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THE EFFECT OF ETHANOLIC SEED EXTRACT OF *SOLANUM LYCOPERSICUM* ON WOUND HEALING

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ABSTRACT

Background: A wound is a trauma to any of the tissues of the body especially that caused by physical means with interruption to continuity which normally heals rapidly without difficulty. The gross wound morphometry and wound healing activity of ethanolic extract of Solanum lycopersicum seeds were studied. **Materials and Methods:** The ethanolic extract of Solanum lycopersicum seeds were prepared using 42.38g of blended S. lycopersicum seeds after drying. 20 male wistar rats separated into two groups; experimental and control (of 10 each) was used in this research. A wound size of 2cm by 2cm which exposed the panniculus adiposus was inflicted on the right dorso-lateral shaved aspect of the thorax after anaesthetizing. The wound sizes were immediately measured using a 4cm by 4cm template of transparent sheet and placed on a graph sheet for counting of the small blocks. The experimental group were administered the extract while control group had distilled water. **Results and Discussion**: The percentage mean wound contraction on day 3, 6, 9 and 12 for the control group were 18.41 $\pm$ 9.86, 42.27 $\pm$ 12.39, 73.63 $\pm$ 11.89 and 81.23$\pm$7.34 respectively, while for the experimental group on day 3, 6, 9 and 12 were 22.35 $\pm$8.65, 57.11 $\pm$ 12.33, 87.50 $\pm$ 3.84 and 95.58 $\pm$ 3.14 respectively. Comparing the two groups for the respective days, day 3 and 9 showed no statistical difference, while day 6 and 12 were found to be statistically significant ($P<0.05$). The mean wound closure day for the experimental and control groups were approximately day 15 and 21 respectively. **Conclusion:** Solanum lycopersicum seeds have been found to accelerate wound healing by facilitating wound contraction and promoting quicker wound closure day.

KEYWORDS: Wound, Contraction, *Solanum, Lycopersicum*, Morphometry.

INTRODUCTION

Animals and humans sustain tissue injuries resulting to wound problems. For many centuries, most wounds have occurred due to trauma or war injury. The time taken for any wound to heal (closure) may be dependent on the size of the wound, extent of damage on the site of injury or the efficacy of the therapeutic drug used in the treatment of such a wound.[1]

Wound Healing

Wound healing is a complex process — in simple terms; it refers to the replacing of new tissues to injured tissues by the body demanding an increased consumption of energy. A wound causes a number of changes that can affect healing processes.[2-10]

Wound healing is a biological process that begins with trauma and ends with a scar formation. It could be defined as an intricate process in which the skin (or another organ) repairs itself after injury.[11-12] It is also the body's natural process of regenerating dermal and epidermal tissues.[13] Wound healing processes are well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a special manner. Healing of wounds involves the activity of an intricate network of blood cells, cytokines and growth factors which ultimately leads to the restoration to normal condition of the injured skin or tissue.[14-20] Once the protective barrier (skin) is broken, the normal (physiologic) process of wound healing is immediately set in motion.

Phases of Wound Healing

For a wound to heal successfully, all phases must occur in proper sequence and lime frame.[21]

As a normal biological process in the human body, wound healing is achieved through three (some authors divide it into four by splitting haemostasis from inflammatory phase) precisely and highly programmed phases.

1. Inflammatory or lag phase

It is the first phase of wound repair that begins at the time of injury and lasts for 2 -5days. This inflammatory response depends on the depth and volumes of tissue lose from the injury.[22] This phase begins with haemostasis and 'platelets plug formation.[23]

(a) Haemostasis — once a wound occurs, the blood vessels in the wound bed contract leading to bleeding, and the collagen of the blood vessel contracts, thus stimulates the platelets to coagulate forming a clot. In wound healing, these cells (platelets) act as "utility workers" to seal off the damaged blood vessels.

(b) Inflammation — this occurs immediately after haemostasis. This step usually last up to 4days post injury and appears as erythema (redness of the skin due to capillary dilatation), swelling and warmth often associated with pain, the classic "*rubor el tumor cum calore et dolore"*.[24]

The substances and factors released by the platelets necessitate the attraction of phagocytic cells (neutrophils, macrophages and basophils). These inflammatory cells and connective tissues (fibroblast) are parameters used in the identification of wound healing processes.

2. Proliferative Phase

This is the second phase of repair, it's also called granulation stage and it starts at about 4 days after wound infliction and usually last until day 21.[25] It is characterized by the following steps;

- Angiogenesis — the pericytes act as 'sub-contractors', these 'plumber' cells (pericytes) regenerate the outer layers of capillaries and the endothelial cells which produce the lining. This process is called angiogenesis. [25] Angiogenesis occurs concurrently with fibroblast proliferation when endothelial cells migrate to the wound site. The tissue in which angiogenesis has occurred typically appears red (erythematous) due to the presence of capillaries.[26]
- Fibroplasia and Granulation — occurs simultaneously with angiogenesis. Here, the fibroblast begin accumulating in the wound site, they enter the wound site 2- 5 days after wounding (just as the inflammatory phase is ending) and their number increases at 1-2weeks post-wounding and they become the main cells at the end of the first week.

3. Remodelling Phase

This is the final phase which occurs once the wound has closed. It involves remodeling of collagen from type III to type I in the ratio 4:1.[27] During maturation, type III collagen which is prevalent during proliferation is gradually degraded and replaced by the stronger type I collagen. The maturation/remodelling phase can last for a year or longer depending on the size of the wound whether it was initially closed or left open.

Wound Contraction

Wound contraction is the centripetal movement of the edges of a wound that facilitates closure. It involves a complex and superbly orchestrated interaction of cells, extracellular matrix and cytokines.[28] Contraction begins approximately a week after wounding when fibroblasts have differentiated into myofibroblasts. In full thickness, wounds, contraction peaks at 5-15 days post-wounding.[29] It is possible that contraction can last for several weeks and continuous after the wound is re-epithelised completely.

Wound closure or healing may be either by primary, secondary or tertiary healing intensions.

Solanum Lycopersicum

The genus solanum lycopesicum refers to tomato plant or the edible red fruit which it bears. Its origin is tracked back to the early Aztecs of central Mexico around 700A.D. it is believed that the tomato is a native of western south America and Central America. The word 'tomato' comes from the Nahuati word-tomati, literally meaning 'swelling fruit'. The tomato belongs to the nightshade family (solanaceae) whose plant typically grows to 1-3 meters (3-loft) in height with a weak stem that often spreads over the ground.

Tomato (*Solamum lycopersicum*) is one of the most widely cultivated vegetable crops. There is a particular interest in tomato so-called "Mediterranean diet" which has recently been associated with a healthier life style. Tomato plays an important role in maintaining health and vigour and is helpful in wound healing as a result of ripe fruit possessing anti-oxidant properties such as carotenoid lycopene, different polypherols, vitamin B and vitamin C (which synthesize mucopolysacharides of basement membrane of epithelial tissues, collagen and also necessary for wound healing.[30-34]

There are a few works on wound healing by some authors.[35-50]

AIM AND OBJECTIVES OF THE STUDY

This research is aimed at demonstrating the wound healing ability of the ethanolic seed extract of Solanum lycopersicum with respect to;

i. Rate of wound contraction
ii. Ascertaining the mean day of complete wound healing (closure)

Scope of Study

This study will be limited to wound morphometry.

MATERIALS AND METHOD

Research Design: This is an experimental and descriptive study.

Procurement of Animals

Twenty (20) male wistar rats weighing about 170g were acquired and brought to the Histology laboratory of the

Department of Human Anatomy, University of Port Harcourt. The animals were afterward separated into two groups of ten animals each by random selection. The two groups were tagged experimental and control groups respectively. The animals were housed two per cage and thereafter allowed to acclimatize for a period of two weeks so as to allow them adapt to their new environment. Throughout the acclimatization and research period, the animals were adequately fed with growers mash and drinking water.

The Preparation of Ethanolic Extract of Solanum Lycopersicum

Fresh ripe *Solanum lycopersicum* was acquired from a fruit store in Choba market, Obio/ Akpor LGA in Rivers State. The seeds were separated from the pulp and skin by water floatation which allowed the seeds sink to the bottom. The seeds were collected, washed with tap water severally and then dried in an oven at 50°c and grinded using a blender and the powder was packaged in a polyethylene bag and kept until use. The blended S. lycopersicum seeds weighed 42.38g. Using a measuring cylinder, 423m1 of absolute ethanol was added to the 42.38g blended Solanum lycopersicum seeds which were allowed to ferment for a period of 3 days in an air right beaker at room temperature and periodically rotated. The mixture was then filtered using filter papers, a funnel and a conical flask. The filtrate showed a pale yellow colouration.

With the aid of a water bath with a thermometer, the filtrate was heated at a temperature range of about 40 — 50°c to allow the evaporation of ethanol until a paste-like yellowish-brown substance was obtained. This yellowish-brown extract was stored in a refrigerator until administered.

Infliction of Wound

At the end of the acclimatization period (2 weeks), the animals were weighed again and their weights fell within a range of 225-300g. Prior to wound infliction, the instruments and dissecting table used were thoroughly sterilized to avoid contamination of any sort.

To reduce pain and place the animals in a subconscious state during the period of wound infliction and wound size measurement, an anaesthetic and a sedative-ketamin and Diazepam were used respectively. Using a normal l ml syringe with an insulin needle, a calculated dose of Ketamin and Diazepam per body weight was administered to each of the animals intraperitoneally.

After the animal had attained a subconscious state, one animal at a time was placed on the dissecting table with the fur shaved on the right dorso-lateral aspect of the thorax using a sharp razor. The skin was thereafter cleaned using methylated spirit. With a pair of scissors a 2cm by 2cm square template was cut out of the transparent sheet, sterilized and placed on the shaved area of the animal and then traced on the skin using a felt pen. Using a scalpel, forceps and a surgical blade, a wound size measuring 2cm by 2cm was made on the animal following the traced line thus exposing the Panniculus adiposus.

Wound Size Measurement and Dressing

The measurement of the wound size was done by placing a 4cm by 4cm square transparent sheet on the wound surface. Using a marker, the shape and extent of the wound was traced on the transparent sheet which was then placed on a graph sheet. The number of blocks that falls within the marked area were counted and then multiplied by $0.04cm^2$ since each small block of a graph sheet is equivalent to $0.04cm^2$. However, each small block not up to half within the marked area was not counted.

After measurement of the wound size, the wound was then dressed for both the Experimental and Control groups. For the experimental group, the ethanolic extract of *Solanum lycopersicum* seeds was topically applied on the wound surface. The wound was then covered with sterilized gauze and a plaster was used to firmly secure both the therapeutic agent and the sterilized gauze. For the control group, same procedure was carried out except that distilled water was used in place of ethanolic extract of S. *lycopersicum* seeds.

Wound Contraction Assessment and Tissue Excision

The wound contraction assessment was done every three days after the plaster was removed. The assessment of wound contraction was done in line with the procedure of wound size measurement after which the wound was dressed. On every third day, the wound contraction rate and percentage wound contraction was determined by using the formula below:

$$\frac{Wound\ size\ at\ day\ zero(0) - wound\ size\ on\ the\ given\ day}{Wound\ size\ at\ day\ zero(0)} \times \frac{100}{1}$$

On day 6, the granulation tissue of the healing wound surface of three (3) animals each from both the experimental and control groups were excised using a surgical blade.

The same procedure was repeated on another three (3) different animals of each group on day nine (9). Therefore, the remaining animals were left until complete wound closure was attained.

RESULTS

Table 1a: Complete Wound Healing (Closure) Day for Experimental and Control.

ANIMALS	EXPERIMENTAL	CONTROL
7	15	21
8	15	18
9	12	21
10	15	18

Table 1b: Mean and Standard Deviation for Complete Wound Healing (Closure) Day for Experimental and Control.

	EXPERIMENTAL	CONTROL
MEAN	14.25	19.50
STANDARD DEVIATION	1.50	1.73

t=4.583, df= 6, P<0.05, (P=0.0038: Statistically significant).

DISCUSSION

Wound healing processes are well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a special manner. Wound contraction is the process of shrinkage of the area of wound. It is a process that occurs throughout the healing process and the primary physical evidence of wound healing.[35-50]

From the analysis of the data obtained from this study, the percentage mean wound contraction of control group treated with distilled water on day 3, 6, 9, and 12 were 18.41 ± 9.86, 42.27 ± 12.39, 73.63 ± 11.89 and 81.23 ± 7.34 respectively. Those treated with ethanolic extract of Solanum lycopersicum seeds on day 3, 6, 9 and 12 were also 22.35 ± 8.65, 57.00 ± 13.33, 87.50 ± 3.84 and 95.58 ± 3.14 respectively. On day 3 and 9, there was no significant difference between the groups, thus ethanolic extract of Solanum lycopersicum tested insignificant. However, the values for day 6 and 12 tested to be significant using students T-test at 95% confident level ($p<0.05$).

The wound-healing models revealed that the both groups showed increased mean wound contraction from day to day. However, on the 12th post wounding day, the control group animals showed 81.23% closure (healing), whereas the experimental group animals showed 95.5 8% healing. This explains that the faster wound contraction rate of the experimental group may be as a result of the topically applied ethanolic extract of Solanum lycopersicum seeds.

It was observed also that the complete wound closure day for both the control and experimental groups had an average (mean) of 19.50 + 1.73 and 14.25 ± 1.50 respectively. The p value was 0.0038. This was shown to be statistically significant at 95% confident level ($p<0.05$). Thus, it could be inferred that the application of S. lycopersicum seeds extract shortened the time of complete wound closure day and epithelialization period for the experimental group to on or before day 15 compared to the control group treated with distilled water with epithelialization period of 21 days. This almost corresponds to study carried out by other authors[35,50] on The Wound healing activity of Crinum zeylanicum L. (Amaryllidaceae) who had mean healing days for control group as 21.75 days and experimental groups with 20.40, 17.60 and 14.4 days (1%, 5% and 10% concentration) respectively.

In comparison with respect to the basis of the cells that play critical roles in wound healing, inflammatory cells were intense in both groups while fibroblasts and blood vessels were low on day 6. This agrees with the findings of stated by previous authors.[35-50]

At day 9, it was observed that the number of inflammatory cells reduced while fibroblasts and blood vessels became dominant. This is in line with the study of Adam and Richard.[1] On the days of complete wound closure, the two groups showed increased keratinization, blood vessels (which were more in the experimental group), fibrosis and remodeling of collagen. This corresponds to the study of Allen.[2]

It is worthy to note that throughout the period of wound treatment, the extract did not cause irritation or pain to the animals as the rats neither showed any signs of restlessness nor scratching/biting of wound site or any toxic effect that could probably be attributed to the irritant effect of the extract. This is important and researchers have proved that the control of microbial infection is necessary for better healing and its management.[35-50]

Between the control and experimental groups, Solanum lycopersicum was found to possess wound healing activity as it facilitated wound contraction faster as well as promoting faster wound closure.

CONCLUSION

This research revealed that the ethanolic extract of Solanum lycopersicum seeds has been found to accelerate wound closure at a moderate statistical significance which could be attributed to its vitamin C and antioxidant properties such as lycopene.

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CONFLICT OF INTEREST

We write to state that there is no conflict of interest.

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