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EFFECT OF WHOLE SNAKE TOMATOE (*TRICHOSANTHES CUCUMERINA*) ON WOUND HEALING USING WISTAR RAT.

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ABSTRACT

Background: Wounds have been part of mankind from the time of creation till date. In this research, titled the effect of snake tomato on wound healing, the wound morphometry and white blood cell count were determined. **Material and Method:** 28 gram concentration of snake tomato (Trichosanthes cucumerina) was prepared. 24 male Wister rats weighing aproximately 180g were used in this research. They were grouped into a control (C) and experimental groups (E) with each comprising 12 rats. A wound size of 2cm by 2cm were inflicted on the dorsolateral aspect of the thorax of all the rats, measured and recorded. The animals in the control group were dressed using distilled water while those in experimental group were dressed using 28g concentration of snake tomato. **Results and Discussions:** The % mean wound contraction on day 3 control group was (9.73±5.01) and experimental group (51.69± 11.64) this was statistically significant ($P<0.05$). The % mean wound contraction on day 6 control group (38.8±8.33) and experimental group (70.75±9.10) also statistically significant. The % mean wound contraction on day 9 control group was (57±9.22) and the experimental group (87.1±4.43) was statistically significant ($P<0.05$). However, the mean healing day of control group (18.75±1.49) as against the experimental group (15.00±0.00) was statistically insignificant ($P>0.05$). The histological analysis of these tissues revealed that there were more neutrophils and macrophages at day 3 then day 9. **Conclusion:** All these observations point towards the fact that 28% concentration of snake tomato has the ability to accelerate wound healing.

INTRODUCTION

Wounds have been part of mankind from the time of creation, till date. As such, wounds can be defined as any injuries to the different body tissues and is considered as a break in the structure of an organ or tissue caused by an external agent. Bruises, grazes, tears, cuts, punctures.[1] Wounds are injuries caused by physical means and involving a discontinuity of normal anatomy 1Types of Wounds: Aseptic Wound, Avulsion Wound, Sucking wound, Tangential wound, Tetanus-prone wound.[1]

Wound Healing: As stated by Michael Mercandetti, wound healing is a complex and dynamic process of restoring cellular structures and tissue layers. The human adult wound healing process can be divided into 3 distinct processes in which the skin (or another organ) repairs itself after injury.[2]

Tomatoe (Lycopersicon Esculentum)

Lycopersicon esculentum is a plant in the solanaceae or might shade family as are its close cousins tobacco, chili peppers, potato and egg plant.[3] Lycopersicon is native to central, South and Southern north America from Mexico to Peru. It often grows in temperatures climates as an animal, typically reaching 1-3m (3 to 10ft) in height with a weak, woody stem that often vines over other plants.[3] The leaves are 10-25cm long with 5-9 leaflets, each leaflet up to 8cm long with a serrated margin; both the stem and leaves are densely granular-hairy. The flowers are 1-2cm across, yellow with five pointed lobes on the corolla.[3] The name (word) lycopersicon esculentum is derived from a word in the nahuatle language, tomatl. The specific name lycopersicon, means "wolf-peach".[3]

Trichosanthes Cucumerina (Snake Tomato)

According to Srinivasan and Kirana[4] trichosanthes cucumerina belonging to family cucurbitagceae is an annual climber and widely distributed in southern parts of India. Traditionally, decoction of the stem learns, and aerial parts were used in the treatment of diabetes and inflammatory diseases. The major active constituents of the drug are triterpenoid saponuns viz, cucurbitacins.

Trichosanthes cucmerina (snake tomato). Where it hails from in south western Nigeria it is also known as the snake tomato because the rope fruit is rich red coloured and supposed tastes like tomatoes.[4] Most of the fruit resemble pale cucumbers.

Constituents of Snake Tomato
The snake tomatoes have the following vitamin C, vitamin A, raw proteins, essential amino acids and calcium far more than the more popular and widespread tomato.[4]

Some researhers have worked on the healing effect of tomato and stated that there findings were positive with respect to the effectiveness in wound healing.[5-8]

AIM/OBJECTIVE
The aim of this work was to find the effect of Trichosanthes Cucumerina L. (whole) as regards wound healing.

LIMITATION OF THE STUDY
This study is limited to microscopic analysis of the fibroblast, neutrophil, microphage and wound morphometry.

MATERIALS AND METHOD
Research Design: Experimental and analytical. Ethical clearance was obtained from the University of Port Harcourt Ethics Committee. Duration of Study: January 10, 2010 –December 1, 2010. Twenty four (24) male wistar rats of average weight 187g were bought from the animal farm at the University of Nigeria, Nsuka, Eungu and brought to the histology laboratory of the College of Health Sciences for acclimatization for a period of two (2) weeks. This was done to enhance better adaptation of the rats to new environment. On arrival of the rats, they were randomly separated into two groups. One group (consisting 12 wistar rats) served as the experimental group while the other group served as the control group. During the period of adaptation, the rats were feed with growers feed and adequate water.

Fresh snake tomatoes were brought from ADP farm in Isiokpo in Ikwerre L.G.A. in Port Harcourt, River State. The fruit was identified to be *Trichosanthes Cucumerina* of the family of Solanaceae in the Department of Plant Science and Biotechnology of the University of Port Harcourt, Nigeria. They were blended using a blender. This was followed by heating of the blended snake tomato in an oven at a very minimum temperature of 30-35^{0}c. Twenty eight grams (28g) of the heated content of snake tomato was measured and then, dissolved in 100ml of distilled water so as to have a 28% concentration.

$$28\%\ \text{concentration} = \frac{28\text{g of snake tomato}}{100\text{ml of distilled water}}$$

Anaesthesia
With the aid of 1ml syringe, 0.5ml of ketamine and 0.5ml of diazepam were mixed. Calculated dose per body weight of the mixture of ketamine and diazepam were administered to each wistar rat.

Infliction of Wound
When the animal attained a subconscious state, it was brought out the cage and placed on the operating board. The animal was carefully shaved at the dorsolateral aspect of the abdomen using a very sharp razor. A 2cm by 2cm square transparent sheet was placed on the shaved part and marked with a felt pen. With the help of a forcep a 2cm by 2cm square wound size was inflicted on the skin following the trace of the felt pen on the skin of the shaved region.

Measurement of Wound Size and Application of Tomatoe. The wound size was measured by placing a 4cm by 4cm transparent sheet on the wound and traced out using a marker. The traced portion on the 4cm by 4cm transparent sheet was placed on a graph sheet. The number of small square boxes within the traced margin were counted and recorded. Each square box measures 0.04cm^2. The total number of square boxes were multiplied by 0.04cm^2 which gave a value equal to the size of the inflicted wound. Already prepared 28% concentrated snake tomato was applied on the inflicted wounds of the 12 experimental animal every three (3) day interval.

Assessment of Wound Contraction
At three days interval, a transparent sheet of about 4cm by 4cm was placed on the wound after the removal of the plaster and gauze. This was followed by the marking of the size of the wound on the transparent sheet using a felt pen. The wound size, represented by the marked part was read by counting the number of square of the graph sheet that fall within the circumference wound size and contraction assessment was done on days zero, three, six, nine, twelve, fifteen.

Histological Analysis of Tissue at Day 3 and 9
Granulation tissues were collected at day 3 and 9 from four wistar rats each in both the experimental and control group. The granulation tissues were then processed using routine tissue processing technique.

Statistical Analysis: Chi square test analysis was done using Statistical Package for the Social Sciences (SPSS 20.0 version).

RESULTS
In table 1 the control and experimental groups showed that on day three (3), animals treated with snake tomato (Trichosanthes Cucumerina) had a percentage mean wound contraction of 51.69±11.64. While those treated with distilled water had a percentage mean contraction of 9.73± 5.01.

In table 2 the rat wounds treated with snake tomato on the day six had a percentage mean wound contraction of

70.75± 9.10. While those treated with distilled water had a percentage mean wound contraction of 38. 8±8.33.

In table 3 day nine (9) the wounds treated with snake tomato had a percentage mean wound contraction of 87.1±4.43 while those treated with distilled water had a percentage mean wound contraction of 57.31±9.22.

In table 4 the wounds treated with snake tomato on day twelve (12) had a percentage mean wound contraction of 97.98±0.993 while those treated with distilled water had a percentage mean wound contraction of 91. 09±2.14.

In table 5 Percentage (%) wound contraction (wc) for Experimental was insignificant and Control Groups was 100% at Day 21.

In table 6 the rats treated with snake tomato had an average wound healing (closure) day of 15.00±0.00 while those treated with distilled water had an average wound heading (closure) day of 18.75± 1.49.

DISCUSSIONS

A comparism of the control and experimental groups of wound contraction at day three (3), six (6) and day nine (9) showed significant differences. A mathematical analysis of % mean wound contraction ratio for the experimental and control animals at the various days showed that the ratio of the wound contraction for experimental animal to the control at day 3 was 5: 1 meaning that the wound contraction for the experimental animals were five times that of the control animals.

In addition, the analysis showed that the ratio of wound contraction at day 6 for the experimental and control animals were 2:1, meaning that the rate of wound contraction for the experimental animals were twice that of the control animals at day 6.

Similarly at day 9, the result indicated that the wound contraction ratio for the experimental animals to that of the control animals were 3:2 meaning that every 3 times wound contraction for the experimental animals, the control animals contracted twice.

The wound healing day of the control and experimental groups showed no significant difference. However, the probability of wound contraction were statistical significant at day three, six, nine and twelve at $p<0.05$. Also probability of complete wound healing (closure) day was statistically insignificant at $P<0.05$. It may be assumed that the ratio of the wound contraction for the experimental group to that of the control group could be attributed to the large quantity of vitamin C in the snake tomato The result of this present study agree with the reports by previous authors.[5,6,7,8]

Furthermore, a histological analysis of the tissue slides with the aid of a photomicrograph microscope showed that certain types of white blood cells were present in large numbers than the others at specific days of the wound healing (contraction). At day 3, during the Inflammatory phase the neutrophils and the macrophages were more in number than the fibroblast and blood vessel; these have been suggested to be a result of the role of the neutrophil and macrophage in wound contraction. The result of this present study is in synergy with the reports by previous authors.[9-11]

In addition, a comparism of the tissues at day 3, between the experimental animals and control animals showed that the experimental animals had more of macrophage than the neutrophils whereas, the control animals had more of neutrophils than the macrophage. These suggests the fact that the experimental animals had a faster wound contraction at day 3 than the control animals and all these points to one direction that the snake tomato accelerated the process of wound contraction in the experimental animals at day 3. The result of this present study agree with the reports by previous authors.[12-15]

Similarly, at day 9, the experimental animals had more of the fibroblast and blood vessels than the control animals, the reason being that the snake tomato administered to the surface of the wound on the experimental animals facilitated the contraction of the wounds while those of the control groups had a very slow contraction rate as a result of the minute number of the fibroblast and blood vessels present at day 9. The result of this present study agree with the reports by previous authors.[12-15]

CONCLUSION

The snake tomato (Trichosanthes Cucumerina) has being found to accelerate wound healing with respect to a statistical significance percentage wound contraction especially on day three, six, nine and twelve (3,6,9,12) respectively.

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Table 1: Percentage (%)Wound Contraction(WC) for Experimental and Control Groups at Day 3.

S/N	% WC Experimental Group	% WC Control Group	Experimental Group		Control Group	
			Mean	STD	Mean	STD
1	56.00	12.90	51.69	±11.64	9.73	±5.015
2	32.28	6.29				
3	49.58	18.33				
4	58.33	3.25				
5	52.00	4.76				
6	33.33	11.60				
7	43.09	18.85				
8	54.03	8.80				
9	75.20	10.93				
10	50.83	6.34				
11	58.67	7.03				
12	57.03	7.69				

P<0.05, Significant.

Table 2: Percentage (%) Wound Contraction(WC) for Experimental and Control Groups at Day 6.

S/N	% WC Experimental Group	% WC Control Group	Experimental Group		Control Group	
			Mean	STD	Mean	STD
1	71.20	35.48	70.75	±9.10	38.80	±8.33
2	67.71	38.58				
3	85.12	40.00				
4	77.68	38.36				
5	76.00	46.03				
6	54.76	52.00				
7	65.85	36.88				
8	67.74	23.20				

P<0.05, Insignificant.

Table 3: Percentage (%) Wound Contraction(WC) for Experimental and Control Groups at Day 9.

S/N	% WC Experimental Group	% WC Control Group	Experimental Group		Control Group	
			Mean	**STD**	**Mean**	**STD**
1	84.00	43.54	87.10	±4.43	57.31	± 9.22
2	82.67	60.62				
3	91.73	62.50				
4	90.00	62.60				

P<0.05, Significant.

Table 4: Percentage (%) Wound Contraction (WC) for Experimental and Control Groups at Day 12.

S/N	% WC Experimental Group	% WC Control Group	Experimental Group		Control Group	
			Mean	**STD**	**Mean**	**STD**
1	97.60	87.90	97.98	±0.99	91.09	± 2.14
2	96.85	92.12				
3	99.17	92.50				
4	98.33	91.86				

P<0.05, Significant.

Table 5: Percentage (%) Wound Contraction (WC) for Experimental and Control Groups at Day 21.

S/N	% WC Experimental Group	% WC Control Group
1	Insignificant	100
2	Insignificant	100
3	Insignificant	100
4	Insignificant	100

P<0.05,

Table 6: Complete Wound Healing(Closure) Days for Experimental and Control Groups.

S/N	% WC Experimental Group	% WC Control Group	Experimental Group		Control Group	
			Mean	STD	Mean	STD
1	15	21	15	*Insig*	18.75	± 1.49
2	15	18				
3	15	18				
4	15	18				

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