

ORIGINAL ARTICLE

Comparative Study of Wound Healing Activity of Topical and Oral *Ocimum Sanctum* Linn in Albino Rats

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Abstract: *Introduction:* *Ocimum sanctum*, when given orally in one of the studies showed wound healing property. Since majority of the agents tried for wound healing are topical, the present study was planned to compare the oral and topical *Ocimum sanctum* for wound healing property. *Methods:* Excision and incision resutured wound models in albino rats were used to study complete epithelisation time, wound contraction, histopathological study and tensile strength of the wounds. *Results:* The animals were divided into four groups of oral test, control and topical test, control with 6 animals in each group. The time taken for 50% wound contraction and complete epithelisation by oral *Ocimum sanctum*, topical *Ocimum sanctum* was significantly ($p < 0.001$) less compared to oral and topical controls. Histopathological studies showed early inflammatory changes, dense collagen and neovascularisation in wounds treated with oral and topical *Ocimum sanctum*, compared to respective controls. Mean tensile strength of oral and topical *Ocimum sanctum* treated wound was significantly great ($p < 0.001$) compared to controls. *Interpretation and Conclusion:* Oral and topical *Ocimum sanctum* promoted better granulation tissue, early and complete epithelisation and better tensile strength compared to both controls.

Key words: *Ocimum sanctum*; Wound healing activity, topical, oral.

Introduction

Wounds are one of the first medical problems faced by mankind since very existence, hence there arises the need to have pharmacological agents which could promote and accelerate the process of wound healing. *Ocimum sanctum* linn, a commonly available plant, belongs to the class **Magnoliopsida**, is found to have antiinflammatory, analgesic, immunostimulatory, free radical scavenging and antimicrobial activity [1]. It is widely distributed throughout India and different parts of the world. The principle constituents of plant are volatile oil, alkaloids and glyctannins. The leaves contain ascorbic acid and carotene. It is used in ayurveda and Siddha system for the treatment of diverse aliment like infectious skin diseases herpetic disorder and as an antidote for snake bite and scorpion sting [2]. A methanol extract and an aqueous suspension of *O. sanctum* leaves were found to have antiinflammatory, analgesic and immunostimulatory properties [3]. Flavonoids isolated from *O. sanctum* scavenged free radicals in vitro and showed anti lipoperoxidant activity in vivo at very low concentration [4]. The free radical scavenging activity of plant flavonoids help in the healing of wounds [5].

Low levels of antioxidants accompanied by raised level by markers of free radical damage play a significant role in wound healing in rats [6]. Free radical scavenging activity is a major mechanism by which *O sanctum* products protect against cellular damage [7]. It acts on various levels of immune system and is an immunomodulator [8]. It has antibacterial, antifungal activity [9]. The stimulus for the present work is a study conducted in which aqueous extract of *ocimum sanctum* orally showed wound healing property. Since majority of the agents tried for wound healing are topical, the present study is planned to compare the oral and topical *Ocimum sanctum* preparation for wound healing activity.

Material and Methods

Source of Data: The study on the wound healing effect of *Ocimum sanctum* leaves in Albino rats was done in the department of pharmacology, JJM Medical College, Davangere, Karnataka, INDIA. Healthy adult Albino rats of either sex weighing 100-150g which were inbred in the Central Animal house, JJM Medical College, Davangere under suitable conditions of housing, temperature, ventilation and nutrition were used. Handling and animal care was done as per the guidelines set by Indian National Science Academy, New Delhi, India. The animals were housed individually in polypropylene cages containing sterile paddy husk (procured locally). The study was undertaken after obtaining the approval of institutional animal ethical committee. Aqueous extract of *Ocimum sanctum* was obtained from Natural Remedies (Bangalore). To study wound contraction, epithelisation, histopathological studies and wound breaking strength, healthy Albino rats of both sex weighing 100-150g were used.

Preparation of animals: The animals were depilated on the dorsal surface before wounding. They were caged individually with free access to food (animal chow) and water. The animals were starved 12hours with only free access to water, prior to wounding. Wounding was performed aseptically under light ether (obtained from Davangere Scientifics) anaesthesia. The animals were grouped 6 each into 12 groups. These 12 groups were arranged into two sets. **Set I:** a. Excision wound model I b. Excision wound model II **Set II:** Incision and resutured wound.

Groups: The above three models of wound healing have four groups. They are as follows.

Group I: Oral Control - These animals received oral normal saline via intragastric tube once daily at 10.00am. **Group II: Oral test-** These animals received oral *Ocimum sanctum* via intragastric tube in a dose of 800mg/kg body weight [10] once daily at 10.00am. **Group III: Topical Control.** These animals were applied 0.5ml of glycerine twice daily at 10.00am and 5.00pm. **Group IV: Topical test.** These animals were applied 1g of aqueous extract of *Ocimum sanctum* mixed in 0.5ml of glycerine twice daily at 10.00am and 5.00pm.

Wound Models: The wound models chosen for the present study were excision and resutured incision wound models. The three attributes were physical, mechanical and histological.

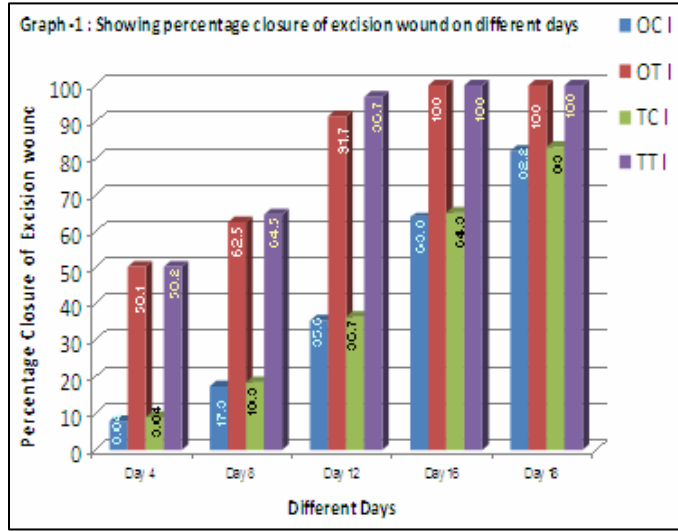
Excision Wound Model-I: Under light ether anaesthesia, the animal was secured to the operation table in its natural position. An impression was made on the depilated dorsal thoracic surface 2cms behind the ears, by using a round seal of 2cms diameter as used by Hunt and his co-workers on either side, 1cm away from the vertebral column [11]. The full thickness of the impressed area was excised to obtain a wound area of 31.4sqmm. The physical attributes of wound healing namely, wound closure (contraction) and epithelisation time were studied in this model. Contraction, which mainly contributes for wound closure in the first two weeks was studied by tracing the raw wound area on tracing paper on the wounding day followed by 4,8,12,16 and subsequently on every alternate day, till complete epithelisation occurred. The criteria for complete epithelisation being the fall of scab without any raw surface. Wound area was measured by retracing the wound on a millimetre scale graph paper. The degree of wound healing was calculated as percentage closure in wound area from original wound area. The mean and standard error values were calculated. The number of days for complete epithelisation was noted.

Excision Wound Model-II: Excision wounds were made to study the histopathological attributes. Wound biopsy was done under light ether anaesthesia. The ulcer along the base and 0.5cm of adjacent normal tissue were excised. The biopsy tissue was fixed in 10% formalin and subjected to histopathological examination. Various cellular elements and collagenisation were quantified microscopically by giving scores to. (1)Inflammatory cells (2) Granulation tissue in which ground substances, (3) Neovascularisation, (4) Fibroblasts, (5) Collagenisation and (6) Epithelisation were quantified.

Resutured Incision Wound Model: Under light ether anaesthesia, two paravertebral linear incisions of 6cms each were made through the entire thickness of skin on either side of the vertebral column with the help of a sharp blade as described by Ehrlich and Hunt. After complete hemostasis, the wounds were closed by means of interrupted sutures placed at equidistant points about 1cm apart, using 4-zero silk thread and straight round body needles. Wounds were then mopped with cotton swabs soaked in 70% alcohol. The animals were caged individually. Removal of the sutures was done on the 8th post wounding day. Wound breaking strength was determined on the 10th post wounding day as described below.

The anaesthetized animal was secured to the operation table. Two Allie's forceps were firmly applied on the lines facing each other, the forceps on one side was hooked to a metal rod fixed firmly to the operation table. The other forceps was tied with a string, which ran over a pulley. To the other end of the string, serial measuring weights in ascending order were added. The basal weight added to the string was 50g and the weight was gradually increased. As soon as the wound gaping was observed, the weights were immediately removed and the total weight was noted down. The wound breaking strength was expressed as the minimum weight at which the wound started to gape. Three such recordings were made for a given incision wound and the procedure was repeated on the other site. Statistical analysis: All the results were expressed as Mean \pm standard deviation (SD). Data was analysed using one-way ANOVA followed by post hoc test. P value <0.01 were considered significant.

Results and Discussion



Wound healing is a complex biologic process that involves integration of inflammation, mitosis, angiogenesis, synthesis and remodelling of extracellular matrix. This study was undertaken to find the effectiveness of aqueous extract of *Ocimum sanctum* by different routes of administration i.e, orally and topically on wound healing, studied under different parameters. As

can be seen from the results, the test drug by both routes of administration has produced highly encouraging effects on wound healing. The results of the present study were grouped under the following headings.(i)Percentage closure of excision wounds on different days (Graph I).(ii)Time taken for 50 percent wound contraction.(iii)Time taken for complete epithelisation.(iv)Histopathological finding of wound biopsy.(v)Wound breaking strength of 10 day old skin incision wound.

Table-I: Showing time taken for 50% wound contraction (WC-50)			
Groups		Number of wounds	50% Wound contraction in days (WC-50) mean ± SE
1	Oral Control	12	13.5 ± 0.25
2	Oral Test	12	4.7 ± 0.40 ^a
3	Topical Control	12	12.5 ± 0.29
4	Topical Test	12	5.7 ± 0.97 ^a
Values are mean ± SD; (n =6); a= p < 0.001 Vs control			
Note: Early achievement of 50% wound contraction in oral <i>Ocimum sanctum</i> treated followed by topical <i>Ocimum sanctum</i> treated groups.			

1. To study the time taken for 50% wound contraction: Oral *Ocimum sanctum* achieved 50% wound contraction by 4.7±0.40 days, with topical *Ocimum sanctum* 5.7±0.97, with oral control 13.5±0.25 and topical control 12.5±0.29 days (Table I). To achieve 50% wound contraction oral *Ocimum sanctum* has taken less than 1/3rd of the time taken by that of control. Time taken for 50% contraction of topical *Ocimum sanctum* is also

significantly less compared to topical control, and values are statistically significant (p <0.001). There is no significant difference between the oral and topical *Ocimum sanctum* to achieve 50% wound contraction.

2. Time taken for complete epithelisation by topical *Ocimum sanctum* was 12.1±0.13, with oral *Ocimum sanctum* 13.0±0.18 and with both control 20.6±0.29 (Table II). Epithelisation with topical *Ocimum sanctum* was early and complete as compared to oral *Ocimum sanctum*. Epithelisation time of both topical and oral *Ocimum sanctum* was statistically significant compared to the controls.

Table II: Showing time taken for complete epithelisation			
Groups	Number of wounds	Complete epithelisation in days mean \pm SE	
1	Oral Control	12	20.6 \pm 0.29
2	Oral Test	12	13.0 \pm 0.18 ^a
3	Topical Control	12	19.7 \pm 0.77
4	Topical Test	12	12.7 \pm 0.27 ^a
Values are mean \pm SD; (n =6); a= p < 0.001 Vs control			
Note: Early and complete epithelisation is achieved by topical <i>Ocimum sanctum</i> followed by oral <i>Ocimum sanctum</i> treated groups compared to oral and topical controls.			

Excision wound model – II

1. *Microscopic findings of 4th day wound biopsy*: Oral and topical *Ocimum sanctum* treated wound, which showed greater degree of neovascularisation and fibroblast proliferation indicates better granulation tissue formation and collagenisation.
2. *Microscopic findings of 7th day wound biopsy*. Oral *Ocimum sanctum* showed maximum collagenisation, moderate in case of topical *Ocimum sanctum* and minimum with controls. Devascularisation seen in test group. Epithelisation was early and complete with topical *Ocimum sanctum* followed by oral *Ocimum sanctum*.
3. *Microscopic findings of 14th day wound biopsy*. Collagenisation was maximum with oral *Ocimum sanctum* followed by topical *Ocimum sanctum*, minimum with both controls. Epithelisation was complete by day 14 with topical and by oral *Ocimum sanctum*.

Incision wound model: Tensile strength of oral *Ocimum sanctum* treated incision wound was 382.7 \pm 5.6g, with topical *Ocimum sanctum* 380.4 \pm 7.77g and with oral and topical control 231.4 \pm 7.63g and 232.4 \pm 7.70g respectively (table III). Oral and topical *Ocimum sanctum* showed high tensile strength compared to both control. The values are statistically highly significant (p<0.001).

Table III: Showing tensile strengths of 10 day old skin incision		
Groups	No. of incision wound	Tensile strength in grams
Oral Control	12	231.4 \pm 7.63
Oral Test	12	382.7 \pm 5.69 ^a
Topical Control	12	232.4 \pm 7.70
Topical Test	12	380.4 \pm 7.77 ^a
Values are mean \pm SD; (n =6); a= p < 0.001 Vs control		
Note: Significant increase in the breaking strength in oral test followed by topical test as compared to oral and topical control groups		

The aqueous extract of *Ocimum sanctum* by both oral and topical route have enhanced wound healing in the animal experimental models studied. It has also increased the strength of the wound. Aqueous extract of *Ocimum sanctum* when given orally had definite prohealing action. [9]. The present study confirms the same. An increase in wound healing strength and hydroxyproline content of treated wounds may be due to increase in collagen and stabilization of fibers [9].

Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in the leaves of *Ocimum sanctum* has been found to be responsible for the therapeutic potentials of Tulsi.

Phytochemical screening revealed the presence of flavonoid in the aqueous extract of *Ocimum sanctum*. Better collagenisation seen under the influence of this plant extract may be because of the presence of flavonoids, which is responsible for the free radical scavenging activity that is believed to be one of the most important components of wound healing. The features suggesting prohealing activity are enhancement of the early inflammatory response, better collagenisation, and an early, complete epithelisation. Since both routes of administration of *Ocimum sanctum* has showed almost similar effect, topical route can be preferred as wound healing agent. Topical route is more advantageous as it has minimal systemic toxicity and is convenient to use.

Ocimum sanctum is ubiquitously and abundantly grown, and hence it could be a fairly economic therapeutic agent for wound management as a prohealer as well as to control abnormal healing. Further study in depth is necessary to probe into the exact mechanism and for clinical correlation.

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