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Research Article

A Comparative Study of the Wound Healing Role of *Lycopersicon esculentum* Fruit and Gentamicin Ointment on Male Wistar Rats

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Abstract: A wound is a trauma to any tissue of the body especially that which is caused by physical means and interruption of continuity which normally heals rapidly without difficulty. Wound healing is an intricate process in which the skin (or another organ) repairs itself. In the course of this study, 24 male wistar rats weighing approximately 175g were used in this research. The rats were separated into two groups with 12 animals each in the *Lycopersicon esculentum* fruit and the gentamicin groups. A wound size of 2cm by 2cm which exposed panniculus adiposus was inflicted on the dorsolateral shaved aspect of the thorax after anaesthetisa. The wound sizes were immediately measured using a 4cm by 4cm square template transparent sheet placed on a graph sheet. The wounds were then dressed with the *L. esculentum* fruit paste and gentamicin ointment. The result showed non statistical significant difference in the % percentage wound contraction excepting at day 8; the wound healing cells at day 8 and the wound closure day. This study has therefore revealed both therapeutic agents to promote wound healing activity.
Keywords: *Lycopersicon esculentum*, Re-epithelization, Wound contraction & fibroblast.

INTRODUCTION

Wound care is a major health care concern that affects many individuals with different types of wounds. Wounds have varying effects on the quality of life of those affected, their families and caregivers. Providing skin and wound care is a major common consideration in the day to day caring of patients with wounds whether in acute, long term or community based environments.

For the past two decades, many changes have occurred in the art of science on how wounds are managed. There has been great advancement in wound technology, research and development of sound policies and standards of care based on research and clinical evidence to achieve positive outcome in wound healing [1].

Our knowledge about wound healing mechanism in man is still incomplete. Valuable information has been obtained by comparing results from animal experiments with clinical observation of human wound healing after surgery and trauma [2].

Many similarities exist in the course of healing of connective tissue in different species of animals. It is not however possible to transfer completely the information from animal experiment to clinical conditions [2]. Substantial progress has been made in understanding the pathophysiology of wound healing and new therapeutic methods have been developed. Research has shown the rate of healing of skin defect and has demonstrated how younger patients' wounds heal faster than the old [3]. In developing countries including Nigeria, different crude drug preparations are used to treat wounds. Overtime, due to substantial progress made in understanding the pathophysiology of wound healing, new therapeutic procedures have been used. The therapeutic efficacies of many plants – vegetable, fruits and herbs for diverse diseases have been described by trado-medical practitioners [4].

It is with the aim of achieving perfect healing that various investigations had been carried out so as to identify various therapeutic options which may accelerate the wound healing process [5]. One of such therapeutic options documented to have been utilized for normal wound dressing by traditional medicine is *Lycopersicon* esculentum (tomato) leaves and fruits [6].

Literature reviews are available on the wound healing process [7-10]. However, literature review is rather scanty with regards to the scientific wound healing effects of *L. esculentum* on wounds.

Gentamicin Sulfate Ointment USP, 0.1% is a wide spectrum antibiotic preparation for topical application [11]. Each gram of Gentamicin Sulfate

Ointment USP, 0.1% contains 1.0mg of gentamicin in a base of white petrolatum with methylparaben and propylparaben [11]. It is active against a wide range of human bacterial infections, mostly gram-negative bacteria that include Pseudomonas, Proteus, Serratia, and the Gram-positive Staphylococcus [12].

Gentamicin sulphate ointment is used to treat minor burns/cuts/wounds in addition to its use in the treatment of minor primary skin infections (impetigo, folliculitis, psoriasis, sycosis barbae and pyoderma gangrenosum) and secondary skin infections (ezematoid dermatitis, pustular acne, pustular psoriasis).

Based on this, gentamicin ointment was then used as a standard therapeutic agent to test and compare the wound healing efficacy of *Lycopersicon esculentum* fruit.

Aim of Study

To compare the effect of *Lycopersicon esculentum* fruit (tomato) and gentamicin ointment on wound healing.

Study Objective

- To compare the percentage mean wound contraction of wounds treated with the different therapeutic agents at the different days
- To ascertain and compare the re-epithelization (wound closure) day of skin wound with respect to the different therapeutic agents.
- To analyze inflammatory cells such as neutrophil, macrophage and the connective tissue cell – fibroblast via cell count.
- To compare the histology of the re- epithelized skin to the normal skin with respect to the different therapeutic agents.

METHODOLOGY

Plant collection, identification and preparation

Lycopersicon essculentum fruit was procured from the fruit garden market port-Harcourt and immediately taken to the Department of Plant Science and Biotechnology of the University of Port Harcourt, Nigeria for identification and authentication. The fruits were identified and authenticated by Dr. N. L. Edwin–Wosu of same department with the species name as *Lycopersicon esculentum*, of the family of Solanaceae with identification number: UPH/P/058. All fruits were washed and the very good and healthy ones were selected and there after oven dried at 60^0C with the Gallen Kamp Hot box Oven. Oven- drying lasted for 48hours after which the dried fruits were then grounded using a blender. Fifty grams (50g) of the blended fruit was mixed with 100g of a formula known as the ointment base whose standard constituents are: white bees wax (2g), hard paraffin wax (3g), propylene glycol (5g), cestostearyl alcohol (5g) and white soft paraffin (85g).These different constituents were placed in beaker and heated using a heating mantle into a liquid form. While heating, the mixture was continuously stirred with a glass stirrer and thereafter allowed to cool.

Animal procurement and preparation

A total of 24 male adult wistar rats weighing between 150 to 200g were used for the study. Female rats were not used because of the hormonal variations associated with them. The animals were bought from the animal farm of the University of Nigeria, Nsuka and brought into the animal house of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt. The rats were divided into two groups of twelve rats:

Group A: tomato fruit group
Group B: Gentamicin ointment

For two weeks, the rats were kept in the animal house for acclimatization and were fed with pellet-Finisher feed and water *ad libatum* throughout the research period.

Anaesthesia

Using a 1ml syringe, a calculated dose per body of ketamine and diazepam were administered subcutaneously via the ventral part of the abdomen of the rats. The process of anesthetizing the rats was done whenever the wounds were to be measured and dressed as well.

Wound infliction

The dorsolateral aspect of the thoracic region of the skin were shaved and thereafter cleaned with methylated spirit. A 2cm by 2cm template sterilized sheet was placed on the shaved skin with its shape traced using a marker. The traced margin of skin (2cm by 2cm) was then excised together with the panniculus adiposus.

Wound morphometry and wound dressing

The wound sizes were measured using a sterilized 5cm by 5cm transparent plastic sheet placed on the skin wound with the wound margins traced using a marker. The traced wound margin was then placed on a graph sheet and the numbers of small blocks in each square block were counted. All small blocks up to half and above lying within the traced margin were also counted as one while those not up to half were not counted. The total numbers of blocks within the traced wound margin were multiplied by $0.04cm^2$ since each small block measures $0.04cm^2$. The outcome therefore gave the mathematical representation of the wound dimension or the wound area. Assessment of the wound sizes were done at every 2 day interval with the percentage wound contraction calculated at these intervals using the formular below [5]:

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

Wound dressing was done once every two days for the different groups using their respective therapeutic agents alongside sterilized gauze and adhesive zinc oxide plaster. Wound dressing continued until complete skin re-epithelialization was achieved with the days of wound closure noted.

The different therapeutic agents used for the respective groups of animals are:

Group A: was dressed using the paste of *Lycopersicon esculentum* fruit.

Group B: was dressed using gentamicin ointment

On day four and eight, four animals were randomly selected from each group for granulation tissue histological analysis with emphasis on neutrophil, macrophage, fibroblast cell counts. The remaining animals were left until complete skin re-epithelization (wound closure day) was achieved. Thereafter, the end scar tissues were harvested from the remaining four animals in each group for a comparative histological analysis.

Tissue processing

The granulation tissue excised on the 4th and 8th days was preserved in 10% formal saline (fixation) at room temperature prior to tissue processing. The healed skin scar was also excised and also processed as well using the routine tissue processing technique involving dehydration, clearing, impregnation, embedding, sectioning and staining.

Microscopy

All the tissues were viewed using a light microscope at 40 and 100 magnification. Macrophage, neutrophil and fibroblast were identified and estimated using oil immersion oil.

Histological analysis

Granulation tissues on days 4 and 8 were analyzed with emphasis on the inflammatory cells (neutrophils, macrophages) and the connective tissue cell- fibroblast using a software known as Motic Images Plus 2.0 Set Up as template. In order to eliminate bias the lap top screen measuring 35cm (Length) by 19.5cm (width) was used as the photomicrograph screen. The software was then superimposed on two photomicrographs per granulation tissue of 8 animals for day 4 and another 8 for day 8. Of the 8 animals each at day 4 and 8, neutrophils, macrophages and fibroblast were identified and counted. The superimposed software been placed on the photomicrograph was then divided into six equal rectangular frames measuring 212.0µm in width, 956.0µm in height, 202672.0 sq.µm in area and 2760.0µm in perimeter. In all, at day 4 and day 8, thirty two photomicrographs of granulation tissues were analyzed.

Gross morphometric analysis of wounds

The wound sizes and the % mean wound contraction per two day interval were determined as healing progresses using the formula:

$$\frac{\text{(Area of Wound at Day 0 - Area of Wound at a given day)}}{\text{Area of wound at Day 0}} \times 100$$

The mean wound healing day or wound closure in each of the group were noted. Wound closure was defined as the day at which the wound bed was completely re-epithelialized.

Statistical analysis

Statistical Analysis was done using Statistical Package for Social Scientist (SPSS) windows where necessary with comparison made among the measured parameters in the two different groups. Results were expressed as mean and standard deviation and were evaluated using student's T-test and the level of significance determined at $p< 0.05$.

From the sections of the granulation tissues, estimates of neutrophils, macrophage and fibroblasts at days 4 and 8 were compared.

RESULTS

Table 1: Mean values of the wound sizes in cm^2

Days	D0	D2	D4	D6	D8	D 10	D12	D14	D16
Therapuetic Agent									
L. esculentum fruit	4.83	4.23	3.05	1.98	1.01	0.45	0.18	0.14	00
Gentamicin	5.15	4.03	3.62	2.29	0.6	0.45	0.23	0.18	00

Table 2: T-test for two independent samples at 0.05 significance level of the % mean wound contraction using the different therapeutic agents at day 2

Therapeutic Agents	N	Mean ± S.D.	SEM	t (Obs)	|t| (Crit)	p-value	Inference
L. esculentum fruit	12	13.41±7.42	2.142	-1.819	2.074	0.083	Not Sig
Gentamicin	12	23.00±16.68	4.815				

Table 3: T-test for two independent samples at 0.05 significance level of the % mean wound contraction using the different therapeutic agents at day 4

Therapeutic Agents	N	Mean ± S.D.	SEM	t (Obs)	|t| (Crit)	p-value	Inference
L. esculentum fruit	12	37.13±9.803	2.830	0.737	2.074	0.469	Not Sig
Gentamicin	12	32.95±17.073	4.929				

Table 4: T-test for two independent samples at 0.05 significance level of the % mean wound contraction using the different therapeutic agents at day 6

Therapeutic Agents	N	Mean ± S.D.	SEM	t (Obs)	|t| (Crit)	p-value	Inference
L .esculentum fruit	12	59.94±11.424	3.298	-1.436	2.160	0.175	Not Sig
Gentamicin	12	67.23±7.500	2.165				

Table 5: T-test for two independent samples at 0.05 significance level of the % mean wound contraction using the different therapeutic agents at day 8

Therapeutic Agents	N	Mean ± S.D.	SEM	t (Obs)	|t| (Crit)	p-value	Inference
L. esculentum fruit	12	79.62±7.314	2.111	-3.749	2.145	0.002	Sig
Gentamicin	12	90.52±2.441	0.705				

Table 6: Mean day of wound re-epithelization (wound closure) involving the different therapeutic agents

Therapeutic Agents	Calculated Mean	Trend Line Mean
L. esculentum fruit	16	before day 12
Gentamicin	15.5	before day 12

Table 7: Test of proportionality difference in Neutrophil count of the *L. esculentum* and gentamicin groups at 0.05 sig level at day 4

Therapeutic agents	Observed population (n)	Total population (N)	Observed proportion	z-value (cal)	|z| crit	p-value (obs)	Inference
*L. esculentum f*ruit	148	223	0.157	6.627	1.96	< 0.0001	Significant difference in proportions
Gentamicin	23	90	0.253				

Table 8: Test of proportionality difference in Macrophage count of the *L. esculentum* and gentamicin groups at 0.05 sig level at day 4

Therapeutic agents	Observed population (n)	Total population (N)	Observed proportion	z-value (cal)	|z| crit	p-value (obs)	Inference
L. esculentum Fruit	35	223	0.157	-1.981	1.96	0.0475	Significant difference in proportions
Gentamicin	23	90	0.253				

Table 9: Test of proportionality difference in Fibroblast count of the the *L. esculentum* and gentamicin groups at 0.05 sig level at day 4

Therapeutic agents	observed population (n)	total population (N)	obsvd proportion	z-value (cal)	|z| crit	p-value (obs)	Inference
L. esculentum Fruit	36	223	0.160	-5.170	1.96	< 0.0001	Significant difference in proportions
Gentamicin	41	90	0.450				

Table 10: Test of proportionality difference in neutrophil cell count of the the *L. esculentum* and gentamicin groups at 0.05 sig level at day 8

Therapeutic agents	Observed population (n)	Total population (N)	Observed proportion	z-value (cal)	|z| crit	p-value (obs)	Inference
L. esculentu Fruit	6	76	0.079	-0.020	1.96	0.9838	No Significant difference in proportion
Gentamicin	4	50	0.080				

Table 11: Test of proportionality difference in macrophage cell count of the *L. esculentum* and gentamicin groups at 0.05 sig level at day

Therapeutic agents	Observed population (n)	Total population (N)	Observed proportion	z-value (cal)	|z| crit	p-value (obs)	Inference
L. eculentum Fruit	4	76	0.049	-0.709	1.96	0.4779	No Significant difference in proportions
Gentamicin	4	50	0.080				

Table 12: Test of proportionality difference in fibroblast cell count of the *L. esculentum* and gentamicin groups at 0.05 sig level at day 8

Therapeutic agents	Observed population (n)	Total population (N)	Observed proportion	z-value (cal)	|z| crit	p-value (obs)	Inference
L. eculentum Fruit	63	76	0.820	0.818	1.96	0.4134	No Significant difference in proportion
Gentamicin	38	50	0.760				

DISCUSSION

Wound healing is the process by which a damaged tissue is restored as closely as possible to its normal state. It also proceeds quickly and efficiently in a physiologic environment conducive to tissue regeneration and repair [13]. 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 [14].

It is important to note that throughout the period of wound treatment, the *Lycopersicon esculentum* paste did not cause irritation or pain to the animals as they neither show any signs of restlessness nor scratching/biting of wound site when the paste were applied. Care was taken to properly cover up the wound with sterile gauge and plaster so as to prevent them from biting off the wound or eating up themselves and probably getting their wounds infected. Researchers had proven that the control of microbial infections is necessary for better healing and its managements [15]. Many studies indicate that plant products are potential agents for wound healing and largely preferred because of the absence of unwanted side effects and their effectiveness. However, the efficacy of medicinal plants that are used for wound healing purpose may be due to their direct action on the wound repair processes, anti-inflammatory and antimicrobial effects or a combination of these effects [16]. Previous studies carried out on wound healing effects of medicinal plants by some researchers [17-19] have shown that these medicinal plants were effective in wound care, facilitate rapid wound healing with minimal pain and discomfort.

Table 1 shows the data of the mean wound size (measured in cm^2) of *Lycopersicon esculentum* and gentamicin treated group at days 0-16. The area of wound decreased as the days progresses.

Tables 2 - 5 show the percentage mean wound contraction in both therapeutic agents at the different days. Day 2, 4 and 6 showed statistically insignificant wound contraction whereas day 8 only, showed a statistically significant wound contraction. This inconsistency is not clearly understood. Contraction has been found to be due to a unique type of cell, the myofibroblasts, derived from fibroblasts in the wound [14]. In wound contraction, the wound is reduced by 80% of its original size by the action of myofibroblasts, which establishes a grip on the wound edges, and contract them. When the cells' roles are close to complete, unneeded cells undergo apoptosis [20-22].

A number of drug therapies have been documented to affect healing rate. Failure to heal is common in patients using corticosteroids, non-steroidal anti-inflammatory drugs, immune suppressants or

chemotherapeutic agents. Ideally, their use is withheld until any wound healing process is complete [23]. Administration of glucocorticoids has anti-inflammatory effect [24]. Chemotherapy used in treating cancer patients, radiations and immunosuppressive drugs prohibit healing [24]. When comparing a standard therapeutic agent like gentamicin to this unprocessed *lycopersicon esculentum* fruit, the insignificant difference observed in the wound contraction rate, mean day of skin re-epithelization (wound closure) and wound healing cells at day 8, could probably suggest the *lycopersicon esculentum* fruit to be a strong competitor to gentamicin. Although there were significant difference in the cell counts at day 4 yet, the calculated mean of wound closure day for both *lycopersicon esculentum* fruit (day 16) and gentamicin (day 15.5) showed no much variation. Infact a more reliable predictor to the wound closure day is better depicted by even the trend line from a linear graph which revealed the *lycopersicon esculentum* fruit and the gentamicin applied groups to have re-epithelized before day 12.

CONCLUSION

In this study, the effect of *Lycopersicum esculentum fruit* (tomato) and gentamicin was screened for wound healing activity on adult male albino wistar rats. This research has therefore showed both therapeutic agents to promote wound healing activity.

REFERENCES

1. Ayello AE, Baranoski S, Kerstein MD, Cuddington I; Wound Care Essentials: Practice Principals. Lippincott, Williams and Wilkins, Springhouse, Pennsylvania. 2004.
2. Eric L, Philip S; Wound Healing in Man: Tensile strength in Healing Wounds in some Patient Groups. Department of Urology and Department of Surgery, University Hospital, Lund, Sweden, 1974.
3. DuNouy PL; Cicatrization of wounds, mathematical expression of representing cicatrization. J Exp Med., 1916; 24(5): 451-460.
4. Natarajan E, Saeb M, Crum C, Woo SB, McKee PH, Rheinwald JG; Co-expression of p16INK4A and Laminin 52 by microinvasive and superficial squamous cell carcinomas in vivo and by migrating wound and senescent keratinocytes in culture. American Journal of Pathology, 2003; 163(2): 477-491.
5. Osuagwu FC, Oladejo OW, Imosemi IO, Adewoyin OO, Aiku A, Ekpo OE *et al.*; Wound healing activities of methanolic extracts of ocimum leaf in wistar rats- a preliminary study. African Journal of Biomedical Science, 2004; 33(1): 23 - 26.
6. Erhardt G; Lycopene, Beta-carotene and Colorectal Adenomas. 6th edition, Am J Publishers, 2003; 1219-1244.
7. Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Griffin GL, Senior RM *et al*.; Platelet-derived growth factor and transforming growth factor-β enhance tissue repair activities by unique mechanisms. J Cell Biol, 1989; 109(1): 429–440.
8. Cai M, Yan WY; Study on iron nutritional status in adolescence. Biomed Environ Sci.,1990; 3(1): 113–119.
9. Dostal GH, Gamelli RL; The differential effect of corticosteroids on wound disruption strength in mice. Arch Surg., 1990; 125(5): 634-640.
10. Bairy KL; Wound healing potentials of plant products. Journal of Natural Remedies, 2001; 2/1: 11-20.
11. Gentamicin **(**Gentamicin Sulfate**)** ointment**.** Available from http://dailymed.nlm.nih.gov/dailymed/archives/fdaDrugInfo.cfm?archiveid=2814
12. Rajawat S, Qureshi MS; Comparative study on bactericidal effect of silver nanoparticles, synthesized using green technology, in combination with antibiotics on *Salmonella typhi*. Journal of Biomaterials and Nanobiotechnology, 2012; 3: 480-485.
13. Nguyen DT, Orgill DP, Murphy GF; The pathophysiologic basis for wound healing and cutaneous regeneration. Biomaterials for treating skin loss. CRC Press (US) & Woodhead Publishing (UK/Europe), Boca Raton/ Cambridge, 2009: 25-57.
14. Badoe MK, Habibipour S, Oswald TM., Zhang F, Joshi P, Zhou X *et al.*; Topical treatment of experimental skin lesions in rats: macroscopic, microscopic and scanning electron- microscopic evaluation of the healing process. Int J Tissue React., 2009; 8(6): 533-544.
15. Muhammad HS, Muhammad S; The use of Lawsonia inermis Linn. (henna) in the management of burn wound infections. Afr J Biotechnol., 2005; 4(9): 934-937.
16. Esimone CO, Nworu CS, Jackson CL; Cutaneous Wound Healing Activity of a Herbal Ointment containing the Leaf Extract of Jatropha Curcas L. (Euphorbiaceae). IJARNP, 2009; 1(4): 1-4.
17. Taranalli AD, Kuppast IJ; Study of wound healing activity of seeds of *Trigonella foenum-graecum* in rats. Ind J Pharm Sci., 1996; 58(3): 117–119.
18. Odimegwu DC, Ibezim EC, Esimone CO, Nworu CS, Okoye FBC; Wound healing and antibacterial activities of the extract of *Dissotis theifolia* (Melastomataceae) stem formulated in a sample oitment base. J Med Pl Res., 2008; 2(1): 11-16.
19. Patil TA, Swami T, Singh TR; Effects of atorvastatin and simvastatin on wound healing in Albino rats. Pharmacologyonline, 2009; 2: 963-974.

20. Peter CM; Honey as a topical antibacterial agent for treatment of infected wounds. Nurs Times, 2001; 49: 96.
21. Harsh M; Textbook of Pathology. 5th edition, Jaypee Brothers Medical Publishers (P) Ltd., 2005: 171-177.
22. Harsh M; Textbook of Pathology. 6th edition, Jaypee Brothers Medical Publishers (P) Limited, 2010.
23. Ethelwynn LS; Judith CB; Nursing practice. Medica-surgical nursing for hospitals and community. African edition, Churchill Livingstone Elsevier; 2007: 721-730.
24. Coutinho AE, Chapman KE; The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell Endocrinol., 2011; 335(1): 2–13.
25. Wound Management - Easing the pain. Available from http://www.inmo.ie/MagazineArticle/PrintArticle/6144