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Wound healing activities of methanolic extracts o*cimum gratissimum* leaf in wistar rats - a preliminary study

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Summary

The wound healing effect of leaf extracts of *Ocimum gratissimum* was investigated in adult male Wistar rats. Two groups of adult male Wistar rats, average body weight 170g, had a 2cm by 2cm square wound inflicted on the dorsolateral aspect of their trunk with Paniculus Carnosus removed. Experimental group had their wound dressed with methanolic leaf extracts of *Ocimum gratissimum* while control group had their wounds dressed with normal saline dressing. All animals had wound dressing done every five days; wound dimension measured and, wound morphometry assessed. Wound biopsy was done by random selection in each group on day 10 and the day of complete re-epithelisation. Routine paraffin wax processing was done, slides stained with haematoxylene and eosin for histological assessment of fibroblast count, neovascularisation and granulation tissue profile. The result revealed significant wound contraction ($P<0.05$) on day 10 in the experimental group (mean 73.40 ± 3.30)cm^2 compared with the control group (mean 53.50 ± 4.32)cm^2. Histology of the healed scar showed non-significant ($P>0.05$) decrease in the mean fibroblast count for the experimental group (83.80 ± 5.70) relative to fibroblast count of 90.20 ± 17.90 in the control group. The mean blood vessel count was also non-significantly lowered ($P>0.05$) in the experimental group (9.20 ± 1.20) relative to the control group (13.40 ± 2.40). Granulation tissue histology on day 10 showed denser inflammatory infiltrate as reflected by increased cellularity in the control group relative to that of the experimental group which though appeared adequate was not as dense as the control group. Thus we suggest that the methanolic extracts of *O. gratissimum* could be a potential wound healing agent due to its ability to enhance wound contraction.

Keywords: *Wound healing. Ocimum gratissimum,*

Résumé

L'effect de la guerrison de la blessure a l'aide des extraits des feuilles d'*Ocimum gratissimum* était évalue a 2 groupes des rats de pods moyen 170g. Des blessures de 2X 2 cm2 etaient afflictees surl'aspect dorsolatéral de leur tronc avecc le carnosus paniculus enlevé. Le groupe expérimental avait des blesures nettoyées aux extraits de feuilles au methanol alorsque celles du groupe de controle était nettoyé avec la solution salée chaque 5 jours. La dimension de la blessure était mesurée et la morphométrie de la blessure évaluée et la biopsie faite par selection au harzard d'un animal dans chaque groupe au 10ieme jour et le jour de la reépithelisation compléte. La routine preparation du paraffin de wax était faite , pour l'évaluation du resultat de fibroblastes néovasculaire et le profile des tissues étaient taintés a l'hématoxyline et l'eosine. Le resulat a revelé une contraction significative de la blessure au 10ieme jour d'experimentation($P<0.005$) moyenne (73.40±3.30)cm2 comparé au groupe de controle(moyene:53.50±4.32)cm.l'histologie de la cicatrice montrait une reduction non –significative dans le nombre moyen des fibroblasts dans le grouped ex[pperimental (683.80±5.70) relatif au nombre des fibroblasts de 90.20±17.90 dans le groupe de controle. Le nombre moyen des vaisseaux sanguine était aussi non-significativement bas ($P>0.05$) dans le groupe experimental(9.20±1.2) relatif au groupe de controle (13.40±2.40). L'histologie de la granulation des tissues au 10ieme jour montrait une dense inflammation reflectant l'augmentation de la cellularité dans le groupe de controle qu'au groupe experimental traité aux extraits des feuilles *O. gratissimum*.Nous indiquons que ces extraits pourraient etre un potential médicament des plaies vu l'effect sur la contraction de la blessure.

Introduction

Wounds occur frequently via numerous agents. The extent of injury, which is dependent on the severity of the agent, ranges from simple cutaneous wheals or blisters to gross tissue death (necrosis) [1]. Therefore, it is necessary to assist the healing process in the body to restore an intact surface as soon as possible [2]. Management of wounds has always been of considerable importance in surgical practice. Many ointments, powders, gel, sprays, irrigating fluids and dressings are promoted for application to enhance wound healing [3]. Due to their exorbitant costs, attention is being turned to cheaper, easily available substitutes.

Ocimum gratissimum Linn. (Family Labiatae), commonly referred to as "Efinrin" by the Yoruba in Nigeria, is a commonly used edible vegetable plant [4] whose medicinal value has been subject to intense scrutiny by numerous investigators. *Ocimum.gratissimum* is used in traditional treatment to stop bleeding of wounds and nose infections [5]. Chemical analyses of its essential oils have revealed antimicrobial activity [6,7]. Analgesic and spasmolytic properties of aqueous extracts of *Ocimum. gratissimum* have also been documented [8]. In view of the importance of this plant as a medicinal plant, this study reports the potential of crude Methanolic extracts of *Ocimum. gratissimum* leaf in promoting wound healing in Wistar rats.

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Materials and methods

Plant collection and identification

The plant *Ocimum gratissimum* was authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan after its collection. The Voucher specimen number was FHI 106412.

Preparation of plant materials

The air dried leaves of *O.gratissimum*(250gm) ground into powdery form and was extracted by maceration in methanol at room temperature for 72hours. After removal of solvent the extract (8gm) was made into a 1.5% suspension in water. (1.5mg/100ml). This extraction was carried out at the Research laboratory of the Department of Pharmacognosy, University of Ibadan.

Animals and creation of wounds

Twenty adult male Wistar rats weighing between 150gm and 200gm (average weights 170gm) were used for the experiment. The animals were obtained from the animal house of the Department of Anatomy, College of Medicine, University of Ibadan. The animals were kept in wire bottomed cages and kept in the laboratory for 7 days prior to the commencement of the experiment; during this period they were screened for pre-existing diseases especially skin lesions. They were fed with standard rat pellet cubes and water provided *ad libitum*. The animals were assigned into two groups (A&B) each group having 10 animals per group.

Group A animals were the experimental group and this group had their wounds dressed with methanolic extract of *Ocimum. gratissimum.*

Group B served as the control group and the animals had their wounds dressed with normal saline.

Each animal was carefully and gently restrained, anaesthetized with chloroform via inhalation. The dorsolateral hair on the trunk was shaved clean with an electric clipper and the skin cleaned with 70% methylated spirit. Under strict aseptic conditions, a $2cm^2$ by $2cm^2$ area of skin was measured and cut with a scalpel; with the paniculus carnosus layer removed. Sterile transparent cellophane was placed on the wound and the outline traced on it to give the wound dimension on day 0 which was determined using a graph paper as described by Billingham *et al.* [9].

The Group A animals had their wounds dressed with sterile gauze soaked in methanolic extract of *Ocimum. gratissimum* leaf, while the Group B animals had their wounds dressed with sterile gauze soaked with normal saline. Dressings were changed every 5 days and wound dimensions measured. Dressing of the wounds continued till full re-epithelisation completed.

One animal was selected randomly from each group on day 10 (granulation tissue) and day of complete re-epithelisation. Biopsy specimen f the wounds and healed scars were taken, fixed in 10% buffered formalin for 48 hours and processed via routine paraffin wax technique for histological observations. The tissues were stained with haematoxylin and eosin and examined under the light microscope. Fibroblast and blood vessel counts were done using graticule affixed to the eyepiece of the light microscope. Granulation tissue pattern was also assessed. The slides were sent to the Department of Pathology, University of Ibadan for histopathological analyses. The data obtained were analyzed using Students t – test where applicable.

Results

No local or systemic adverse effects of the dressing agent used were noted. The gross appearance of the wounds of the animals in both groups appeared remarkably clean and showed no feature suggestive of infection throughout the duration of the experiment. The histopathological analyses of the granulation tissue in the experimental group versus control group revealed adequate inflammatory infiltrate and neovascularisation. However, the, control group had denser inflammatory cells as shown by increased cellularity. The photomicrographs are shown in Plates 1 and 2 respectively.

Fig. 1: Photomicrograph showing an H&E Section of the granulation tissue from experimental animal on day 10 x 160.

Fig. 2: Photomicrograph showing an H & E Section of the granulation tissue from control animal on day 10 x 160

The measurements of wound dimension on days 0 and 10 and the percentage wound contraction on day 10 in both groups are as shown in Table 1. Wound contraction on day 10 in the experimental group A ($73.40 \pm 3.30)cm^2$ was very significantly more pronounced ($P<0.05$) than that observed in the control group of rats ($53.50 \pm 4.32)cm^2$.

Table 1: The wound dimensions on days 0 and 10 and the percentage wound contractions on day 10 in both the experimental and control groups.

	Wound dimension Day (cm2)	Wound dimension Day 10 (cm2)	Wound dimension (%)
Group A Experimental	4.99±0.17	1.50±0.28	73.40±3.30
Group B (Control)	5.38±0.23	2.38±0.28	53.50±4.32
P-Value	P>0.05 (0.180)	P<0.05 (0.04)	P<0.05 (0.002)

Values: Mean ± standard error of mean.

Table 2: Fibroblast and blood vessel counts per high power field in both the experimental and control groups.

	Mean fibroblast count/high power field	Mean blood vessel count/high power field
Group A (Experimental)	83.80 ± 5.70	9.20 ± 1.20
Group B (Control)	90.20 ± 17.40	13.40 ± 2.40
P-Value	*P*>0.05 (0.78)	*P*<0.05 (0.15)

Values: Mean ± standard error of mean.

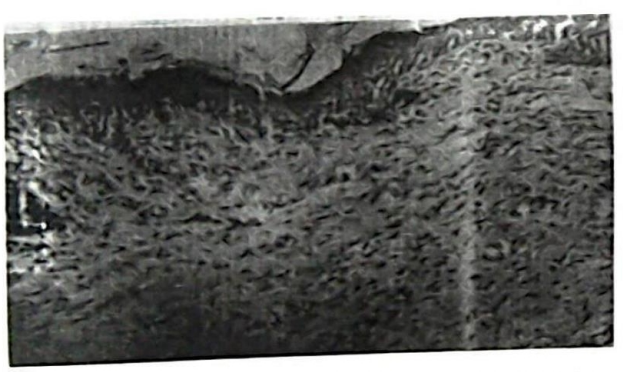

Fig. 3: Photomicrograph showing an H & E Section of the healed scar tissue from control animal X 160

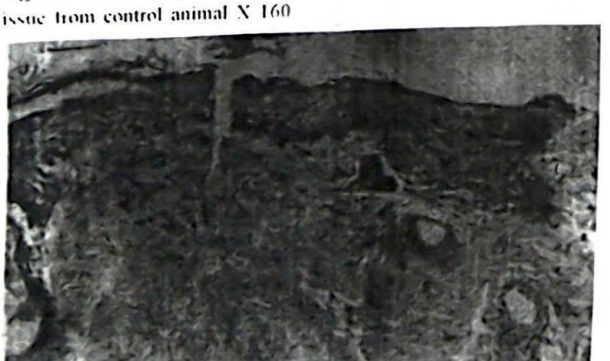

Fig. 4: Photomicrograph showing an H & E section of the healed scar tissue from experimental animal X 160.

The mean fibroblast and blood vessel counts in the experimental and control groups of rats are as shown in table 2. Wound dressing with *Ocimum. gratissimum* reduced the mean blood vessel count as well as the mean fibroblast count, albeit non-significantly, (*P*>0.05) in the rat relative to the control group.

Discussion

The suggestion that the extract of *O. gratissimum* might play a role in wound healing was derived from its wide spread use in traditional medicine an analgesic and antispasmolytic agent [8]. Its possible wound-healing role was further strengthened by reports in the literature ascribing antifungal and antimicrobial activities to the plant [6,7,10,11,12,13,14]. Some of these pathogens are known to infect wounds and delay healing. Furthermore oil extracts of this plant species on topical application reduced both the number of skin papillomas in affected mice and the incidence of mice bearing papillomas [15]. This was ascribed to the ability of *O.gratissimum* to modulate xenobiotic enzyme systems. Atal and co-worker [16] described the ability of *O.gratissimum* to enhance phagocytic activity during immune modulation experiments. One of the key events in wound healing involve invasion of the wound by phagocytes, which remove wound debris [1,2].

In the light of the foregoing, the enhanced wound contraction observed in animals whose wound were dressed with extracts of *O.gratissimum* leafs on day 10 as compared with the control group suggests that the extracts of *Ocimum gratissimum* promoted wound healing by enhancing percentage wound contraction. Although several theories have been proposed for the exact mechanism of wound contraction, majority implicate the fibroblasts called myofibroblast cell lines due to it's possession of contractile elements that enhance wound contraction [17]. Since the alpha smooth muscle action which confers contractile ability to myofibroblast is nondetectable until day six and then expressed for the next fifteen days of healing [18], we timed our measurements to coincide with this period. In view of the reported antimicrobial activity of *O. gratissimum* it was not necessary to do microbiological study [6,7,13,14].

Finally, the caveat to the foregoing is that for all its uses in other settings, *O. gratissimum* leaf extracts wound healing properties could be ascribed to its promotion of wound contraction. Thus it might be worthwhile to further carry out a concentration based study to evaluate the effects of the extracts of *O.gratissimum* leafs on specific cell culture lines of myofibroblast and keratinocytes.

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