a milling machine. One kilogram of the powdered sample was macerated with distilled ethanol for five days with occasional stirring. The ethanol extract obtained was filtered using Whatman filter paper No. 1 and concentrated *in-vacuo*. The extract was reserved for wound healing assay.

Experimental animal

Fifteen Wistar rats (150-200 g) used for the evaluation of wound healing activity of the plant extract were purchased from the Animal house, Department of Physiology, University of Ibadan. The animals were acclimatized for two weeks and fed with standard pellets and water *ad libitum* prior to the commencement of the experiment. The animals were divided into three groups of five animals each.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on the powdered sample of *V. paradoxa* stem bark according to standard methods (Sofowora, 1993; Trease and Evans, 1989).

Ethical approval

An ethical approval was obtained from the ethical committee of the Faculty of Veterinary medicine, University of Ibadan, Nigeria before commencement of the experiment.

Incision Wound Model

The rats in each group were anaesthetized with intravenous Ketamine Hydrochloride (10 mg/kg body wt.). Diazepam injection (1 mL) was given to the animals as preclinical prior to Ketamine Hydrochloride administration. The animals were shaved prior to incision to avoid contamination of the wound site. Chlorhexidine was used as disinfectant. One paravertebral 5 cm long incision was made with a sterile blade through the shaved skin and cutaneous muscle at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. After the incision was made, the parted skin was kept together and sutured with Nylon suture material (2.0) at 0.5 cm intervals using surgical thread (No. 000) and a curved needle (No. 11) for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. The acute toxicity studies of *Vitellaria paradoxa* stem bark had been reported by Ayankunle *et al.* (2012). The LD_{50} of the ethanol extract was reported to be 115 mg/kg therefore a 100 mg/kg dose was used as treatment dosage in this experiment.

The treatment started on the day of wound creation in the same manner:

Group I: Negative control (Normal saline): 1 mL

Group II: Standard (Mupirocin Ointment): 8 mg/mL

Group III: *Vitellaria paradoxa* ethanol leaf extract: 100 mg/mL

The drugs were applied once daily after cleaning with surgical cotton wool.

Measurement of wound area

The gradual changes in wound area were measured in cm at three days interval. Gradual decrease in the wound size was monitored periodically. Wound size measurements of each animal were used to calculate % wound contraction as:

$$\% \text{ wound contraction} = [(A_0 - A_t)/A_0] \times 100$$

Where A_0 is the original wound area, and A_t is the area of wound at the time of biopsy.

Statistical Analysis

Reduction of wound area (cm) was statistically analyzed using paired t- test for test of significance at 5% confidence level. The mean values of wound area (cm) of incision wound were also determined using Microsoft excel 2013 version.

RESULTS AND DISCUSSION

Preliminary phytochemical screening result of *Vitellaria paradoxa* stem bark showed the presence of tannins, saponins, alkaloids, flavonoids, steroids, cardiac glycosides while anthraquinones were absent (Table 1). The result of the study showed significant wound healing activity of the crude ethanol extract of *Vitellaria paradoxa* in incision wound model (Table 2). The size of the wound reduced from 5.0±0.5 on day 0 to 0.4±0.05 on day 9 of incision in rats treated with *Vitellaria paradoxa* crude extract, the positive control (Mupirocin ointment) reduced to 0.5±0.3 while the negative control (normal saline) reduced to 2.3±1.1. All the groups had 5 cm as their original size on day 0. The percentage reduction of wound size of *Vitellaria paradoxa* crude were 69.2%, 76.8%, and 92.0% on days 3, 6 and 9 while that of the positive control (Mupirocin ointment) were 62.8%, 74.0%, and 90.8% on days 3, 6 and 9, respectively. The negative control in percentage reduction in wound size gave 32.8%, 48.0% and 63.6% on days 3, 6, and 9 of incision model, respectively. This clearly showed that *Vitellaria paradoxa* crude gave the best result, followed by the positive control.

Table 1: Preliminary Phytochemical Screening results of *Vitellaria paradoxa* stem bark

Test	Inference
TANNINS	
Ferric Chloride Test	+++
SAPONINS	
Frothing Test	+++
Emulsification	+++
ALKALOIDS	
Dragendorff's reagent	+++
Mayer's reagents	+
Wagner's reagent	++
BORNTRAGER'S TEST	
Free anthraquinones	-
Combined anthraquinones	-
FLAVONOIDS	
Ferric Chloride solution	+
Reaction with NaOH	+

+++ = Highly positive
+ = moderately positive
++ = Positive
- = negative

Table 2: Incision Wound Model: Reduction of Wound area (cm) in rats treated with crude ethanol extract of *Erythrophleum suaveolens* leaves

	Wound area (cm)/day			
Treatment group	0	3	6	9
Control	5.0±0.0 (0.0)[a]	3.4±0.6 (32.8)[a]	2.6±0.6 (48.0)[a]	2.3±1.1 (63.6)[a]
Mupirocin ointment	5.0±0.0 (0.0)[a]	1.9±0.5 (62.8)[b]	1.3±0.5 (74.0)[b]	0.5±0.0 (90.8)[b]
Vitellaria paradoxa stem bark	5.0±0.0 (0.0)[a]	1.5±0.1 (69.2)[bc]	1.2±0.1 (76.8)[b]	0.4±0.0 (92.0)[b]

Wound area on day 0 = 5 cm. Values are mean ± S.D. at $p < 0.05$ as compared to control.
In parenthesis is the percentage of wound reduction as compared to control. Means with the same superscript are not significantly different from each other. Doses of *Vitellaria paradoxa* (100 mg/mL), Reference drug (8 mg/mL).

The present study showed the role of *V. paradoxa* in wound healing. The stem bark extract of the plant demonstrated better wound healing activity in rats in the incision wound model than Mupirocin ointment included in the study as the positive control drug. The closure of wound in incision wound model indicated enhanced collagen maturation. The demonstration of high biological activity seen in *V. paradoxa* in this study is in agreement with the study of Ahmed *et al*. (2009) who worked on the antifungal profiles of extracts of *V. paradoxa* bark. Their work proved that the Shea butter plant is very effective against dermatophytes. The result of the present study is also in line with the reports of several studies, where plant extracts had shown promising wound healing activities. Sumanta *et al*. (2012) who worked on the wound healing activity of *Cleome rutidosperma* roots revealed that the animals treated with methanol and aqueous extracts of *Cleome rutidosperma* showed faster rate of wound healing

compared to the chloroform extract. Narendra *et al.* (2009) reported that the latex of *Calotropis gigantea* significantly promoted collagen as compared to that of control.

The wound healing properties of most of these plants have been linked to the presence of various complex chemical substances of different composition which are found as secondary metabolites in one or more parts of plants (Kumar *et al*., 1993). These bioactive compounds in plants include alkaloids, flavonoids, tannins and phenolic compounds. Of these plant constituents, tannins and flavonoids play a major role in the wound healing process by preventing and protecting oxidative damage from free radicals (Nayak *et al*., 2009, Okuda *et al*., 2005). In the present study, the preliminary phytochemical screening result of *Vitellaria paradoxa* stem bark showed the presence of tannins, saponins, alkaloids, flavonoids, steroids and cardiac glycosides in line with the findings of El-Mahmood *et al*. (2008).

Wound healing occurs in three principal phases namely: inflammatory, proliferative, and remodeling. Inflammation sets in at the time of injury and lasts for 24 to 48 hours. The proliferative stage is predominated by fibroblasts proliferation leading to the production of collagen. Collagen provides structure to the wound and replaces the fibronectin–fibrin matrix by cross-linking to form fibres. Collagen synthesis and degradation reach equilibrium in the remodelling phase. The high collagen turnover in the wound healing process resulting from the activities of phytoconstituents like flavonoids are known to reduce lipid peroxidation by preventing or slowing the onset of cell necrosis and by improving vascularity (Getie *et al.*, 2002).

In our study, the acceleration of wound healing demonstrated by *Vitellaria paradoxa* stem bark extract provides support for its traditional use. Further studies are required to explore the detailed mechanism of the wound healing effect of the plant and to isolate bioactive compounds responsible for the activity of the plant to become a candidate for future drug discovery and development.

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