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Effect of 28% Concentration of Ethanolic Extract of *Trichosanthes Cucumerina* (Snake Tomato) Fruit on Wound Healing using Male Wistar Rats

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ABSTRACT

In this study the wound healing property of 28% concentration of ethanolic extract of *Trichosanthes cucumerina* fruit was determined by considering its effect on gross wound morphometry, histological analysis of the granulation tissue (with respect to neutrophils, macrophages, fibroblast and blood vessel estimation). 28% concentration of ethanolic extract of *T. cucumerina* fruit was prepared by adding100ml of absolute ethanol to every 28g of blended *T. cucumerina* fruit and then allowed to ferment in an air tight container in a period of three days. The mixture was filtered and the filtrate heated using a water bath at a temperature range of 40°c-50°c until a dark-brownish paste-likesubstance (extract) was obtained. Prior to its topical usage, 28g of the extract was added to 100ml of water so as to obtain the 28% concentration of the extract. 24 male wistar rats of two groups; experimental and control (of 12 each) weighing approximately 200g were used in this study. A wound size of 2cm by 2cm which exposed the *panniculusadiposus* was inflicted on the dorsal-lateral shaved aspect of the thorax after anaesthesia. The wound sizes were immediately measured using a 4cm by 4cm square template transparent sheet which was subsequently placed on a graph sheet for wound morphometry. The experimental and control groups were dressed using 28% concentration of the extract and distilled water respectively. The % mean wound contraction on day 3, 6, 9, and 12 for the control group were 10.28 ± 5.58, 37.74 ± 7.30, 56.01 ±8.99 and 70.74 ± 9.93 respectively. While for the experimental group on the same days were 28.42 ± 11.37, 61.03 ± 12.86, 83.64 ± 12.28 and 95.27 ± 5.04 respectively. The two groups at these respective days were found to be statistically significant ($P<0.05$). The mean day for complete wound closure for both the control and experimental groups were 18.75 ± 2.87 and 14.25 ± 1.50 respectively, and this was found to be statistically significant ($P<0.05$). On day 3and 9, the granulation tissue was excised and the histological analysis revealed generally, more neutrophils and macrophages than fibroblast and blood vessels at day 3 and vice versa at day 9. Comparing the experimental and control groups on these days revealed more neutrophils in the control as against the experimental group, more macrophages in experimental than in control groups (vice versa at day 9),more fibroblast in experimental than in control groups, and more blood vessels in experimental than in control groups. This study showed *Trichosanthes cucumerina* fruit to accelerate wound closure (healing) to a reasonable statistical significance which could be attributed to its phytochemical constituent especially lycopene which is described to be a powerful anti-oxidant.

Key Words : *Trichosanthes cucumerina,* Wound healing and Fibroblast

INTRODUCTION

Tissue injury and the resulting wound problems have existed for as long as humans have walked the earth.[1] 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.[2] Wound healing is thus the restoration of that continuity. Wound healing (closure) is a biological process that begins with trauma and ends with scar formation.[3] Wound healing process is divided into phases;Inflammatory or Lag phase, Proliferative phase, and Remodeling and Maturation phase.[4]

So many therapeutic agents have been topically applied on wounds and in this study *Tricosanthes cucumerina* would be used. *Tricosanthescucumerina* is a well known plant, the fruit of which is mainly consumed as a vegetable. It is commonly known as snake gourd, viper gourd, snake tomato or long tomato. It is very red in colour 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.[5] The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids, vitamin A, C and E, and lycopene.[6,7]

Trichosanthescucumerina falls under the scientific classification of

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Mangnoliopsida
Order:	Curcubitales
Family:	Curcubitaceae
Genus:	Trichosanthes
Species:	Cucumerina

Snake tomato is widely distributed, and so, has diverse names for identification in diverse locations.

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.[8] 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. [9] In Nigeria, it is known as snake tomato.

The genus Trichosanthes isa native to Southern and Eastern Asia, Australia and Islands of the western Pacific. Trichosanthescucumerina is found wild throughout these areas. It was probably domesticated in ancient times in India.[10] It is grown as a minor vegetable in many countries of tropical Asia. It is locally grown as a vegetable in home gardens in Africa. Commercial growers around big cities in East Africa occasionally grow cultivars of snake gourd imported from India for people of Indian origin. It is also imported from India through Malaya to tropical Australia.

Trichosanthescucumerina is a newly introduced crop of increasing importance in several parts of Africa, including Ghana and Nigeria. The genus Trichosanthes comprises about 100 species, of which a few have been domesticated in Asia, snake gourd being the most important. Two varieties are distinguished within Trichosanthescucumerina. They are the wild var. cucumerina occurring from India, Sri Lanka and China, through South-East Asia, to northern Australia, and the cultivated var. anguina (L.). Only traditional landraces of Trichosanthescucumerina are used in West and Central Africa, whereas improved cultivars from India are grown in East Africa.[11] It is distributed in temperate Asian regions like china, tropical regions of Bangladesh, India, Nepal, Pakistan Sri Lanka, Myanmar; Vietnam, Indonesia; Malaysia; Philippines, in Australia it is found in Northern Territory, Queens land and in Western Australia.[5]

Trichosanthescucumerina is a rich source of nutrition. It is highly constituted with proteins, fat, fibre, carbohydrates, vitamin A and E. The total phenolics and flavonoids content is 46.8% and 78.0% respectively.[11] The fruit is rich in Vitamin C and E. The crude protein content is 30.18%.[12] The predominant mineral elements were potassium (121.60mg 100-1g) and phosphorus (135.0mg 100-1g). Other elements found in fairly high amounts are Sodium, Magnesium and Zinc.[7]

Trichosanthescucumerina is used in the treatment of headache, alopecia, fever, abdominal tumors, bilious, boils, acute colic, diarrhoea, haematuria and skin allergy. T.cucumerina is used as an abortifacient, vermifuge, stomachic, refrigerant, purgative, malaria, laxative, hydragogue, hemagglutinant, emetic, cathartic, bronchitis and anthelmintic. [13]

The constituents of this invaluable plant and its tribal uses informed the basis for this virgin research using T. cucmerinaas a therapeutic option in wound treatment.

AIM OF STUDY

This study was carried out to determine the wound healing property of 28% concentration of the ethanolic extract of Trichosanthescucumerina on the gross wound morphology histology and haematologic profile of Wistar rats.

STUDY OBJECTIVES

These include the following:

- 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 studies.

MATERIALS AND METHODS

Experimental Animals:

Twenty four (24) male Wister rats weighing 200g procured from the Enugu State University animal farm and later transferredto the animal house of the Department of Human Anatomy, University of Port Harcourt were used for this study.

The animals were immediately separated into two groups of twelve (12) each by random selection. The two groups were tagged experimental and control respectively. Each animal group was kept in six cages (two animals per cage) and then allowed to acclimatize for a period of two (2) weeks. This was done to enhance better adaptation of the animals to their new environment. Throughout the period of acclimatization and research the animals were fed with guinea pig feed and adequate water.

Plant Material:

Preparation of 28% Concentration of Ethanolic Extract of Trichosanthescucumerina Fruit

Trichosanthescucumerina fruit weighing 905.2g were

procured from farmers at Omoku (Onelga), Isiokpo, and Omuanwa towns (Kelga) all in Rivers State of Nigeriaand allowed to ripe. The rippedTrichosanthes*cucumerina*was blended using a blenderand its approximate weight came to 864.64g. Using a measuring cylinder, 100ml of absolute ethanol was added to every 28g of the blended *Trichosanthescucumerina* and allowed to ferment for a period of three (3) days in an air tight beaker. The mixture was then filtered using a filter paper, funnel, and conical flask. Unlike the colourless absolute ethanol, the filtrate gave a golden yellow or *amber* colouration.

Using a water bath with a thermometer dipped into it, the filtrate was heated at a temperature range of about 40°C - 50°C (so that the active ingredient will not be destroyed) until a dark-brownish paste-like substance (extract) was obtained. The extract was stored in a refrigerator. During application, 28g of the extract was added to 100ml of water so as to obtain 28% concentration of the *Trichosathescucumerina*fruit extract. The 28% concentration was adopted with the intent of comparing its efficacy in the future with the tin or canned tomato whose tomato concentration is also 28%.

Infliction of Wound

After the two (2) weeks of acclimatization, the animals were weighed using a weighing balance and the animal weight increased to approximately 300g.

In other to reduce pain and keep animals in a subconscious state during wound infliction and measurement of wound sizesanaesthesia was used. The anaestahetics used were Diazepam and Ketaminof which 0.3ml to 0.45ml of each of them were administered intra-peritoneally according to their body weight. Following a subconscious state of the animals, each animal was then thoroughly shaved at the dorsolateral aspect of the thorax using a very sharp razor and then cleaned using methylated spirit. Using a pair of scissors, a 2cm by 2cm square template was cut out from a transparent sheet, sterilized and then placed on the shaved region of the animal, and subsequently traced on the skin using a felt pen. With the help of a scalpel, surgical blade and forcep,the traced path of the skin of the animals were excised thus creating a wound measuring 2cm by 2cm square that exposed the Panniculusadiposus.

Measurement of Wound Size and Mode of Dressing

The wound size was measured by placing a 4cm by 4cm square transparent sheet on the wound. Using a marker,the shape and extent of the outer wound margin was traced or marked on the transparent sheet and immediately transferred or placed on a graph sheet. The number of blocks the graph sheet 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. For the experimental group, the 28% concentration preparation of ethanolic extract of *Trichosanthescucumerina*fruit was applied topically on the wound surface using a 5ml syringe. The wound was then covered with sterilized gauze soaked in the same preparation, and firmly secured with a plaster. The control group was dressed in a similar way except that distilled water was used in place of 28% concentration preparation of ethanolic extract of *Trichosanthes. cucumerina*fruit.

Assessment of Contraction (Wound Closure) and Excision

The wound contraction assessment was done every three (3) days (that is, on day 3, 6, 9, 12, 15 etcetera) after the removal of plaster. This was carried out following the procedure of wound size measurement after which wound dressing followed as explained above. The wound contraction rate and % wound contraction was determined on each of these days by using the formula below :[14, 15]

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

On day 3 and 9, the granulation tissue of the healing skin wound of four (4) animals each from both the experimental and control groups were excised using forceps and surgical bladeor razor blade. Usually excision was done afterthe measurement of wound size.

Tissue Processing and staining

The excised healing wound (granulation tissue) was then processed using routine tissue processing technique and stained using Haematoxylin and Eosin stain to show the general architectural pattern of the tissue cells.

Microscopy and Cell Identification

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

RESULTS
Observations made during wound closure (healing) are summarized in the table below:

Table 1: Percentage (%) mean wound contraction and standard deviation for both experimental (E) and control (C) groups at day 3, 6,9 and 12.

Days	Mean ± SD Control (C)	Mean ± SD Experimental (E)
3	10.28± 5.58	28.42± 11.37
6	37.74 ± 7.30	61.03 ± 12.28
9	56.01 ± 8.99	83.64 ± 12.28
12	70.74 ± 9.93	95.27 ± 5.04

P < 0.05: Statistically significant

Table 2: Mean day of complete wound closure and standard deviation for both experimental (e) and control (c) groups.

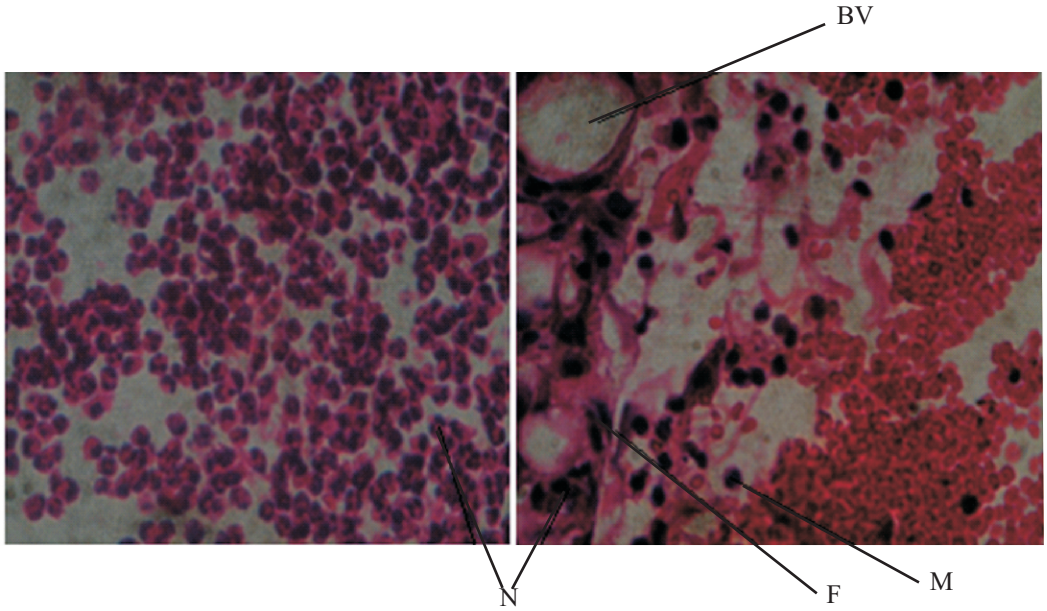
	Control (C)	Experimental (E)
Mean ($\bar{x}$)	18.75	14.25
Standard deviation (S.D)	2.87	1.50

P < 0.05: Statistically significant

The table below shows cell and blood vessel count (estimate) as either, low, moderate, or high at day 3 and day 9.

Table 3: Cell and blood vessel count (estimate) at day 3.

Group / Cell	Control (C)	Experimental (E)	Remark
Neutrophils (N)	High	High	C higher than E
Macrophages (M)	High	High	E higher than C but less than N of C and E
Fibroblast (F)	Low	Low	E higher than C
Blood Vessel (BV)	Low	Low	E higher than C but less than F of C and E

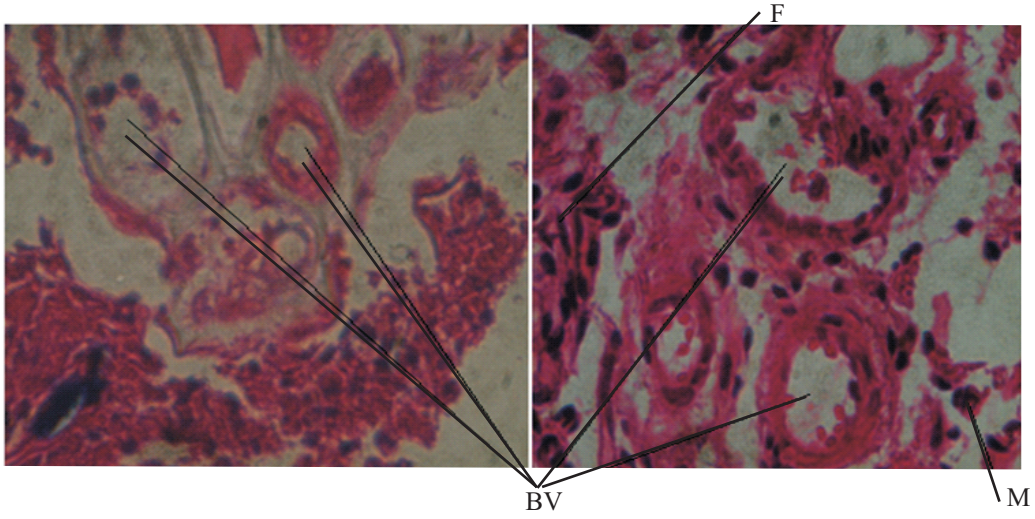


(a)Control (b) Experimental

Figure 1: Photomicrograph of both control and experimental animals at day. Mag. X1000

Table 4: Cell and blood vessel count (estimate) at day 9.

Group / Cell	Control (C)	Experimental (E)	Remark
Neutrophil (N)	Moderate	Low	C lower than M of C but E higher than M of E
Macrophages (M)	Moderate	Low	C higher than N of C but E lower than N of E
Fibroblast (F)	High	High	E higher than C
Blood Vessel (BV)	High	High	E higher than C but BV of C higher than F of E



(a) Control (b) Experimental

Figure 2: Photomicrograph of both control and experimental animals at day 9. Mag. X 1000

DISCUSSION

From the analysis of the data obtained from this study, the percentage mean wound contraction of the control group treated with distilled water on days 3, 6, 9, and 12 were 10.28 ± 5.58, 37.74 ± 7.30, 56.01 ±8.99 and 70.74 ± 9.93 respectively. While those treated with 28% concentration of ethanolic extract of *Trichosanthescucumerina*fruit on days 3, 6, 9, and 12 were also 28.42 ± 11.37, 61.03 ± 12.86, 83.64 ± 12.28 and 95.27 ± 5.04 respectively(Table 1). When the two groups were compared at these respective days,they were all found to be statistically significant using students T- test at 95% confident level (P<0.05).

It was also observed that the complete wound closure day for both the control and experimental groups had mean day of 18.75 ± 2.87 and 14.25 ± 1.50 respectively(Table 2). This was found to be statistically significant at 95% confident level (P<0.05). This corresponds with the study carried out by Shivanada*et al.,*[16]using ethanolic extract of *Morindacitrifolia* leaf (Indian mulberry).

Comparing the control and experimental groups on the basis of the cells which play a critical role in wound healing, it was observed from the photomicrographs and charts that neutrophils and macrophages were generally high in both groups at day 3 (Table 3) while fibroblast and blood vessel were low thus agreeing with the findings of Keast and Heather.[17]

However, there were relatively more neutrophils in the control group than the experimental group. But there were more fibroblast and blood vessel in the experimental group when compared to the control group which could be attributed to the rich nutrient content of *Trichosanthescucumerina*fruit applied.This agrees with the study of Fawehinmi and Ligha[18] where a wound treated with plant exudates of Jatrophacurcas Linn had higher number of fibroblast at day 3 when compared to the control group. As regards to the macrophages, their findings also agrees with the observation that there were more macrophages in the experimental than in the control at day 3 but less in number when compared to neutrophils in the control group.

At day 9 fibroblast and blood vessel were seen to be generally high in both groups (but more in experimental) while neutrophils and macrophages were moderate in number in control group as against the experimental group where they became reduced(Table 4). The reduced amount of neutrophils and macrophages could probably be as a result of the application of 28% concentration of ethanolic extract of *Trichosanthescucumerina*fruit. This therapeutic agent could probably have triggered early migration of neutrophils and macrophages to the wound site and eventually reduced chances of infection hence, accelerating inflammatory phase of wound healing. This corresponds to the study of Ojiako and Igwe [7]that*Trichosanthescucumerina*has anti- infective and wound healing properties. The moderate amount of neutrophils and macrophages found in the control group at this period would have delayed fibroplasia and angiogenesis thus, slowing down wound healing.[4]

On the other hand it was also observed that the number of neutrophils and macrophages reduced as the wound advances in days while fibroblast and blood vessel became dominant. This observation agrees with the study of Adam and Richard.[4]

CONCLUSION

Evidence of wound healing processes is the presence and amount of inflammatory cells (neutrophils, and macrophages), connective tissue and blood vessels at the wound site. From this study it is clear that 28% concentration of ethanolic extract of *Trichosanthescucumerina*fruit has accelerated the migration of these inflammatory cells particularly macrophages to the wound site for quick phagocytosis, fibroplasias and angiogenesis (which are essential processes of wound healing) in respect to the control and experimental groups.

Conclusively, this study reveals that *Trichosanthescucumerina*fruit has been found to accelerate wound closure (healing) to a reasonable statistical significance which could be attributed to its phytochemical constituent especially lycopene which is described to be a powerful anti-oxidant.

RECOMMENDATION

More research should be done on *Trichosanthescucumerina* and other *Trichosanthes* species like watermelon(*Citrilluslanatus*), pumpkins (*Cucurbita maxima* and *Cucurbitapepo*), and fluted pumpkin (*telfaireaoccidentalis*) since they all have lycopene and vitamin C to ascertain their wound healing properties as these invaluable plants could be developed into therapeutic agents for effective wound healing.

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