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Study of wound healing activity of topical *Ocimum sanctum Linn* in albino rats

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ABSTRACT

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 know the effect of topical Ocimum sanctum for wound healing property. 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. The animals were divided into two groups of of topical test, control with 6 animals in each group. The time taken for 50% wound contraction and complete eipthelisation by topical Ocimum sanctum was significantly ($p<0.001$) less compared to topical control. Histopathological studies showed early inflammatory changes, dense collagen and neovascularisation in wounds treated with topical Ocimum sanctum, compared to control. Mean tensile strength of topical Ocimum sanctum treated wound was significantly great ($p<0.001$) compared to control. Topical Ocimum sanctum promoted better granulation tissue, early and complete epithelisation and better tensile strength compared to control.

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

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 know the effect of topical *Ocimum sanctum* preparation for wound healing activity.

EXPERIMENTAL SECTION

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 6 groups. These 6 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 two groups. They are as follows.
Group I: Topical Control. *These animals were applied 0.5ml of glycerine twice daily at 10.00am and 5.00pm.*
Group II: 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

impressioned area was exicised 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 haemostasis, 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 8^{th} post wounding day. Wound breaking strength was determined on the 10^{th} post wounding day as described below.

The anaesthetized animal was secured to the operation table. Two Allies 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 ± standard deviation (SD) . Data was analysed using one-way ANOVA. 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 topically on wound healing, studied under different parameters. As can be seen from the results, the test drug by topical route 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 (**Table 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 percentage of closure of excision wound on different days						
Groups		Day 4	Day 8	Day 12	Day 16	Day 18
1	TC I	9.04±2.8	18.3±2.1	36.7±2.40	64.9±2.25	83.0±7.76
2	TT I	50.2±2.60	64.5±2.77	96.7±1.65	100	100

On	4th day	8th day	12th day	16th day	18th day
1 α 2	P < 0.01	P < 0.01	P < 0.01	P < 0.001	P < 0.001

Significant enhancement of wound closure in topical Ocimum sanctum (TT) compared to topical control (TC)

1. *To study the time taken for 50% wound contraction*: Topical *Ocimum sanctum* achieved 50% wound contraction by 5.7±0.97 days, and with topical control 12.5±0.29 days (**Table II**). Time taken for 50% contraction of topical *Ocimum sanctum* is significantly less compared to topical control, and values are statistically significant (p <0.001).

Table II:Showing time taken for 50% wound contraction(WC-50)			
Groups		Number of wounds	50% Wound contraction in days (WC-50) mean ± SE
1	Topical Control	12	12.5 ± 0.29
2	Topical Test	12	5.7 ± 0.97[a]
Values are mean ±SD; (n =6); a= p < 0.001 Vs control			

Early achievement of 50% wound contraction in topical Ocimum sanctum treated followed by topical control treated group

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

Table III: : Showing time taken for complete epithelisation			
Groups		Number of wounds	Complete epithelisation in days mean ± SE
1	Topical Control	12	19.7 ± 0.77
2	Topical Test	12	12.7 ± 0.27[a]
Values are mean ±SD; (n =6); a= p < 0.001 Vs control			

Early and complete epithelisation is achieved by topical Ocimum sanctum followed topical control.

Excision wound model – II

1. *Microscopic findings of 4th day wound biopsy*: 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*. Topical *Ocimum sanctum* showed maximum collagenisation and minimum with control. Devascularisation seen in test group. Epithelisation was early and complete with topical *Ocimum sanctum*.

3. *Microscopic findings of 14th day wound biopsy*. Collagenisation was maximum with topical *Ocimum sanctum*, minimum with control. Epithelisation was complete by day 14 with topical *Ocimum sanctum*.

Table IV. Showing tensile strengths of 10 day old skin incision		
Groups	No. of incision wound	Tensile strength in grams
Topical Control	12	232.4 ± 7.70
Topical Test	12	380.4 ± 7.77[a]
Values are mean ±SD; (n =6); a= p < 0.001 Vs control		

Significant increase in the breaking strength in topical test as compared to topical control group.

Incision wound model: Tensile strength of topical *Ocimum sanctum* treated incision wound was 380.4±7.77g and with topical control 232.4±7.70g (**Table IV**). Topical *Ocimum sanctum* showed high tensile strength compared to control. The values are statistically highly significant ($p<0.001$).

The aqueous extract of *Ocimum sanctum* by 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 its effectiveness by topical route. 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 Ocimum sanctum has showed statistically significant wound healing activity by topical route, this route can be preferred 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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