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Original Research Article

The Effect of Hydro- Extract of the Seed of *Lycopersicion esculentum* (Tomato) on Wound Healing Using Wistar Rats

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Abstract

Background: The word "wound" is as old as man's creation. Wound can be defined as an injury to the body in which the skin, tissue or organ is broken by some external force such as a blow or surgical incision, with damage to the underlying tissue. *AIM:* This study was aimed at evaluating Wound Morphometry with respect to wound size and rate of wound contraction using tomatoe seed. ***Materials and Methods:*** The Solanum lycopersicum seeds were prepared using 28.0g of blended S. lycopersicum seeds after drying. 24 male wistar rats separated into two groups; experimental and control (of 12 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 seed extract while control group was merely dressed without seed extract. ***Results and Disccusion:*** The rats treated with L. esculentum (experimental) had a mean wound healing (closure) day of 15.75 ± 1.49 while those treated without seed extract had a mean wound healing (closure) day of 19.50 ± 1.73. ***Conclusion:*** The seed of Cherry tomato (Lycopersicon Esculentum) was concluded to have accelerating contracting/ healing (closure) effect with respect to the statistical significant mean value on wound healing (closure) day.
Keywords: Seed; Contraction; Closure; Wound and *Lycopersicon Esculentum*.

INTRODUCTION

The word "wound" is as old as man's creation. Wound can be defined as an injury to the body in which the skin, tissue or organ is broken by some external force such as a blow or surgical incision, with damage to the underlying tissue. It can also be defined as an emotional injury or a damage to plant tissue caused by an external agent such as wind or frost [1]. Wounds could be classified as acute and chronic [2]. They have also been identified as incised, puncture, lacerated and contused wounds [3].

However Wound healing, or wound repair, is an intricate process in which the skin (or another organ) repairs itself after injury [3]. In normal skin, the epidermis (outermost layer) and dermis (inner or deeper layer) exists in steady-state equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, the normal (physiologic) process of wound healing is immediately set in motion [3].

Phases of wound healing

Hemostasis and Inflammatory Phase

Just before the inflammatory phase is initiated, the clotting cascade takes place in order to obtain hemostasis, or stop blood loss by way of a fibrin clot. Tissue trauma stimulates the inflammatory response. Immediately after injury intense local vasoconstriction occurs, mediated by circulating catecholamines and prostaglandins released by injured cells [4].

Clotting Cascade

Fibrin and fibronectin cross-link together and form a plug that traps proteins and particles and prevents further blood loss. This fibrin-fibronectin plug is also the main structural support for the wound until collagen is deposited. Migratory cells use this plug as a matrix to crawl across, and platelets adhere to it and secrete factors. The clot is eventually lysed and replaced with granulation tissue and then later with collagen [5, 6].

Platelets

The highest number of cells present shortly after a wound occurs, is the platelets. They release

ECM proteins and cytokines, including growth factors. Growth factors stimulate cells to speed their rate of division. They also release other proinflammatory factors like serotonin, bradykinin, prostaglandins, prostacyclins, thromboxane, and histamine, which serve a number of purposes, including to increase cell proliferation and migration to the area and to cause blood vessels to become dilated and porous [7, 8].

Vasoconstriction and vasodilation

Vasoconstriction lasts five to ten minutes and is followed by vasodilation, a widening of blood vessels, which peaks at about 20 minutes post-wounding. Vasodilation is the result of factors released by platelets and other cells. The main factor involved in causing vasodilation is histamine. Histamine also causes blood vessels to become porous, allowing the tissue to become edematous because proteins from the bloodstream leak into the extravascular space, which increases its osmolar load and draws water into the area [9, 10].

Polymorphonuclear Neutrophils

Within an hour of wounding, polymorphonuclear neutrophils (PMNs) arrive at the wound site and become the predominant cells in the wound for the first two days after the injury occurs, with especially high numbers on the second day. Neutrophils phagocytise debris and bacteria and also kill bacteria by releasing free radicals in what is called a 'respiratory burst'. They also cleanse the wound by secreting proteases that break down damaged tissue. Neutrophils usually undergo apoptosis once they have completed their tasks and are engulfed and degraded by macrophages [9, 10].

Macrophages

Macrophages are essential to wound healing. They replace PMNs as the predominant cells in the wound by two days after injury. They are attracted to the wound site by growth factors released by platelets and other cells, monocytes from the bloodstream enter the area through blood vessel walls. The main role of the macrophage is to phagocytize bacteria and damaged tissue, debride damaged tissue by releasing proteases, and secrete a number of factors such as growth factors and other cytokines, especially during the third and fourth post-wounding days [11]. Decline of inflammatory phase inflammation plays roles in fighting infection, clearing debris and inducing the proliferation phase, it is a necessary part of healing. However, inflammation can lead to tissue damage if it lasts too long. Thus the reduction of inflammation is frequently a goal in therapeutic settings. Inflammation lasts as long as there is debris in the wound. Thus the presence of dirt or other objects can extend the inflammatory phase for too long, leading to a chronic wound [12].

Proliferative Phase

The onset of the proliferative phase is marked by the entering of fibroblasts into wound site, about two or three days after injury occurs, sometimes even before the end of the inflammatory phase. The proliferative phase is also called the reconstruction phase.

Angiogenesis

Angiogenesis is stimulated by raised lactate levels, decreased PH and tissue hypoxia, capillaries sprout from existing venules. These capillary sprouts grow by proliferation of endothelial cells and in primarily closed wounds. The sprouting vessels soon meet their counter parts from the other side of the wound thus re-establishing blood flow across the wound. In unclosed wounds, the new capillaries fuse only with neighbours migrating in the same direction and so forming granulation tissue cytokines including FGF, TGF-a, EFG, TGF-b, PDECGF, VEGF, angiogenin, interlukin-8, wound fluids, P.Gs and adipocytes lipids stimulate angiogenesis [13].

Fibroplasia and Granulation Tissue Formation

During Granulation, fibroblasts lay bed of collagen, fills defects and produces new capillaries Tamara, 1995. Granulation tissue functions as rudimentary tissue, and begins to appear in the wound already during the inflammatory phase; two to five days post wounding, and continues growing until the wound bed is covered. Granulation tissue consists of new blood vessels, fibroblasts, inflammatory cells, endothelial cells, myofibroblasts, and the components of a new, provisional extracellular matrix (ECM) [14].

Collagen Deposition

One of fibroblasts' most important duties is the production of collagen. Collagen production continues rapidly for two to four weeks, after which its destruction matches its production and so its growth levels off. Collagen deposition is important because it increases the strength of the wound; before it is laid down, the only thing holding the wound closed is the fibrin-fibronectin clot, which does not provide much resistance to traumatic injury [15].

Epithelialization

Epithelialization alone can provide total healing in partial thickness wounds. Incisional wounds are usually completely re-epithelized in 24-48hrs. Epithelialization can be divided into cell differentiation, mitosis, migration and proliferation, which begins within hours of injury and results in resurfacing any denuded area. Thickening of the basal cell layer at the wound edge is the earliest aspect of epithelialization [16]. In epithelialization, cell travel about 3cm from point of origin in all directions, Epithelialization crosses moist surface [16].

Contraction

Contraction is a key phase of wound healing. If contraction continues for too long, it can lead to disfigurement and loss of function [17]. Contraction is simply the pulling together of wound edges to reduce defect [17]. Contraction starts 4-5 days after injury, and is represented by centripetal movement of wound edges towards the centre of the wound. The average rate of wound contraction is 0.6-0.75mm/day. Myofibroblasts in the injured area are thought to be responsible for wound contraction [17].

Maturation and Remodeling

The remodeling phase last for 3 weeks to 2 years. In this phase, new collagen forms which increases tensile strength to wounds and the scar tissue is only 80% as strong as original tissue [16]. When the levels of collagen production and degradation equalize, the maturation phase of tissue repair is said to have begun. During maturation, type III collagen, which is prevalent during proliferation, is gradually degraded and the stronger type I collagen is laid down in its place [16].

The age long phenomenon of wound healing is attributed to the use of crude wound dressing agents on wounds by the Ancient Egyptian Physicians and other Physicians in other parts of the world [17].

Types and forms of wound healing

There are three (3) major types or forms of wound healing: Primary or First intention healing:

The Primary or first intention healing occurs when tissue is cleanly incised and re-approximated and healing occurs without complications. The incision defect re-epithelizes rapidly and matrix deposition seals the defect [18].

The Secondary or second intention healing occurs in open wounds. When the wound edges are not approximated and it heals with formation of granulation tissue, contraction and eventual spontaneous migration of epithelial cells [18]. The wound is allowed to granulate which results in a broader scar.

Tertiary or Third intention (delayed primary) healing occurs when a wound is allowed to heal open for a few days and then closed as if primarily. Such wounds are left open because of gross contamination [18].

Factors affecting wound healing

The following are the factors that affect wound healing: Age, Temperature and Wound dressing/Wound care

Age

Wounds heal faster in the young than in the old individuals. The wounds in young have increased vigour of repair and more likely to have hyper tropic scars – a raised, erythematous, pruritic lesion that remains within the confines of the original scar [18].

Temperature

Wounds on the exposed parts of the body heal more slowly in cold weather. Experiments on animals that hibernate support the observation that cold temperature inhibits healing [18].

Wound dressing/Wound care

Undisturbed wounds heal best and promote formation of new capillaries and cells. The hormone effect can be easily demonstrated in animals that are sensitive to those compounds. However, humans are relatively resistant and glucocorticoid does not apparently impair wound healing [18].

Lycopersicon esculentum (tomato)

The tomato is a savoury, typically red, edible fruit, as well as the plant (*Solanum Lycopersicum*) which bears it [23]. The tomato is a member of the *Solanaceae*, or Nightshade family [23]. The name that this fruit was given in various languages reflects some of the history and mystery surrounding it. *Lycopersicon* means “Wolf Peach” in Latin and refers to the former belief that, “like a wolf, this fruit is dangerous”. The French call it “Pomme d’amour”, meaning “love apple” since they believed it to have aphrodisiacal qualities, while the Italians call it “Pomodoro” or “golden apple”, owing to the fact that the first known species with which they were familiar may have been yellow in colour [23].

Fig-1: Cherry tomato fruits (Slideshare, 2018) [26]

Medicinal (healing) properties of tomatoes

Tomatoes constitute an excellent food for those who want to adopt a natural diet [23]. Tomato is a rich source of vitamins. The vitamins A, B and C contents in tomatoes are more than that of orange and grapes and also more useful than those fruits [23]. Vitamins in tomatoes are not destroyed even when heated or cooked. Tomato is also rich in iron, twice more than in eggs. Tomato is digestive, diuretic, invigorating, germicidal, appetizer, stimulates blood circulation and enriches red corpuscles in the blood. It gives energy and strength to the muscles of the heart and bones. It clears constipation and activates liver [23].

Tomato prevents/lowers the risks of cancer. People who ate tomatoes regularly have a reduced risk of contracting cancer diseases such as lung, prostate, stomach, cervical, breast, oral, colorectal, esophageal, pancreatic and many other types of cancer [23]. Tomatoes and garlic taken together, lycopene and the newly discovered bioflavonoid in tomatoes, raw tomatoes and also cooked or processed tomato products are responsible for cancer fighting agents [23].

Tomato seeds

Fig-2: The arrangement of tomato seeds in a slice of tomato (slideshare, 2018)

Varieties of tomato seeds

More than 10,000 varieties of tomato seeds exists [23]. Heirlooms alone have about 600 varieties. The other popular varieties are beefsteak, Brandywine, cherry and Roma. Beefsteak, Brandywine and Heirloom grow to be quite large yet still maintain a full flavor. Roma tomatoes are popular for pasta and pizza sauces as they are a pastier variety, and Cherry tomatoes are a popular choice for salads or snacks. Tomatoes are most often coloured red, but they may also be pink, yellow, orange or purple depending on the variety [23].

Features of tomato seeds

Each tomato seeds can yield one plant, which can yield at least 15 tomatoes. Tomato seeds can also be eaten by people. Unlike the seeds from other fruits (example, apples), tomato seeds are harmless to the body. Tomato seeds are located in an important area of the tomato. The thick gel-like material where the seeds grow is the most nutrient-dense part of the tomato [23].

The rat (rattus norvegicus)

White albino rats, Rattus Norvegicus are representatives of a major lineage of animals, the deuterosomes. Deuterosomes include such diverse organisms as starfish, sea urchins, and all vertebrates. Deuterosomes are classified together because all share a similar developmental pattern. Cleavage is radial, the anus forms before the mouth, and the mesodermal lined body cavity is formed from out-pockets of the gut [27].

Fig-3: Wistar rat [27]

Rats are homoeothermic and regulate their body temperature by generating heat through metabolic processes and controlling heat loss. Heat loss is reduced by a covering of hair and a layer of subcutaneous fat. Rats are also capable of regulating the amount of blood flowing to the skin, where heat is exchanged. They can also pant and sweat to help relieve heat build-up. Like other mammals young develop in a uterus and are nourished through the placenta. After birth nourishment is provided through the mammary glands [27].

There have works done on wound healing using different things including tomatoe to investigate rate of contraction, factors promoting or delaying wound healing [1-25].

Scope of study

This work is limited to Gross Morphometric study and Microscopic analysis of certain parameters such as white blood cells (fibroblast, macrophage and neutrophil) count and blood vessel count.

Aim of the study

This study was aimed at evaluating Wound Morphometry with respect to wound size and rate of wound contraction using the hydro-extract of the seed tomatoe.

In ancient times, till date, different methods and materials have been used in the treatment and in healing of wounds. Examples of these include the use of herbs, water, fire, modern therapeutic measures like the use of drugs though tomato and its seeds have multiple medicinal values including wound healing. As regards its wound healing value, much work has not been done to carry it out. Therefore in an attempt of exploring and contributing to knowledge as touching its wound healing medicinal value, the seeds of *lycopersicon esculentum* would be used in this research as the therapeutic option in treating incised wounds on wistar rats.

MATERIALS AND METHODS

Twenty-Four (24) male albino rats with weight ranging 120g to 180g were bought from the animal farm at the University of Nigeria, NSUKA and the animal farm at the University of Port Harcourt and brought into the Histology laboratory of the College of Health Science at the University of Port Harcourt. The animals were afterward separated into two groups of twelve 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 feed and drinking water.

Fresh tomatoes were acquired from Choba daily market, Port Harcourt, Rivers State and identified in the Department of Plant Science and Biotechnology. The seeds were extracted from the tomatoes using a manual sieve and blended with Keenwood electric blender. The blended seeds were heated using an electric heater to boiling temperature to remove excess water as to obtain a paste. Furthermore, twenty eight grams (28g) of the heated content of the L. esculentum was measured using a sensitive weighing balance and then added to 100ml of distilled water, so as to have a 28% concentration. Below is the equation for preparation:

Fig-4: Photographs showing the preparation of tomato seeds paste

$$\textbf{28\% concentrations} = \frac{\textbf{28g of L.esculentum seeds}}{\textbf{100ml of distilled water}}$$

Infliction of Wound

The experimental animals were brought to a subconscious state via application of 1ml syringe, 0.5ml of ketamine mixed with 0.5ml of diazepam. Calculated dose per body weight of the mixture of ketamine and diazepam were administered to each experimental animals following the standard procedure. The animals were brought out the cage and placed on the operating board, 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. This gave a square shape on the hairless skin. The marked area of the skin was excised using a surgical blade, leaving the paniculus adiposus still intact.

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 was 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.

Fig-5: The process of wound infliction

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.

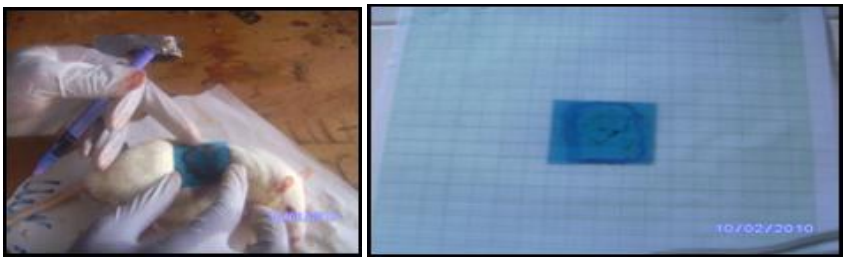

Fig-6: Wound measurement

Table-1: Complete wound healing (Closure) day for control group (C) and experimental group (E)

S/N	Wound closure Day for Control Group	Wound Closure for Experimental Group
1	18	15
2	21	15
3	18	18
4	21	15

Table-2: Mean ($\bar{X}$) and SD of Complete Wound healing (closure) day for control group (C) and experimental group (E)

Statistics	Control	Experimental
Mean ($\bar{x}$)	19.50	15.75
S.D	1.73	1.49

P-Value ($P < 0.05$), Significant

RESULTS

The results obtained during wound healing (closure) experiment are presented in this chapter.

DISCUSSION

The result of this study showed that the experimental group (E) had a mean wound healing (closure) day of 19.50 ± 1.73 while the control (E) had mean wound healing (closure) day of 15.75 ± 1.49. It goes on to indicate that the wound of the experiment group closed earlier than the control group. It also indicated that the wound contraction rate was higher (2:1) in the experimental group (E) than the control (C). The difference in the wound closure day between the experimental and control were seen to be statistically significant ($P<0.05$).

Edibamode *et al.*, [24] reported in their study a similar result when they stated that the mean wound closure day for the experimental and control groups were approximately days 15 and 21 respectively. This indicates that the experimental group had their wounds closed several days ahead of that of the control. This confirms the result of this present study and suggests that tomotoe in its constituent has wound healing effect depicted by the significant difference ($P<0.05$) in wound closure day between the experimental and control groups. We speculate that the difference in wound contraction for the experimental group may be attributed to the large quantity of vitamin C in the Cherry Tomato and its seed.

The result of this present study again confirms the report of the study done by Paul *et al.*, [25] where they stated that the mean healing (closure) day for the

control group was 18.75±1.49 and while that of the experimental group was 15.00±0.00. The difference in the wound closure day was three days, meaning that the experimental group had their wound closed three days ahead of the control group.

CONCLUSION

It suggests that the seeds of Cherry tomato (*Lycopersicon Esculentum*) have the tendency to accelerate wound healing (closure) given the statistically significant difference in mean wound healing (closure) day between both groups.

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Conflict of interest

The authors declare that there is no Conflict of Interest.

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