

Wound healing activity of *Crinum zeylanicum* L. (Amaryllidaceae)

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Abstract

Crinum zeylanicum is a medicinal plant used in Western part of Nigeria for management of skin trouble, injuries and on refractory ulcers. The aim of the current study was to evaluate its wound healing effect in Wistar rats using excision wound model. Acute dermal toxicity of the methanolic extract of *Crinum zeylanicum* was evaluated in rats using acute toxic class method described in OECD 404 guideline. *Crinum zeylanicum* methanolic bulb extract formulated into 1%, 5% and 10% ointment was tested for pro-wound healing activity. Excision wound measuring about 200 mm² was created on 25 male Wistar rats randomly divided into 5 groups of 6 rats each. The ointment was applied topically on the wounded area for 7 consecutive days, measured at 3 days intervals for 21 days. The acute dermal toxic dose (LD₅₀) was greater than 2000 mg/kg body weight. Topical application of *Crinum zeylanicum* based- ointment significantly (P<0.01) increased rate of wound retraction and reduced the epithelialization period from 21.75 days in control to 20.40, 17.60 and 14.4 days at 1%, 5% and 10% concentration respectively. These results strongly suggest that *Crinum zeylanicum*-based ointment accelerated wound healing process and may therefore be developed as a safe and effective herbal-based cream for management of wounds, ulceration and bruises

Keywords: Wounds; *Crinum zeylanicum*; Formulation; Topical

Introduction

The use of herbal medicines for management of various ailments is increasingly being accepted by general populations in both eastern and western countries as medicines and dietary supplements, along with modern chemotherapeutic agents (Agrahari et al, 2010). This is because of general acceptability that phyto-based health products have fewer side effects.

In developing countries including Nigeria different crude drugs preparation are used to treat various skin diseases including wound. Some medicinal plants reported to possess wound healing effect and are used in folk medicine for wound care include aqueous extract of pulp and seeds of *Moringa oleifera* (Rathi et al., 2004), *Tridax procumbens* extracts (Udupa et al., 1995), ethanol extract of *Wrightia tinctoria* bark (Veerapur et al., 2004), seeds of *Trigonella foenumgraceum* (Taranalli and Kuppast, 1996), *Dissotis theifolia* (Odumegwu et al, 2008) *Saussurea lappa* Clarke root extracts (Patil et al., 2009) and methanolic leaf extract of *Jatropha curcas* (Esimone et al., 2009).

Crinum zeylanicum (Linn.), (Family; Amaryllidaceae) is a bulbous plant, widely distributed in tropical Africa. The Hausas of Northern Nigeria calls it “Albasaar Kwaadii” (frog’s onion), while Yorubas of the Southern part of Nigeria calls it “Isumeri”. It is used in folk medicine for various ailments in different ethnic settings. In Sierra Leone, the leaves cold infusion are used as a stimulant for bathing young children suffering from general debility, rickets etc. The bulb is used in Ghana as a vermifuge. In Western part of Nigeria, the bulb is used externally for skin trouble, injuries and on refractory ulcers. In the present study the wound healing activity of the methanolic bulb extract of *Crinum zeylanicum* was investigated in Wistar rats.

Materials and methods

Plant material

The whole plant (bulb, leaves and flower) of *Crinum zeylanicum* was collected by Mr. Goodluck Jaiyeoba, a traditional herbal medicine practitioner from Rafin sayan, a village in Suleja, Niger state of Nigeria. The plant was identified and authenticated by Mrs. Jemilat Ibrahim, a taxonomist with the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu- Abuja where a voucher sample (NIPRD/H/6258) was prepared and deposited for future references.

Animals

Male Wistar rats weighing 180- 200 g obtained from the Animal Facility Centre of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria, was used for the study. They were housed in stainless steel cages at a temperature of $25\pm 2^{\circ}\text{C}$ and observed under a 12-hour light/dark cycle in a well ventilated room. They were fed with growers mash and water *ad libitum*. The rats were used in accordance with NIH Guide for the Care and Use of Laboratory Animals; NIH Publication (No 83-23) revised (1985) and NIPRD’s Standard Operation Procedures (NIPRD-SOPs).

Extraction of plant material

The bulb of *Crinum zeylanicum* was crushed and air-dried at room temperature. About 100 g of the dry plant material was macerated in 70% methanol for 48 hours. The resulting mixture was filtered using muslin cloth followed by Whatman filter paper (No. 1). The

aliquots obtained was dried on water bath and stored at -4°C until required for use. The yield of the extract was 23%.

Phytochemical analysis

The methanolic *Crinum zeylanicum* bulb extract was screened phytochemically for the presence of secondary metabolites using standard conventional protocols (Sofowora, 1993). *Formulation of liposomal ointment of methanol extract of Crinum zeylanicum* The solvent evaporation method was adopted (Bendas & Tadros 2007). A 2.5 g quantity of phospholipon 90H (phospholipid GmbH koln) was dissolved in a mixture of 7.5 ml in chloroform (BDH) and 2.5 ml methanol (BDH) in a 100 ml round-bottomed flask. Using a rotary evaporator, the solvents were evaporated at 55°C and the thin film formed allowed to stay for 12 hrs for complete evaporation. The film was then hydrated with 1% solution of the *Crinum zeylanicum* (CZ) extract in phosphate buffer solution (PBS) of PH 6.5 and mixed well. The dispersion was made up to 100ml with further adding of PBS PH 6.5 and mixed well using vortex mixer. The liposome was then made into ointment to increase resident time on topical application, by mixing with a base composed of 5 ml sorbitan monolaurate (Aldrich) and 1g soft white paraffin (BDH). The same procedure was followed to formulate two more batches of the ointment containing 5 and 10% of CZ extract which were then used for the pharmacological studies. Similar procedure was followed in formulating 1% gentamycin ointment.

Acute dermal toxicity test (limit test)

This test was performed on rats based on OECD guideline number 404 (OECD, 1981a) with slight modification. Hair was clipped from the back of each rat (weighing 180–200 g), approximately 10% of the total surface area, and then each rat was caged individually and left undisturbed for 24 h. Thereafter, the extract (300, 300 and 2000 mg/kg) moistened with water for test groups and water alone for controls evenly applied on the shaved area. The rats were singly housed in a cage made of stainless steel. Following 24 h after application; the covering was removed carefully and cage side observation was made daily for 7 days. Observation included evaluation of skin and fur, eyes, respiratory effects (salivation, diarrhoea and urination), and central nervous system effects (tremors and convulsion, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength and stereotyped or bizarre behaviour) and mortality resulting from systemic toxicity was recorded.

Wound model

The method described by Saha et al., (1997) was adopted for the study. Back of six rats in each group was depilated and the areas were cleaned with 70% alcohol under Ketamine (25 mg/kg intraperitoneally) anesthesia. Excision wound was inflicted by cutting away a 200 mm² full thickness of skin from a predetermined area; the wound was left open. Then the test ointments and the blank ointment base for the purpose of control were topically applied for 7 days and thereafter the progressive changes in wound area were measured by manual vernier caliper every alternate day. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as percent reduction in wound area.

Statistical analysis

The statistical analyses were carried out using Graph Pad Prism version 5.02 and performed by one-way ANOVA, followed by Tukey's multiple comparison tests. $P < 0.05$ was considered significant. The results of statistical analysis were expressed as the mean \pm SEM.

Results

Phytochemical screening

Phytochemical analysis of the crude methanolic bulb extract of *Crinum zeylanicum* revealed presence of balsam, sterols, terpenes, resins, carbohydrate, tannins, phylobatannins, saponins, flavonoids, alkaloids and volatile oils.

Acute dermal toxicity limit test

At 24 h after dermal application of *Crinum zeylanicum* formulated ointment, treated animals did not show signs of adverse reaction. The animals appeared normal and were active. There was no death recorded in any of the treated groups. The acute dermal toxic dose was estimated to be greater than 2000 mg extract/kg body weight.

Discussion

The yield of 23% of the crude extract from the starting plant material could be taken as one form of standardization parameter because it is indicative of the amount of active ingredient expected in possible productions. The data obtained from this study showed that *Crinum zeylanicum* methanolic bulb extract is safe acutely when applied topically and possesses wound healing effect in excision wound model in Wistar rats. The extract-treated rats did not show any toxic effects that could probably be attributed to the irritant effect of the extract. The absence of acute toxic effect of *Crinum zeylanicum* at the maximum dose of 2000 mg/kg tested showed that its application was not associated with measurable systemic toxicity (Kimber et al, 2003; OECD, 1981).

Daily topical application of *Crinum zeylanicum* based cream for 7 days produced significant and concentration-dependent reduction of wound area (Figure 1) and epithelialization period. Application of the highest concentrations of *Crinum zeylanicum* extract (10% ointment) used in this study shortened the time for complete wound healing and epithelialization period to 14.4 days compared to the control treated with sham ointment with epithelialization period of 21 days.

The efficacy of medicinal plants that are used for wound healing purpose may be due to their direct action on the wound repair processes, anti-inflammatory and antimicrobial effects or a combination of these effects (Shetty et al, 2006). Previous studies carried out on wound healing effects of medicinal plants by Mackay and Miller, (2003), Odimegwu et al., (2008) and Esimone et al., (2009) have shown that these preparations were effective in wound care, facilitated rapid wound healing with minimal pain, discomfort, and scarring to the p-

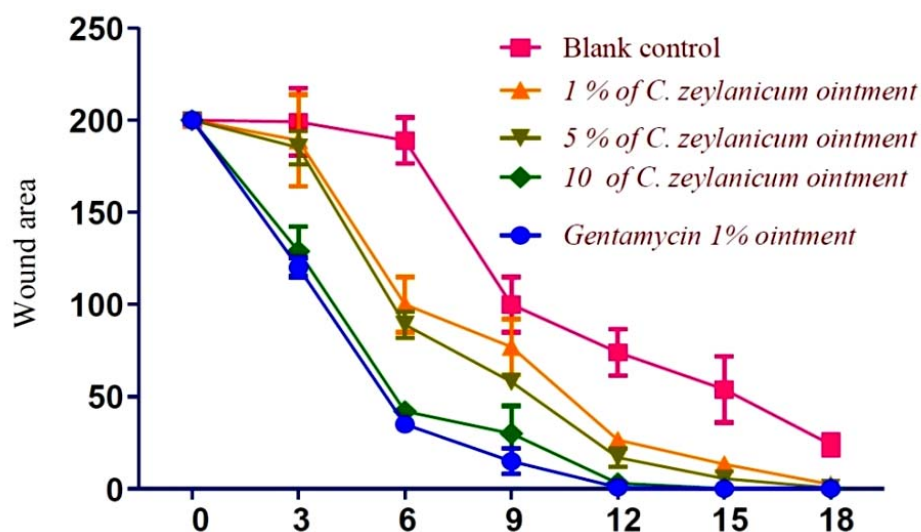


Figure 1. Effect of methanolic extract of *Crinum zeylanicum* ointment (1%, 5% and 10 %) and Gentamycin 1% ointment Control received ointment base. The Y- axis showed the wound area while the X-axis stands for time in days. (F4, 175 = 59.333, P <0.0001, n = 6).

atient. Wound healing is a natural response of injured skin, consists of complex interactive phases of inflammation, proliferation, and remodeling. The first response of the healing period is inflammation as a defence mechanism of the tissue, which provides a resistance to the microbial contaminations (Kondo, 2007; Saha et al., 1997; Mukherjee and Suresh, 2000).

In the inflammatory phase, bacteria and debris are phagocytosed and removed and cytokines and mediators are released that cause the migration and division of cells involved in the proliferative phase. Angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction occur in the proliferative phase (Swamy et al., 2007). During epithelialization, the epithelial cells crawl across the wound bed to cover it (Rao et al, 1991), thus forming a new epidermal cover. The wound is eventually closed by a combination of all these and by the process of wound contracture. During wound contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis.

The stage of wound repair process that is affected by *Crinum zeylanicum* is yet to be determined, it may be suggested that it exert significant effect on one or some of the stages resulting in faster rate of wound closure when compared to the untreated group. The Phytochemical screening of the methanolic extract of *Crinum zeylanicum* used for the formulation of the cream revealed the presence of balsam, sterols, terpenes, resins, carbohydrate, tannins, phylobatannins, saponins, flavonoids, alkaloids and volatile oils. Biological activities of medicinal plants have been attributed to the abundant distribution of phytochemicals in their various parts. Verapuur et al., (2004) and Esimone et al, (2009) have demonstrated that alkaloids, sterols, carbohydrates and tannins in ethanol extract of *Wrightia tinctoria* bark and leaf extract of *Jatropha curcas* respectively may be responsible for their wound healing effects. Tannins have been reported by Deter et al., (2001) and Fernandez et al., (2002) to promote

wound healing via scavenging of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels. Similar findings have been reported with the extracts of the plants containing tannins by Rane and Mengi, (2003). The results of this study also revealed that tannins are one of the important phytoconstituents responsible for wound healing due to their astringent and antimicrobial property. Thus, wound healing potency of *Crinum zeylanicum* may be attributed to the phytoconstituents present in it, which may be due to their individual or additive effect that fastens the process of wound healing (i.e. fasten the wound contraction and process of epithelization).

The results of the present study experimentally showed the effectiveness of *Crinum zeylanicum*-based ointment in wound healing as popularly used in traditional folk medicine among the Yoruba people in Western part of Nigeria. Further studies are warranted in order to explore the detailed mechanism of the wound healing effect of the plant.

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Conflict of Interest

The authors have declared that there is no conflict of interest

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