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WOUND HEALING POTENTIALS OF WIRE WEED (SIDA ACUTA BURMAN, 1768) IN GUINEA PIG

(CAVIA PORCELLUS LINNAEUS, 1758)

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Abstract

Injuries on humans and animals are common in the tropics and the conventional curative drugs are expensive. The objective of the study was to find out the wound healing capability of the methanolic extract of leaves of *Sida acuta*. *S. acuta*, a shrub belonging to Malvaceae family is widely distributed in the tropical regions where it is use as antimicrobial, antibacterial, antihelmintic and antiplasmodial agent. One excision wound was inflicted on each guinea pig (*Cavia porcellus*) by cutting away a 100 mm² full thickness of skin. The wound was daily treated with the reference standard drug (Penicillin skin ointment, positive control) and varied concentrations of *S. acuta* extract ointment (treatment groups). Animals in the negative control received no treatment. Animals were closely monitored till the wounds were completely healed. Effects of topical administration of methanolic extract of *S. acuta* were studied daily measuring the wound area in two types of wound models on guinea pigs: namely the excision and the incision. The methanolic extract of *S. acuta* produced significant healing in both types of wound treated. Compared to the negative control, the extract-treated wounds were found to epithelialize faster and the rate of wound contraction was higher. The extract facilitated the healing process as evidenced by increase in the tensile strength in the incision model and the histological study of the scar tissue. The results were also comparable to those of standard drug. Conclusively, the methanol extract of *S. acuta* is an efficient herbal-drug for treatment of wounds.

Keywords: Wound healing, Sida acuta, Penicillin, Guinea pigs, Tensile strength, Reepithelization.

Introduction

Wound is most commonly used when referring to injury to the skin or underlying tissues or organs by a blow, cut, missile, or stab. Wound also includes injury to the skin caused by chemical, cold, friction, heat, pressure and rays, and manifestation in the skin of internal conditions, for example, pressure sores and ulcers [1]. Wounds have a tremendous impact on the healing healthcare economy. Chronic wounds represent a major health burden and drain on the healthcare resources in the world including Nigeria [2].

A major problem with wounds is the high risk of infection; hence, if an agent active against these microorganisms causing the infection is used in the healing process, it will then help to reduce the risk of infection and the overall time for wound healing can be reduced significantly. For example, it is very easy for bacteria to enter through the broken skin and penetrate the rest of the body. Bacteria colonize wounds within 48 hours after injury and bacteria such as Staphylococcus aureus. Pseudomonas aeruginosa and Streptococcus species may cause infection and this may prolong inflammatory phase of wound healing. Therefore suitable antimicrobial agents can be used either topically or systematically to prevent infection of wounds and speed up wound healing process [3].

The process of inflammation normally leads to the release of biologically active mediators to attract neutrophils, leucocytes and monocytes, to the wound area and these attack foreign debris and microorganisms through phagocytosis. This then leads to the production of oxygen-free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl anion and excess of these agents causes tissue damage in man or animal if they overwhelm the natural antioxidants of the host such as catalase, superoxide dismutase, and glutathione peroxidase. Therefore, antioxidants prevent the activity of the free radicals and thereby prevent the damage to cells and tissues, providing protection to human and animal subjects, and also enhance healing of infected and non-infected wounds [4].

In Nigeria several studies have been carried out on efficacy of plant extract as wound healing agents. These studies includes the use of *Crinum zeylanicum* in Western Nigeria to manage skin trouble, injuries and on refractory ulcers [5], the use of herbal ointment containing methanol leaf extract of *Jatropha* curcas [6] and comparative evaluation of wound healing effect of Ocimum gratissimum, Vernonia amygdaline and Zingiber officinalis extracts in Eastern Nigeria [7], among many others.

The plant *S. acuta* is a malvaceous weed, home to the tropics that traditionally have been used to cure various ailments such as malaria, ulcer, fever, gonorrhea, abortion, breast cancer, poisoning, inflammation, feed for livestock, stops bleeding, treatment of sores, wounds, antipyretic etc. [8-11]. The pharmacological properties of *S. acuta* include antimicrobial [10], antioxidant [11], antiplasmodial [12], cytotoxic and thrombolytic activities [13] and many other properties. The present research is focused on the wound healing activities of *S. acuta* using the excision and incision wound models on guinea pigs.

Materials and Methods

Collection of plant materials

The leaves of *S. acuta* were obtained from the environment of University of Nigeria, Nsukka, Enugu State, Nigeria using a sickle. The plant was identified [14] and authenticated by Prof. M. Nwosu of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Preparation of plant extract

The leaves were washed, air dried under shade in the Physiology Laboratory of the Department Zoology and Environmental Biology, pulverized to powder using blender. Extraction was carried out by dispersing 250 g of the ground *S. acuta* plant material in 1.25L of methanol and soaking was done for 48 hours. This was followed by a vacuum filtration and also concentrated by open air evaporation for 3 days under room temperature. The *S. acuta* methanolic extract was then stored in vials under room temperature.

Phytochemical screening of wire weed

The phytochemical analysis of bioactive constituents of the methanolic extract of *S. acuta* was carried out in the Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka using standard phytochemical procedures [15].

Experimental guinea pig (Cavia porcellus)

Guinea pig weighing 450 to 600 grams were obtained from Genetics and Animal Breeding

Laboratory in the Zoological Garden, University of Nigeria, Nsukka, Enugu State, Nigeria. Three guinea pigs were used for each group. They were acclimatized for two weeks with each animal housed separately in clean and dry metal cages under 12 hours conditions of light, temperature (24 \pm 3°C) and relative humidity (55 \pm 5%), with weed straw as beddings. The guinea pigs were fed with fresh grasses and water ad libitum. The university ethical committee approved the study protocol prior to commencement of the study. The study was carried out in accordance with the guidelines for ethical conduct in the care and use of nonhuman animals in research [16]. The guinea pigs were grouped into different categories as shown in Table 1.

Acute dermal toxicity test

The acute dermal toxicity testing of the methanolic extract was carried out by applying the different does of the extract on the shaved and wounded part of the guinea pigs. The OECD Guidelines No. 434 [17] and a slightly modified Lorkes method [18] were used for the study. Fifteen guinea pigs (*Cavia pocellus*) weighing 0.45 to 0.6 kg were randomized into five groups of three guinea pigs each and were given 1000, 2000, 3000, 4000, 5000 mg/Kg body weight of the extract topically. They were observed for signs of redness, irritation, toxicity and mortality for 48 hours. The dermal median dose LD_{50} was then calculated.

Evaluation of wound healing properties

Excision wound model

Fifteen guinea pigs belonging to five groups (three animals per group) were anaesthetized with ketamine. Each animal represented a replicate. The guinea pigs were shaved and sterilized with methylated spirit before 10 x 10 mm excision wound was created on the back. One excision wound was inflicted to each animal on a predetermined shaved area on the back of each animal by cutting away a 100 mm² full thickness of skin from the shaved area using a surgical blade, the wound was left undressed [19]. The reference standard drug (Penicillin skin ointment - positive control) and S. acuta extract ointments (0.0, 3000, 4000, 5000 mg/kg body weight) in 100 g white paraffin wax was applied once a day. The o.o mg/kg of S. acuta had only the paraffin wax applied to the wound (negative control). The wounds were

closely monitored till the wounds became completely healed. This model was used to monitor wound contraction and wound (epithelization) closure time. Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored plan metrically by tracing the wound margin on graph paper every alternate day [20].

Incision wound model

Another set of fifteen guinea pigs belonging to five groups (three animals per group) were anaesthetized with ketamine. Each animal represented a replicate and one paravertebral 2 cm long incision was made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the shaved back of each guinea pig. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. After the incision was made, the reference standard drug (penicillin skin ointment) and S. acuta extract ointments (0.0, 2500, 3500, 4500 mg/kg body weight) in 100 g white paraffin wax was inserted into the incised wound and the parted skin were kept together and stitched with black silk at 0.5 cm interval using the simple interrupted method. The o.o mg/kg of S. acuta had only the paraffin wax inserted into the incised wound (negative control). Surgical threads and a curved needle were used for stitching. The continuous threads on both wound edges were tightened in the same manner as has already described above. The extract ointments and the penicillin skin ointment were no longer administered daily till when wound was cured thoroughly. The sutures were removed on the 9th day and the tensile strength was measured with a tensiometer [21].

Histological evaluation of healed wounds

The skin specimen from wounds healed areas were recovered, fixed in 10% buffered formalin, histologically processed and embedded in paraffin. Histological sections (5µm thickness) were prepared using a rotary microtome (4060 cut SLEE). Sections were stained by routine Haematoxilin-Eosin (H&E) method. Stained sections were mounted on slides using DPX and viewed under light microscope [22]. Sections were photomicrographed using motic camera.

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA). Statistical difference among treatment means were separated using Duncan new multiple range test [23]. Differences between treatments were considered significant at p<0.05. Results were expressed as the mean \pm SEM of at least triplicate determinations (n = 3). All data were analyzed using GraphPad Prism 5 software (Graph Pad Software, Inc., San Diego, California).

Results

Phytochemical composition of S. acuta

Alkaloids, steroids, terpenoids and flavonoids were detected in moderate levels in the methanolic extract of *S. acuta*, while saponins, glycosides, tannins were absent (Table 2).

Toxicity of S. acuta extract on guinea pigs

Median lethal concentration (LC_{50}) is the most widely accepted basis for acute toxicity test. For the present study, it is the concentration of S. acuta methanolic extract which kills 50% of the guinea pig after a particular length of exposure (6 - 96 hour). Generally in toxicity tests, mortality is a decisive criterion because it is easy to determine and has apparent biological and ecological significance. The acute toxicity of S. acuta extracts on guinea pigs (C. porcellus) at 6h and 12 h respectively indicated that the Probit kill was inversely correlated with the log of concentration (Figures 1 and 2). Low mortality was recorded at high doses of the extract at 6 hour interval and a similar trend was observed at the 12 hour. No mortality was recorded between 18 - 96 hours of the experiment. The probit regression model results were negative in both 6 hour (y = -30.50x + 22.49) and 12 hour (y = -28.34x + 22.49) with low percentages (28.7 and 31.8%) of R^2 respectively. The median lethal (LD₅₀) dose was determined to be 0.29 and 0.386 mg/kg of S. acuta methanolic extract in 6 and 12 hour. The acute toxicity test of S. acuta methanolic extract indicated that the extract was safe, with a LD₅₀ of above 5000 mg/kg body weight.

Decrease in wound area (excision model)

Equal wound area (100 \pm 0.00) was recorded on day 0 before the administration of *S. acuta* extracts (Table 3). In the first four days of treating the guinea pigs wound areas of the groups treated with different dosages of *S. acuta* extracts were

statistically similar (p>0.05) and higher than the standard and the negative control groups but with no observed significant differences (p>0.05). On 5th and 6th day post treatment, a significantly higher (p<0.05) difference in wound area of the extract treated groups than their respective control groups was observed. From days 7 to 12 of the experiment, no significant (p>0.05) differences in wound area was recorded between the extract treated groups and the control groups. However, the groups treated with high dosages (4000 and 5000 mg/kg) of S. acuta extracts recorded a very high decrease in wound area than other extract concentration and the control groups (Table 3). In both the extract treated groups, the negative and the standard control groups, a significant (p<0.05) dose-dependent decrease in wound area were recorded from days 1 to 12 of the experiment, the wound area of the group treated with 4000 mg/kg of the extract healed completely (Figure 3).

Tensile strength after 9 days for incision wound model

Equal wound length (2 cm) was recorded on day o before the administration of *S. acuta* extracts (Table 4). After the 9th day of administration of *S. acuta* extract, group 2, 3500 mg/kg methanolic extract of *S. acuta* appeared to have greater tensile strength (126.47 ± 68.29) compared to the standard drug (0.00 ± 0.00) and the negative control (35.30 ± 17.66).

Histological evaluation of healed wounds

Histological evaluation was carried out for the extract treated healed wounds. There were few inflammatory cells, and more collagen fiber, fibroblasts and proliferating blood capillaries as a result of treatment with methanolic extract of S. acuta. There was full thickness reepithelialization, in which epidermis was thin and well organized, comparable to the adjacent skin which was not involved in the wound generation and healing process. The granular layer was well formed and one cell in thickness. There was a full thickness epidermal regeneration which covered completely the wound area. Early dermal and epidermal regeneration in treated guinea pig also confirmed that the extract had a positive effect towards cell proliferation, granular tissue formation and epithelialization (Figure 5a, b and c).

Discussion

The process of wound healing is aimed at restoring the damaged cellular structures and tissues close to their original state [24]. This helps in the restoration of the disrupted anatomical continuity and functional structure of the skin. The healing process of wound involves four stages viz: coagulation of the blood vessels, inflammation and debridement of the wound, re-epithelialization and collagen deposition and remodeling [25]. Wounds require treatment to either shorten the time for healing or to minimize the undesirable activities of pathogenic of microbes [26]. Treatment requires that attention be focused on agents that can suppress wound progression like corticosteroids, anti-neoplastic or non-steroidal anti-inflammatory agents [27].

The methanolic S. acuta extract exhibited significant wound healing activity compared to the negative and positive control in both excision and incision wound healing model. It was observed that the wound contracting ability of the extract treated groups was significant (p<0.05) from day 3 onwards compared to wound healing using Dissotis theifolia that showed complete healing by 8-11 days [28]. The wound closure rate of the extract treated group decreased as the treatment days increased. Patil and Joshi demonstrated that chloroform and methanol extracts of Mussaenda frondosa root significantly healed wounds and increasing the tensile strength of skin (545.16 ± 12.30) of Wister rats [29]. This is comparable to the tensile strength of the incision wound model of the methanolic extract of S. acuta after 9 days.

The proliferative phase of wound healing, which involves wound contraction, occurs through the centripetal movement of the tissues surrounding the wound. This process is mediated by myofibroblasts, which establish a grip on the wound margins and contract themselves in a manner similar to that of smooth muscle cells [30]. Wound shrinking process relies on a number of factors such as the reparative abilities and general health state of the tissues, and the type and extent of the damage to the tissues [31]. In both excision and incision wounds, the wound healing progressions were monitored, thus, a comparison could be made between the two types of wounds [19]. The period of re-epithelialisation was expressed as the number of days required for the falling of the scar (dead-tissue remnants) without any residual raw wound [32]. Epithelialisation is necessary in the repair of all type of wounds [32]. When compared with the positive control, the reepithelialisation time was lower in the methanolic *S. acuta* extract treatment group. This is consistent with a previous study [31]. The shorter period needed for wound contraction and reepithelialisation in the group treated with the standard drug could be attributed to the antimicrobial activity of the drug [33].

The wound healing activity observed in the extracts could be attributed to the presence of secondary metabolites in the leaves. In the preliminary photochemical analysis of the methanol extract from *S. acuta*, we found that it contains active constituents e.g. flavanoid and terpenoids that are needed by the body for wound healing. *S. acuta* extracts may have exerted their wound healing activity due to the presence of flavonoids which protect tissues from oxidative damage [34].

The flavonoids present in the methanol extract of the leaves may be responsible for its wound healing ability [35]. Tannins have been reported to possess wound healing action by improving the regeneration and organization of the new tissue [36].

The presence of terpenoids in the extract could be attributed for the contraction of wound and accelerated rate of epithelialisation. The radicalscavenging property of flavonoids enhances its antioxidant enzyme levels in granuloma tissue [37]. The results revealed the presence of flavonoids, alkaloids, terpenoids, in the leave. This was consistent with our findings, considering that water and methanol exhibit similar relative polarity.

Conclusion

The present study has shown that the extracts of *S. acuta* had significant wound healing activity which could be attributed to the secondary metabolites like flavonoids with reported antioxidant and immune-stimulating activities.

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Group	Doses	Excision model	Doses	n model
1	Positive control (Penicillin skin ointment, Drugfiled Pharmaceuticals Limited, Lagos, Nigeria)	3 guinea pigs	Positive control (Penicillin skin ointment)	3 guinea pigs
2	Negative control (no form of treatment)	3 guinea pigs	Negative control (no form of treatment)	3 guinea pigs
3	3000mg/kg methanolic extract of S. acuta	3 guinea pigs	2500mg/kg methanolic extract of S. acuta	3 guinea pigs
4	4000mg/kg methanolic extract of S. acuta	3 guinea pigs	3500mg/kg methanolic extract of S. acuta	3 guinea pigs
5	5000mg/kg methanolic extract of <i>S. acuta</i>	3 guinea pigs	4500mg/kg methanolic extract of S. acuta	3 guinea pigs

Table 1. The guinea pigs grouping for study on wound healing capability of wire weed

Table 2. Ph	vtoconstituents of methanolic extract of S. acuta
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Relative abundance
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-
-
++
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Key: - = absent; + = low in abundance; ++ = moderate in abundance; +++ = high in abundance

Table 3. Wound area contraction per day for excision wound model

Days	Experimental Groups (mg)			Controls	
	3000	4000	5000	Standard drug	Negative
0	100.00 ± 0.00 ³⁴	100.00 ± 0.00^7	100.00 ± 0.00 ⁴	100.00 ± 0.00^7	100.00 ± 0.00 ⁶
1	113.00 ± 18.68 ^{a4}	71.00 ± 4.93 ^{a6}	80.00 ± 40.00 ^{a34}	65.00 ± 2.89 ^{ª6}	64.50 ± 0.87 ^{a5}
2	84.00 ± 16.70 ^{a234}	60.00 ± 8.66 ^{a56}	66.00 ± 33.00 ^{a234}	53.00 ± 1.73 ^{a5}	60.00 ± 0.00 ^{a45}
3	72.00 ± 16.00 ^{a1234}	60.00 ± 8.66 ^{a56}	45.00 ± 22.64 ^{a1234}	50.00 ± 3.46 ^{a45}	47.00 ± 1.15 ^{a34}
4	58.33 ± 6.17 ^{a1234}	46.33 ± 5.78 ^{a45}	39.67 ± 19.94 ^{a123}	45.00 ± 5.20 ^{a4}	34.50 ± 8.37 ²²³
5	66.67 ± 9.39 ^{b1234}	41.33 ± 3.18 ^{ab4}	37.00 ± 18.67 ^{ab123}	37.00 ± 2.89 ^{ab3}	31.00 ± 6.35 ^{a12}
6	65.33 ± 17.68 ^{b1234}	41.33 ± 3.18 ^{ab4}	31.00 ± 15.52 ^{ab123}	18.00 ± 0.00 ^{a2}	31.00 ± 6.35 ^{ab12}
7	41.67 ± 10.14 ^{a123}	34.00 ± 4.00 ^{a34}	16.00 ± 16.00 ^{a12}	15.00 ± 1.73 ^{a12}	31.00 ± 6.35 ^{a12}
8	41.67 ± 30.04 ^{a123}	22 . 33 ± 5.36 ^{a23}	8.00 ± 8.00^{a12}	14.00 ± 2.31 ^{a12}	30.67 ± 6.36 ^{a12}
9	36.33 ± 31.94 ^{a12}	12.00 ± 0.00 ^{a12}	6.00 ± 6.00^{a12}	12.00 ± 2.31 ^{a12}	26.50 ± 4.91 ^{a12}
10	19.67 ± 15.39 ^{a1}	10.33 ± 0.88 ^{a12}	4.00 ± 4.00 ^{a12}	11.00 ± 1.73 ^{a12}	$24.00 \pm 3.46^{a_{12}}$
11	19.67 ± 15.39 ^{a1}	5.33 ± 2.40 ^{a1}	1.67 ± 1.67 ^{a1}	9.00 ± 1.73 ^{a2}	19.00 ± 0.58 ^{a1}
12	19.67 ± 15.39 ^{a1}	0.00 ± 0.00^{a1}	1.67 ± 1.67^{a1}	8.50 ± 2.02^{a1}	17.00 ± 0.58 ^{a1}

Mean values with different alphabets as superscripts in a row differ significantly (p<0.05) Mean values with different numbers as superscripts in a column differ significantly (p<0.05)

Table 4. Tensile strength of incision wound model after 9 days					
Group	Tensile strength after 9 days				
1. (2500mg/kg methanolic extract of S. acuta)	0.00 ± 0.00^{a}				
 (3500mg/kg methanolic extract of S. acuta) 	126.47 ± 68.29 ^b				
3. (4500mg/kg methanolic extract of S. acuta)	7.67 ± 7.67ª				
4. (standard drug)	0.00 ± 0.00 ^a				
5. (negative control)	35.30 ± 17.66 ^{ab}				

Mean values with different alphabets as superscripts in a row differ significantly (p < 0.05) Mean values with different numbers as superscripts in a column differ significantly (p < 0.05)



Figure 1: Median lethal (LC₅₀) concentration of S. acuta for Cavia porcellus exposed for 6 hour



Figure 2: Median lethal (LC₅₀) concentration of S. acuta for Cavia porcellus exposed for 12 hour





Figure 4: Pictorial progress of wound healing (incision model) in guinea pig treated 4000 mg/kg of methanolic extract of *S. acuta.* **Key:** (a) scraped skin of the guinea pig, (b) anaesthetized guinea pig, (c) start of the incision using a surgical blade (d), 2 cm long incision made, (e) first simple stitch, (f) second simple stitch, (g) third simple stitch, (h) incised wound after day 9, (i) incised wound after scar has been removed using a tensiometer.







Figure 5: Healed wounds (excision model) in guinea pig treated with varied concentrations of methanolic extract of *S. acuta* showing glandular tissue (gt) inflammatory cells (ic), collagen fiber (cf), fibroblasts (f) and proliferating blood capillaries (bc). Key: (a) 3000 mg/kg of *S. acuta* leaf extract, (b) 4000 mg/kg of *S. acuta* leaf extract and (c) 5000 mg/kg of *S. acuta* leaf extract.