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Research Article

Effect of 28% Concentration of *Trichosanthes cucumerina Linn.* (Snake Tomato) Seed on Wound Healing Using Male Wistar Rats

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Abstract: Various plants and medications have been used in the treatment of wounds. In this study, the efficacy of *Trichosanthes cucumerina Linn.* seed on wound healing was evaluated using male wistar rats. 24 male wistar rats (of 12 for control and 12 experimental) were used for this study. A 2 by $2cm^2$ area of cutaneous wound was made at the dorso-lateral aspect of the Thoracic Region of rats and 28% concentration of *Trichosanthes cucumerina Linn.* seed was topically applied on the wound of experimental rats while distilled water was used on the control rats. The wounds were dressed on a three day interval and granulation tissue was excised at days 3 and 9. The excised tissues were processed for histological analysis. Healing was assessed by wound contraction rates, complete day of wound closure and histological analysis of excised tissues. Wound contraction rates were calculated on days 3, 6, 9, and 12 post-wounding. The wound contraction rates of experimental group on days 3 and 9 were insignificant ($p > 0.05$) as compared to control group but that of day 6 was significant with the control animals having higher contraction rates than experimental animals. *Trichosanthes cucumerina Linn.* seed significantly increased ($p < 0.05$) the day of complete wound closure in experimental group as compared to the control group. The mean wound closure for experimental was 10.5 ± 1.73 as against control group which was 15 ± 2.45.Concentration of neutrophils and macrophages were more intense in experimental than control group in tissue samples excised on day 3.Also, experimental group exhibited more intense concentration of neutrophils, macrophages, fibroblasts and blood vessels on day 9 than control group. Although, no much significant increase in wound contraction rates was observed in experimental group as against the control; however, shorter period of total wound closure and increased inflammatory response was observed while using 28% concentration of *Trichosanthes cucumerina Linn.* seeds topically on treatment of wound.
Keywords: *Trichosanthes cucumerina Linn.*, Wound Healing, Granulation tissue.

INTRODUCTION

A wound refers generally to a cut, a graze or any surgical incision that heals rapidly without difficulty. It could also be regarded as a defect or breach in the continuity of the cells, tissues/ or organs (skin) which presents an opportunity for micro-organisms to invade the body [1].

Tremendous advancements have been made in understanding the processes of wound healing. The cell types and the order in which they appear in the wound have been established; many growth factors and their functions have been elucidated [2].

Wound healing is the process of repair that follows injury to the skin and other soft tissues. An incision created by a scalpel, trauma resulting from a bullet, or tissue death caused by a myocardial infarction all undergo a similar and predictable reparative process. Healing is the body's response to injury in an attempt to restore normal structure and function [3]. The process of healing involves two distinct processes-regeneration and repair [4].

Wound closure (healing) could occur via three categories- primary, secondary, and tertiary intensions [5].

Naturally, wound healing is achieved through three precisely and highly programmed phases: inflammatory, proliferative, and remodeling phase. For a wound to heal successfully, all three phases must occur in the proper sequence and time frame [6, 7]. Wound healing is triggered by the migration of cells like neutrophil, macrophages, and fibroblast [8]. So many therapeutic agents have been topically applied on wounds and in this study *Trichosanthes cucumerina* L. seed would be used.

Tricosanthes cucumerina is a well known plant, the fruit of which is mainly consumed as a vegetable due to its good nutritional value [9]. It is commonly known as snake gourd, viper gourd, snake tomato or long tomato. It is an annual climber (belonging to the family Cucurbitaceae) which is well suited to growth in the humid lowland tropics [9]. *Trichosanthes cucumerina*is a newly introduced crop of increasing importance in several parts of Africa. It is very red in color when ripe and can be used to improve the appearance of food as it can be blended and used to produce a paste for stew which tastes like, and serves the role of tomatoes, hence justifying the name of the plant [9]. The plant is richly constituted with a series of chemical constituents like proteins, fat, fibre, carbohydrates, flavonoids, carotenoids, phenolic acids, vitamin A, C and E, and lycopene [10, 11]. The predominant mineral elements were potassium and phosphorus. Other elements found in fairly high amounts are Sodium, Magnesium and Zinc [12].

The regional names of snake gourd or snake tomato are as follows : in Bengali, it is known as Chichinga/ Chichinge, in Telugu as potlakaaya, in Tamil as pudalankaai, in Canada as aduvalakaayi, in Malayalam as padavalanga, Galartori in Punjabi, padavali in Gujarathi, Chachinda in Hindi [12]. In other nations it is commonly called serpent vegétal in France, Schlangengurke in Germany, Karasu-uri-zoku in Japan, Patola in Srilanka, Zucchettacinese in Italy, Abóbora-serpente in Portugal, Käärmekurkku in Finland, Buapnguu Ma noi in Thailand, Yilankabagi in Turkey and Calabazaanguina in Spain [13]. In Nigeria, it is known as snake tomato. The genus Trichosanthes is native to Southern and Eastern Asia, Australia and Islands of the western Pacific. *Trichosanthes cucumerina* is found wild throughout these areas. It was probably domesticated in ancient times in India [14].

The seeds are half-ellipsoid, somewhat compressed, undulate, hard, rugose, nearly one centimeter long, greyish-brown, sculptured, margin undulate and imbedded in a soft foetid with red pulp [15]. Analysis has shown that the seed of *Trichosanthes cucumerina* L. have high oil content up to 42.5±5% [16].

During the past two decades, substantial progress has been made with respect to our understanding of the pathophysiology of wound healing; and new therapeutic methods have been developed. However, enhancement of wound healing and reduced scar formation remains a common challenge in the fields of plastic and reconstructive surgery.

The therapeutic efficacies of many indigenous plants (vegetables, fruits and herbs) for various diseases have been described by traditional herbal medicine practitioners [17]. Available literatures reveal that the therapeutic effect(s) of *T. cucumerina* have been investigated by some researchers with respect to its anti-inflammatory, cytotoxicity, and antidiabetic activities among others [11, 18]. But its effect on wound healing (and scar formation) has not been established. It is in the light of the above that 28% concentration of *Trichosanthes cucumerina L.* seed was used in this study. The 28% concentration was adopted because of the future plans of comparing this finding with that of caned or tinned tomato whose tomato concentration is also 28%.

Aim

Determination of the wound healing role of 28% of *Trichosanthes cucumerina L.* seed on the gross wound morphology, histology and haematologic profile of Wistar rats.

Study Objectives

- To consider its effects on the gross wound morphometry.
- To analyze the granulation tissue with respect to macrophage, neutrophils, fibroblast and blood vessel count.
- To determine the significance of findings.
- To compare results with previous findings.
- To observe and compare the level of scar formation.

MATERIALS AND METHODS

Experimental Animals

24 male albino Wistar rats weighing averagely 180g were used for this study. They were grouped into two groups of 12 experimental and 12 control animals and thereafter housed in twos per cell cage. The animals were left to acclimatize for a period of two weeks and were well fed with guinea pig feed and adequate water throughout the period of the research.

Plant Material and Seed Preparation

Trichosanthes cucumerina L. fruits were locally collected from Omoku in Ogba, Egbema, Ndoni Local Government Area of Rivers State. The fresh Fruits were opened up to expose the seeds which were carefully selected. The retrieved seeds were washed and sun-dried for three days before the final drying in an oven at 65°C for 10 minutes.

The dried seeds were broken to expose the soft, oily intact seed. The shells were removed and discarded. The intact seeds were grounded with dry blender and further grounded with manual grinder to produce a paste-like dark green substance. The fine paste was weighed (43g) and approximately 153.6ml of water was added to produce a 28% concentration. This solution, which was used for the study, was stored in syringes and refrigerated.

Fig. 1: Photograph of *Trichosanthes cucumerina L.* fruit and seed

Infliction of Injury and Dressing

The animals were anaesthetized with Ketamine and Diazepam prior to the infliction of the injury. 0.3Ml to 0.45ml of both anaesthesia was administered intraperitoneally according to their body weight. The dorso-lateral fur of thorax was shaved using lancet (blade). The anticipated area of the wound to be created was outlined using permanent marker by placing a template of 2cm by 2cm squared transparent sheath on the shaved area. A full thickness of sub-cutaneous wound was created along the markings using toothed forceps and a surgical blade. The wounds of control animals were topically treated with distilled water, covered with sterilized gauze and properly plastered and labeled. The experimental animals were topically treated with the already prepared 28% concentration of *Trichosanthes cucumerina L.* seed, covered with sterilized gauze, plastered and labeled as well. Treatment and dressing of the wounds were carried out on a three-day interval.

Wound Healing Activity

Wound contraction rate and histological analysis of granulation tissue were used to evaluate the wound healing activity of the 28% concentration of *Trichosanthes cucumerina L.* seed.

Assessment of Wound Contraction Rate

The wound contraction rate was assessed by tracing the wound sizes on day 3, 6, 9, 12, 16 and 18 of post-wounding using a transparent 4cm by 4cm transparent sheath and permanent marker. Using the marker, the shape and extent of the wound was traced or marked on the transparent sheet and immediately transferred or placed on a graph sheet. The number of blocks that falls within this marked area were counted and then multiplied by $0.04cm^2$ [since each small box (block) of a graph is equivalent to $0.04cm^2$] so as to get the wound size. Thereafter, the wounds were dressed with the different therapeutic agents. The traced areas were recorded and measured by using a graph paper to count the number of blocks covered by the wound as traced and multiplying by $0.04cm^2$. This was also done on day 0 before dressing using the formula below [19, 20].

$$\frac{wond\ size\ at\ day\ zero\ (0) - wound\ size\ on\ the\ given\ day}{wound\ size\ on\ day\ zero\ (0)} \text{ X } \frac{100}{1}$$

Excision of Wound (Granulation Tissue) for Histological Analysis

The animals were anaesthetized prior to excision of wound tissue. The already injured and anaesthetized animals were un-plastered. The wound sizes were traced as described earlier. Excision was done by excising the granulation tissue from the surface of the wound using toothed forceps and surgical blade. The excised tissue was preserved using 10% formal saline and stored in well labeled specimen bottles. Wound excision was carried out on day 3 and day 9 involving four control and four experimental animals.

Histological Examination

The granulation tissues excised on the 3rd and 9th days were preserved in 10% formal saline at room temperature prior to tissue processing. The preserved granulation tissues were then processed using routine tissue processing technique (dehydration, clearing, impregnation, embedding, and sectioning) and stained using Haematoxylin and Eosin stain to show the general architectural pattern (cells) of the tissue.

Microscopy and Cell Identification

The tissues were viewed using the photomicrograph microscope and the following were identified and estimated using oil immersion objective; Macrophage, Neutrophil, Fibroblast and Blood Vessels.

RESULTS

Table 1: Percentage (%) Mean Wound Contraction Rates of Control and Experimental Groups at Day 3, 6 and 9

Days	Control Mean ± SD	Experimental Mean ± SD	p- Value	Remark
3	33.83 ± 16.64	24.45 ± 15.19	P > 0.05	*
6	65.37 ± 6.37	43.46 ± 10.04	p < 0.05	**
9	90.83 ± 2.77	85.96 ± 11.80	p > 0.05	*

*Not Significant, ** Significant

Table 2: Mean Day of Complete Wound Healing Day of Experimental and Control Groups

Control Group Mean ± SD	Experimental Group Mean ± SD
15 ± 2.45	10.5 ±1.73

p-value < 0.05, hence, significant

Table 3: Neutrohil and Macrophage Cell Analysis of Control and Experimental Groups at Day 3 Pper High Power Field

Cells	Rats	Control	Experimental
Neutrophils	1	++	+++
	2	++	+++
	3	++	+++
	4	+	+++
Macrophage	1	++	+++
	2	++	+++
	3	+	+++
	4	+	+++

Key: (+) = Moderate accumulation of cells, (++) = Intense accumulation of cells, (+++) = Very intense accumulation of cells

Table 4: Neutrophil, Macrophage and Fibroblast Cell Analysis of Control and Experimental Groups at Day 9 per High Power Field

Cells	Rats	Control	Experimental
Neutrophils	1	+	++
	2	+	++
	3	+	+
	4	+	++
Macrophage	1	+	+
	2	+	+
	3	+	++
	4	+	+
Fibroblast	1	++	++
	2	++	+++
	3	+++	+++
	4	+	+++

Key: (+) = Moderate accumulation of cells, (++) = Intense accumulation of cells, (+++) = Very intense accumulation of cells

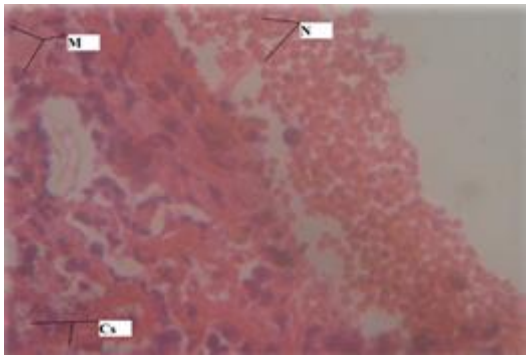

Fig. 2a: Control: H&E X400: A section of the tissue showing infiltration of neutrophils, areas of hemorrhage and necrosis. The tissues are large and edematous

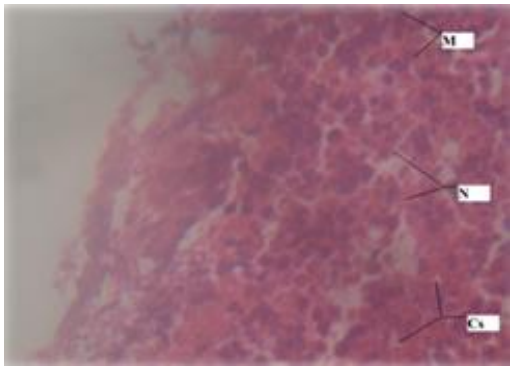

Fig. 2b: Experimental: H&E X400: A section of the tissue showing intense infiltrate of neutrophils and necrosis.
Key: N = Neutrophil, M = Macrophage, Cs = Small capillaries
Fig. 2 (a &b): Micrographs Showing Wound Healing Activity at Day 3

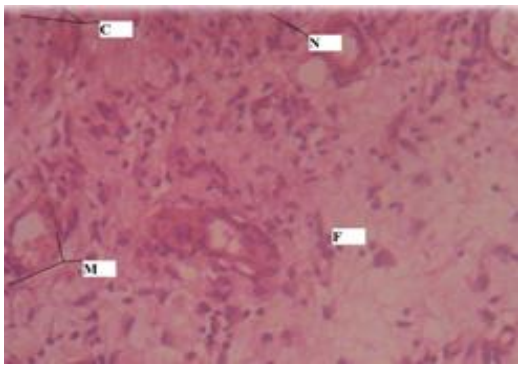

Fig. 3a: Control 8: H&E X400: A section of the connective tissues at the site of the injury. Note the edema, neovascularization and less intense infiltrate of inflammatory cells.

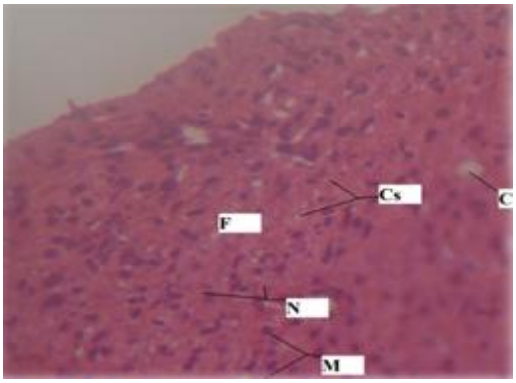

Fig. 3ab: Experimental 8: H&E X400: A section of the excised tissue. Note the intense infiltration by neutrophils and macrophages, edema and numerous small caliber blood vessels.
Key- N = Neutrophil ,C = Capillary, F = Fibroblast, Cs = Small capillaries
Fig. 3: Micrograph Showing Wound Healing Activity at Day 9

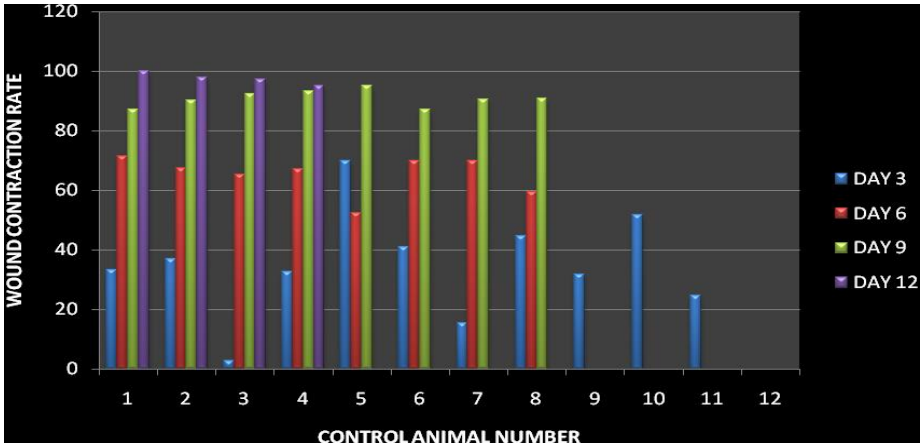

Fig. 4: Chart showing the Rate of Wound Contraction of Control Group at Days 3, 6, 9, and 12

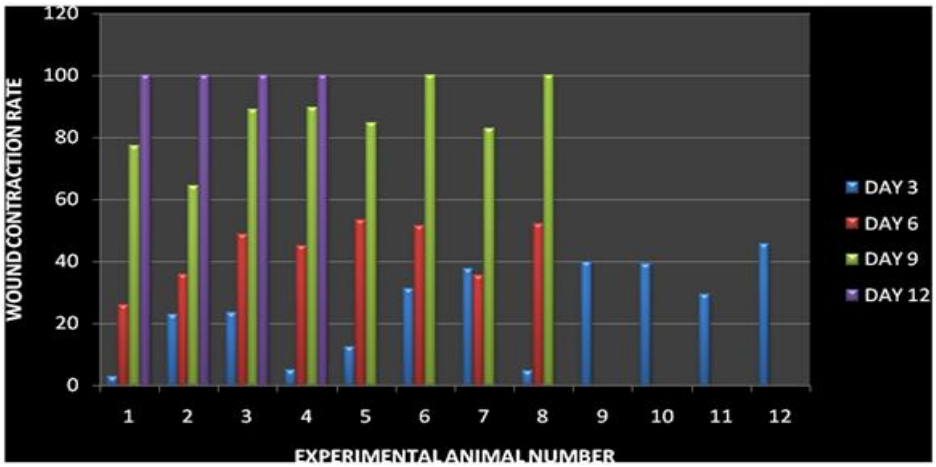

Fig. 5: Chart showing the Rate of Wound Contraction of Experimental Group at Days 3, 6, 9, and 12

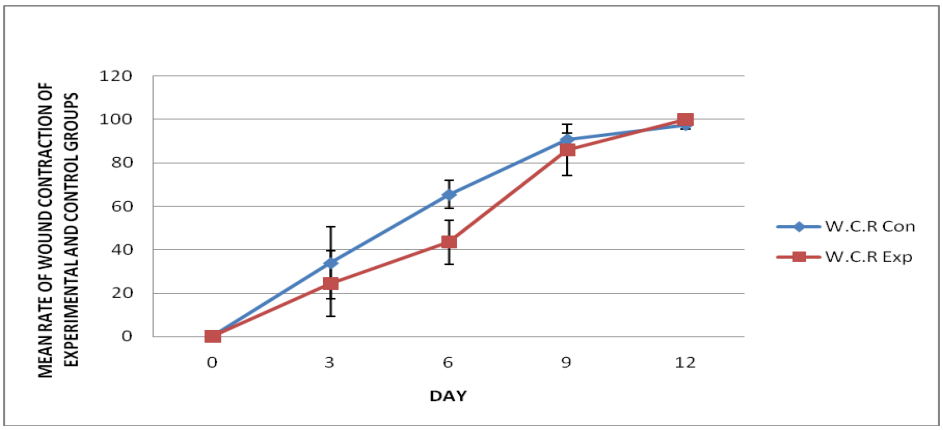

Fig. 6: Graph showing the Mean Wound Contraction Rate of Control and Experimental Groups at Days 3, 6, 9 and 12

This line graph indicates the mean wound contraction rate of control and experimental rats. The error bars indicate the standard deviation. Day 6 shows statistical significant difference at $p< 0.05$ while days 3, 9 and 12 do not show any statistical significant difference.

DISCUSSION

Wound healing, or wound repair, is an intricate process in which the skin (or another organ) repairs itself after injury [4]. It is also, the body's natural process of regenerating dermal and epidermal tissue. Approximately 80% of the wound's original size is reduced by wound contraction. In order to explain this mechanism of wound contraction, factors proposed are: dehydration as a result of removal of fluid; contraction of collagen and discovery of myofibroblasts appearing in active granulation tissue [3]. Wound contraction which shrinks the area of wound begins after two to three (2-3) days but reaches its peak at about the 14th

day post wounding, and continue throughout the healing process [3].

The data depicted in Table 1showed that there was no significant difference in the rate of wound contraction between experimental and control groups in days 3 and 9 ($p > 0.05$). But day 6, on the other hand, showed a significant difference in wound contraction, with the control group having higher contraction rates than the experimental group. These differences observed remains is unclear on the bases of this research.

Cutaneous wound healing process requires interaction between cells in the dermis and epidermis and the release of chemical mediators from inflammatory cells, fibroblasts and keratinocytes. Influx of macrophages into granulation tissue serves to replace the dermal defect and provide substrates and inducers for re-epithelialization. From the histological analysis, it was observed that wounds treated with *Trichosanthes cucumerina Linn* seed (Experimental group) demonstrated a higher intensity of inflammatory response than those treated with distilled water (control group). This was evident in the high concentration of neutrophils, macrophages and fibroblasts in the experimental group as compared to the control group (table 3 and 4).

Furthermore, neovascularization is a crucial step in wound healing process [21]. It is necessary to sustain newly formed granulation tissue and ensure the survival of keratinocytes. High capillary density also suggests increased angiogenesis. In this study, day 9 tissue samples from the rats treated with 28% concentration 0f *Trichosanthes cucumerina Linn* seed showed intense infiltration by neutrophils and macrophages, edema and numerous small caliber blood vessels (Fig. 3). Hence, the promoting of neovascularization may be considered as one of the factors which accelerate wound healing by *Trichosanthes cucumerina Linn* seed.

It is important to note that the complete wound healing day or day of complete wound closure of control rats and experimental rats was statistically significant ($p < 0.05$). The experimental animals started showing complete wound closure from day 9 and all wounds were totally closed at day 12. This is in contrast to the control animals which started complete closure at day 12 and completely closed at day 15(table 2). The experimental animals left little or no scar tissue at day 15 while the control animals had obvious scar tissue at the site of wounding, hence, a possible inference that *Trichosanthes cucumerina Linn* seed reduces scar tissue formation to the barest minimum.

Wound-healing property of *Trichosanthes cucumerina Linn* seed may be attributed to the phytochemical constituents present in it, which may be either due to the individual constituents acting separately or the additive effect of all the constituents that fastens the process of wound healing.

CONCLUSION AND RECOMMENDATION

In conclusion, this research showed that topical application of 28% concentration of *Trichosanthes cucumerina Linn* seed promotes wound healing activity via increased inflammatory response and neovascularization. It may be tested for treating various types of wounds in human beings. More research has to be done in that regard using various concentrations of *Trichosanthes cucumerina Linn* seed.

At this point it is difficult to say which component(s) of the seed are responsible for this wound healing activity. Therefore, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities with respect to wound healing.

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