

Effect of *Ocimum sanctum* Linn. on normal and dexamethasone suppressed wound healing

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Ethanollic extract of leaves of *O. sanctum* was investigated for normal wound healing and dexamethasone depressed healing using incision, excision and dead space wound models in albino rats. The extract of *O. sanctum* significantly increased the wound breaking strength in incision wound model. The extract treated wounds were found to epithelialize faster and the rate of wound contraction was significantly increased as compared to control wounds. Significant increase in wet and dry granulation tissue weight, granulation tissue breaking strength and hydroxyproline content in dead space wound model was observed. The extract significantly decreased the antihealing activities of dexamethasone in all the wound models. The results indicated that the leaf extract promotes wound healing significantly and able to overcome the wound healing suppressing action of dexamethasone. Histological examination of granulation tissue to determine the pattern of lay-down for collagen confirmed the results.

Keywords: Dead space wound, Dexamethasone, Excision wound, Incision wound, *Ocimum sanctum*, Wound healing

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Ocimum sanctum Linn. (Sanskrit: Tulasi; family: Labiaceae), is found throughout the semitropical and tropical parts of India. Different parts of the plant are traditionally used in Ayurveda and Siddha systems for the treatment of diverse ailments like infections, skin diseases, hepatic disorders and as an antidote for snake bite and scorpion sting¹. The ether extract and essential oil of the leaves exhibited antibacterial activity against a number of bacterial species²⁻⁵. A methanol extract and an aqueous suspension of *O. sanctum* leaves were found to have anti-inflammatory, analgesic⁶ and immunostimulatory properties. The essential oil of *O. sanctum* was observed to have anti-inflammatory activity in rats⁷. *O. sanctum* fixed oil and linolenic acid found to possess significant anti-inflammatory activity against PGE², leukotriene and arachidonic acid induced paw edema⁸. *O. sanctum* extract protects against radiation induced lipid peroxidation and GSH and the antioxidant enzymes appear to have an important role in the protection⁹. Pharmacologically important active principles of *O.*

sanctum are a large group of polyphenolic flavonoids like apigenin, vicenin-1 and vicenin-2, caffeic and ursolic acid^{10,11}. Flavonoids isolated from *O. sanctum* scavenged free radicals *in vitro* and showed antilipoperoxidant activity *in vivo* at very low concentration¹². The free radical scavenging activity of plant flavonoids helps in the healing of wounds¹³. Low levels of antioxidants accompanied by raised level of markers of free radical damage play a significant role in wound healing in rats¹⁴. Free radical scavenging activity is a major mechanism by which *O. sanctum* products protects against cellular damage¹⁵. *O. sanctum* may act at various levels in the immune mechanism, such as antibody production, release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs in modulating the humoral immune responses¹⁶. Thus it appears different mechanisms like free radical scavenging, metal chelation as well as immune modulation may act at different levels individually or in combination to bring about the wound healing effects of this medicinal plant. The present study provides a scientific rationale for the traditional use of this plant in the management of skin diseases.

Till today, there are not many agents which are able to successfully overcome the antihealing effects of corticosteroids. Dexamethasone is a very potent anti-inflammatory glucocorticoid used in organ transplantation and skin allografts¹⁷. Glucocorticoids are known to suppress wound healing^{18,19}. Dexamethasone treatments strongly interfere with both the synthesis and degradation of type I and type III collagen²⁰. It is also a potent transcriptional inhibitor of human type VII collagen promoter activity in dermal fibroblasts, which leads to decreased anchoring of fibril formation²¹. Significant inhibition of growth of pathogenic microorganisms was observed *in vitro* by traditional drugs like *O. sanctum*, *Azardicta indica* and *Annona squamosa*. Healing of the wound by indigenous ointment formulation was comparable to that of nitrofurazone and propamidine cream in mice infected by the organisms²². Oral administration of the *O. sanctum* extract significantly increased the tensile strength of the incision wound and promoted epithelization in the excision wound²³. Keeping in view the tremendous pharmacological activities and a wealth of available literature, *O. sanctum* may be utilized to alleviate the symptoms of a variety of diseases as evident from pre-clinical data²⁴. The present study has been undertaken to investigate the effects of ethanol extract of *O. sanctum* on the different parameters of wound healing alone and in the presence of dexamethasone induced suppression of wound healing in rats.

Materials and Methods

Plant material—Leaves of *O. sanctum* were collected during September from the local areas of Udupi district, Karnataka, India, and were authenticated by Professor Gopalkrishna Bhat, Department of Botany, Poorna Prajna College, Udupi. A voucher specimen (No.pp531) has been deposited at the Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, India.

Preparation of ethanol extract—Leaves of *O. sanctum* were dried in shade and powdered. The powder (75 g) was extracted with 700 ml of 95% ethanol in a soxhlet apparatus at 60°-75°C and concentrated. The yield was 10-15%.

Animals—Healthy albino rats of either sex and of approximately the same age, weighing 150-250 g were used for the study. They were individually housed, maintained in clean polypropylene cages and fed with commercially pelleted rat chow (M/s Hindustan Lever Ltd. Mumbai) and water *ad libitum*.

The experimental protocol was subjected to scrutiny of Institutional Animal Ethical Committee for experimental clearance (No.IAEC/KMC/UA/2000).

Acute toxicity studies—Healthy albino rats of either sex (n=6) were orally fed with increasing doses (1,2,4,8 and 16g/kg body weight) of ethanol extract for 14 days. The doses of up to 4g/kg body weight did not produce any sign of toxicity and mortality.

Experimental procedure—The animals were divided into 4 groups of 6 animals each.

Group I: Control group with wound alone;

Group II: Test group with wound and treated with extract;

Group III: Test group with wound and dexamethasone;

Group IV: Test group with wound, dexamethasone and treated with extract.

The wounding procedures were carried out using ketamine (1ml/kg body weight) anaesthetized rats in three different wound models, at the dose level of 800mg/kg body weight. The extract was given daily to the rats orally in the case of incision and dead space wound for ten days. In the case of excision wound, extract was given everyday to the rats until the day of epithelization. Dexamethasone was used in the dose of 0.3mg/kg, im; full dose on the day of operation and half the dose thereafter on alternate day till the end of the study period²⁵.

Incision wound—Two paravertebral incisions (6cm long) were made through the full thickness of the skin on either side of the vertebral column of the rat²⁶. Wounds were closed with interrupted sutures, 1cm apart. The sutures were removed on 7th day. Wound breaking strength was measured on 10th post-wounding day²⁷.

Excision wound—A circular piece of full thickness (approximately 500mm²) was cut off from a predetermined area on the back of rat²⁸. Wounds were traced on 1mm² graph paper on the day of wounding and subsequently on the alternate days, until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction. Number of days required for falling of eschar without any residual raw wound gave the period of epithelization.

Dead space wound—These wounds were created by implanting two polypropylene tubes (0.5cm×2.5cm each), one on either side in the lumbar region on the dorsal surface of each rat. On the 10th post-wounding day, the granulation tissue formed on the implanted

tubes was carefully dissected out. The wet weight of granulation tissue was noted. The breaking strength of granulation tissue was measured by the method of Lee²⁷. Later, these granulation tissues were collected, dried at 60°C for 24hr and weighed and the weight was noted. The dried granulation tissue was then utilized to estimate hydroxyproline content²⁹. A section of wet granulation tissue was subjected to histopathological examination so as to determine the lay-down of collagen using Haematoxylin and Eosin stains.

Statistical analysis—The results were analyzed using one way analysis of variance (ANOVA) with post hoc Scheffe's test. *P* values <0.05 were considered statistically significant.

Results and Discussion

In the incision wound model, a significant increase in wound breaking strength in the extract treated group was observed. A significant decrease in the wound breaking strength in group III (dexamethasone treated group) was observed. Suppression of the wound breaking strength in the group III was reversed

when treated along with the extract of *O. sanctum*.

In dead space wound model, the ethanol extract treated animals showed significantly increased level of hydroxyproline content (which is a reflection of increased collagen content), granulation tissue weight (wet and dry) and granulation tissue breaking strength. The dexamethasone treated animals showed depressed wound healing, as evidenced by significant decrease in breaking strength, wet and dry tissue weight of granulation tissue, hydroxyproline and granulation tissue strength. In the same wound model, administration of extract of *O. sanctum* the plant extract reversed the antihealing effect of dexamethasone (Table 1).

In excision wound model, the extract treated animals showed significant decrease in the epithelization period (Table 1) and increased per cent wound contraction as compared to controls (Table 2). In dexamethasone treated group significant increase in the epithelization period and decreased per cent wound contraction were observed when compared to controls. These effects were reversed when treated with extract of *O. sanctum* (Table 2).

Table 1—Effect of ethanol extract of *O. sanctum* in absence and presence of dexamethasone in incision, excision and dead space wound model

[Values are mean ± SD of 6 replications]

Treatment	Incision wound breaking strength (g)	Excision wound Epithelization period (days)	Dead space wound			
			Wet tissue weight (mg/100g rat)	Dry tissue weight (mg/100g rat)	Breaking strength (g)	Hydroxyproline (mg/g tissue)
Wounded control	274.16±22.89	21.5±1.5	240.5±20.5	30±2.5	285±15.5	14.72±4.02
<i>O. sanctum</i>	465.5±34.76 ^a	15±1.0 ^a	335.8±16.5 ^a	42.55±2.0 ^a	395±16.5 ^a	46.38±6.1 ^a
Dexamethasone	210±26.78 ^c	33.6±4.76 ^{ax}	192±12.5 ^{bx}	24±3.8 ^x	190±10.5 ^{ax}	10.35±3.65 ^x
Dexamethasone + <i>O. sanctum</i>	335.5±16.78 ^c	24±1.5 ^{xp}	255±18.5 ^{xp}	32±4.5 ^{yr}	270±12.5 ^{xp}	19.5±5.5 ^x

P values: ^a:<0.001, ^b:<0.01, ^c:<0.05 vs control; ^x:<0.001, ^y:<0.01, ^z:<0.05 vs *O. sanctum*; and ^p:<0.001, ^r:<0.05 vs dexamethasone

Table 2—Effect of ethanol extract of *O. sanctum* in absence and presence of dexamethasone in excision wound model [Values are mean ± SD of 6 replications]

Treatment	Percent wound contraction in days									
	4	8	12	16	20	22	24	28	32	34
Wounded control	12.46±2.46	48.43±2.4	71.4±1.84	84±4.2	96.5±4.3	99.1±1.5				
<i>O. sanctum</i>	27.4±3.8 ^a	56.2±4.2 ^{cx}	81±3.5	99.5±1 ^{bx}						
Dexamethasone(DM)	-15.8±3.2 ^{ax}	1±5.8 ^{ax}	18±4.1 ^{ax}	39.9±5.5 ^{ax}	61±6.5 ^{bx}		70.2±4.4	81.3±3.2	96.8±4	99.5±1.5
DM + <i>O. sanctum</i>	1.5±1.1 ^{ax}	18.5±3.5 ^{ax}	37.5±2.7 ^{ax}	74.6±5.5 ^{cy}	86±9.5 ^f		98.5±3.5			

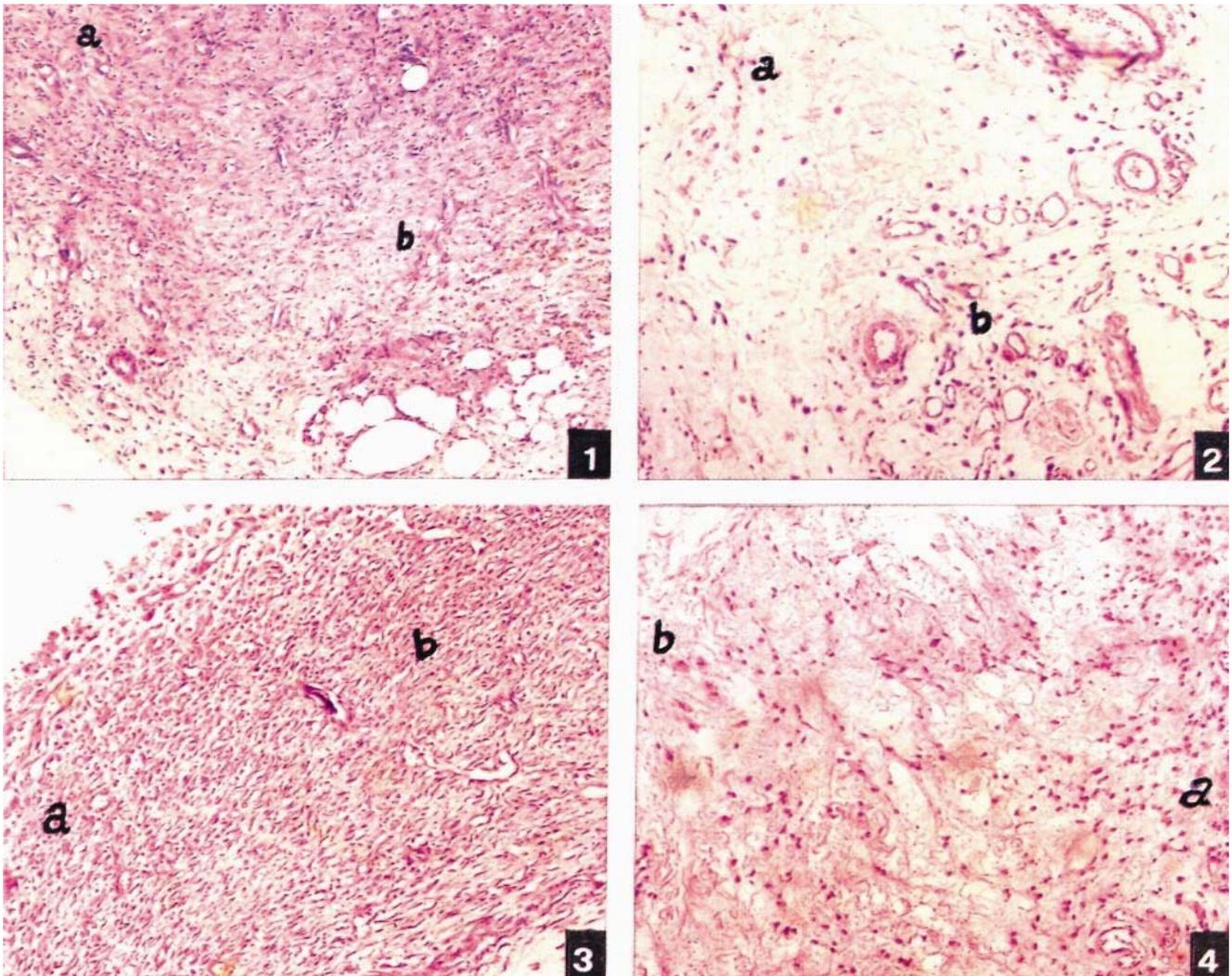
P values ^a:<0.001, ^b:<0.01, ^c:<0.05 vs control; ^x:<0.001, ^y:<0.01, ^z:<0.05 vs *O. sanctum*; and ^r:<0.05 vs dexamethasone

Histological observations of the 10 days old granulation tissue in extract treated group (Fig. 2) revealed a well developed matrix. Collagen was well organized and formed bundles between the cells. There was better neovascularization in the extract treated group as compared to all other groups. Due to the better formation of ground substance the cells appeared to be spread apart in extract treated group when compared to control group (Figs 1 and 2). Moderate cell population with some matrix formation and neovascularization can be seen in group IV (dexamethasone given along with the extract) (Fig. 4) when compared to group III (dexamethasone treated alone) (Fig. 3).

Granulation, collagen maturation and scar formation are some of the many phases of wound

healing, which run concurrently, but independent of each other. The use of a single model is inadequate and no reference standard exists that can collectively represent the various phases of wound healing. Hence, three different models have been used in the present study to assess the effect of *O. sanctum* on the various phases of wound healing.

The results of the present study clearly demonstrate that the ethanolic extract of *O. sanctum* possesses a definite prohealing action in normal healing as well as in the steroid depressed wound healing. An increase in wound breaking strength and hydroxyproline content of treated wounds may be due to increase in collagen concentration and stabilization of fibres³⁰. Increase in wet and dry granulation tissue weight indicated high protein concentration and collagen



Figs 1-4 — Histopathology of 10 days old granulation tissue. 1 – Control (a) Maximum number of fibroblasts, (b) Collagen bundles are indistinguishable. 2 – Extract treated (a) Few cells with well organized collagen bundles, (b) Well developed blood vessels. 3 – Dexamethasone treated (a) Poorly developed matrix shows maximum number of cells, (b) Poor collagen formation. 4 – Dexamethasone with extract treated (a) Moderate cell population with some matrix formation, (b) Developing neovascularisation. [H and E, taken with Olympus PM 20 photomicroscope 20 X magnification]

bundle formation. The increased granulation mass is predominantly contributed by fibroblasts. The plant extract also antagonized the action of dexamethasone to some extent on collagen synthesis, maturation and organization to form bundles. Thus, it has the potential for antagonizing the antihealing effect of steroids in patients receiving steroid therapy.

In recent years, oxidative stress has been implicated in a variety of degenerative processes and diseases. These include acute and chronic inflammatory conditions such as wound healing³¹. Oxygen free radicals play a important role in the failure of ischemic wound healing and antioxidants improve the healing in ischemic skin wounds³². Phytochemical screening revealed the presence of flavonoids in the ethanol extract of *O. sanctum*. Better collagenation, 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. Since *O. sanctum* is ubiquitous and abundantly grown, it could be a fairly economic therapeutic agent for wound management as a prohealer as well as to control abnormal healing.

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