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Research Article

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## Preclinical evaluation of burn wound healing property of *Ocimum sanctum* in rabbits – A histopathological study

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### Abstract

Burns remain a major public health problem all over the world, especially in developing countries. It can be defined as tissue damage caused by heat, chemicals, electricity, sunlight or nuclear radiation. Every year, about two million people receive medical treatment for burn injury. *Pseudomonas aeruginosa* is a major cause of infection and a contributing factor in the death of patients with burns. So, there is a growing need to develop drugs which will prevent infections and complications more effectively than the presently used drugs. Plants have immense potential for the treatment of burns. *Ocimum sanctum* L. (Holy basil in English and Tulsi in Hindi) has been shown to have antioxidant properties, which may be responsible and favorable for faster wound healing. The present study was carried out to evaluate the wound healing property of *Ocimum sanctum* in thermal burns with and without supportive treatment of Ointment Silver Sulfadiazine. After histological evaluation, Control group showed healing score of  $9.66 \pm 5.39$  which revealed poor status of healing of wound. Standard group and group treated with *Ocimum sanctum* showed score of  $15.67 \pm 4.89$  (p-value 0.110,  $p > 0.05$  Not Significant)  $13.83 \pm 5.04$  (p-value 0.416,  $p > 0.05$  Not Significant) respectively, revealed fair status of wound healing. Group treated with Ointment Silver Sulfadiazine + *Ocimum sanctum* showed score of  $18.83 \pm 2.99$  (p-value 0.005,  $p < 0.05$  Significant), revealed good status of wound healing. So, the most effective treatment for burn wound healing in our study was Ointment Silver Sulfadiazine + *Ocimum sanctum*.

### 1. INTRODUCTION

Ayurveda remains one of the most ancient and yet alive tradition practiced widely in India, Sri Lanka and other countries that have a sound philosophical and experiential basis <sup>1</sup>.

Burn can be defined as tissue damage caused by a variety of agents such as heat, chemicals, electricity, sunlight or nuclear radiation. The most common are burns caused by scalds, building fires and flammable liquids and gases. Every year, about two million people receive medical treatment for burn injury <sup>2</sup>.

Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues. It involves continuous cell-cell interaction and cell-matrix interactions that allow the process to proceed in different overlapping phases and processes including inflammation, wound contraction, reepithelialization, tissue remodelling, and formation of granulation tissue with angiogenesis <sup>3</sup>.

Plants have the immense potential for the management and treatment of wounds. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body <sup>3</sup>.

The gold standard in topical burn treatment is Silver sulfadiazine a useful antibacterial agent for burn wound treatment <sup>4</sup>.

*Ocimum sanctum* L. Holy basil in English and Tulsi in Hindi, in addition to its antibacterial, analgesic, antioxidant, anti-inflammatory and immune-stimulating properties, has been used in Ayurveda system for the treatment of skin diseases and infections. *Ocimum sanctum* has been shown to have antioxidant properties, which may be responsible and favorable for faster wound healing <sup>5</sup>.

*Pseudomonas aeruginosa* is a major cause of infection and death or a contributing factor in the death of patients with severe burns <sup>6</sup>.

So, there is a growing need to develop drugs which will decrease the complications and prevent infections more effectively than the presently used drugs.

The present study was carried out to evaluate the wound healing property of *Ocimum sanctum* with and without supportive treatment of standard drug Ointment Silver Sulfadiazine on healing of thermal burn wound in Rabbits

## 2. MATERIALS AND METHODS

This is a Preclinical experimental study on Rabbits. The Study protocol was approved by Institutional Animal Ethics Committee of the institute vide letter no. DMIMSU/IAEC/2011-12/011, dated 28/12/2011.

### 2.1 Procurement of Plants Material

The Plant material of *Ocimum sanctum* Leaves Powder was obtained from Mahatma Gandhi Ayurved College, Wardha, Maharashtra state, India

### 2.2 Procurement of Ointment Silver Sulfadiazine

Ointment Silver Sulfadiazine was procured from Rexcin Pharmaceuticals Private Limited, Solan, Himachal Pradesh, India

### 2.3 Procurement of Anesthetic Agent

Injection Aneket Ketamine Hydrochloride Injection I.P. was procured from Neon Laboratories Limited, Thane, Maharashtra State, India.

### 2.4 Animals

24 Rabbits were obtained from Central Animal House of D.M.I.M.S. Deemed University, Wardha, Maharashtra state, India. Rabbits of both sex either male or female, age group of 6-8 months and weight 1.5 kg -2 kg were included. Unhealthy and pregnant rabbits were excluded.

### 2.5 Grouping of Animals

24 rabbits with age group of 6-8 month and weight 1.5 to 2 kg were divided randomly into 04 groups of 06 animals each.

**Table-1: Grouping of Animals**

Sr. No.	Group	Animals
1.	Control Group : Not receiving any treatment	06
2.	Standard Group: Treated with Silver Sulfadiazine	06
3.	Test Group 1: Treated with <i>Ocimum sanctum</i>	06
4.	Test Group 2: Treated with Silver Sulfadiazine + <i>Ocimum sanctum</i>	06

### 2.6 Preparation of Animals

Animals were acclimatized for 8 days before experiment. Animals were housed in separate cages under standard condition of light, temperature and humidity. They were fed with standard laboratory chow and provided with water ad libitum.

**2.7 Infliction of Burn Wound** - The area on the back of the rabbit was shaved and animals were kept for fasting overnight. The next day the animals were anaesthetized using Ketamine in the dose of 50 mg/Kg of body weight I.M. 1 ml/kg of body weight. A metal disc of diameter 22 cm, thickness 5 mm and area 380.2 mm square was heated in the blue portion of the flame for 5 minute and then immediately kept on the shaved part for 30 seconds with minimal pressure <sup>7</sup>.



Fig. 1. Shaving of Back of Animal



Fig. 2. Anesthetizing Animal



Fig. 3. Infliction of Burn Wound

## 2.8 Administration and Application of Drugs

Ointment Silver Sulfadiazine was applied daily on the burn wound. *Ocimum sanctum* was administered orally in the form of distilled water suspension in the dose of 500 mg/Kg body weight once daily.

## 2.9 Estimation of Healing by Histopathological Evaluation

Histopathology was done to compare the healing status of wounds<sup>8</sup>. Samples were collected by punch biopsy on 14<sup>th</sup> day. Sample was fixed in 10% neutral buffered Formalin. Histological evaluation was performed by staining with Haematoxylin and Eosin stain and Masson's Trichome stain. Healing status of wound was assessed by following Scoring parameters.

Table-2: Parameters for Scoring of Wound Healing Status

S. No.	Parameters	Score
1.	Amount of granulation tissue	Profound – 1, Moderate – 2, Scanty – 3, Absent - 4
2.	Inflammatory infiltrate	Profound – 1, Moderate – 2, Few – 3
3.	Collagen fiber orientation	Vertical – 1, Mixed- 2, Horizontal - 3
4.	Pattern of Collagen	Reticular – 1, Mixed – 2, Fascicle - 3
5.	Amount of early collagen	Profound – 1, Moderate – 2, Minimal – 3, Absent – 4
6.	Amount of mature collagen	Profound – 1 Moderate – 2 Minimal – 3

Total healing score of each case was calculated by adding the score of individual criteria. Healing status was graded as follows:

Table-3: Status of Wound Healing

S. No.	Score Range	Status
01.	16 - 19	Good
02.	12 - 15	Fair
03.	08 - 11	Poor

## 2.10 Statistical Analysis

Results were reported as mean  $\pm$  S.D. Standard Deviation. The data were analyzed by student's 't' test, one way ANOVA followed by Dunnett's test. P values  $<0.05$  were considered statistically significant.

## 3. RESULTS AND DISCUSSION

Histopathological examination was done on day 14 of wound production to assess the healing status of wound.

**Table-4: Healing Status of Wound**

Group	Score Mean $\pm$ S.D.	Healing Status of Wound
Control	9.66 $\pm$ 5.39	Poor
Standard	15.67 $\pm$ 4.89	Fair
Os	13.83 $\pm$ 5.04	Fair
SS+Os	18.83 $\pm$ 2.99	Good

S.D.: Standard Deviation, Os: *Ocimum sanctum*, SS+Os: Silver Sulfadiazine + *Ocimum sanctum*

**Table-5: Comparison of Healing Status of Wound**

Group	Comparison of Healing Status of Wound By One Way ANOVA and Dunnett's Test			
	Mean Difference	Standard Error	P-Value	Level of Significance*
Standard	6.00	2.52	0.110	p>0.05 Not Significant
Os	4.16	2.52	0.416	p>0.05 Not Significant
SS+Os	9.16	2.52	0.005	p<0.05 Significant

\* : as compared to control group.

The most effective group was Ointment Silver Sulfadiazine + *Ocimum sanctum* and probable reason of high effectiveness was the combined effect of Ointment Silver Sulfadiazine and *Ocimum sanctum*, broad spectrum antimicrobial activity of Silver Sulfadiazine and anti-oxidant and wound healing property of *Ocimum sanctum*.

Study conducted by Seyed Jalal Hossenimehr et al<sup>9</sup> on Effect of Aloe Cream versus Silver Sulfadiazine for Healing Burn Wounds in Rats, reported that the antimicrobial effect is the major mechanism of Silver Sulfadiazine in wound healing. The silver ion binds to the organism DNA and consequently releases the sulfonamides that kill the microbes.

Another study conducted by Hoekstra MJ et al<sup>10</sup> on Effect of Silver Sulfadiazine on histopathological parameters of burn wound in pig was reported that Silver Sulfadiazine causes rapid healing through stimulating of re-epithelization, formation of granulation tissue and increase in fibroblasts.

Study conducted by Asha B et al<sup>11</sup> on Study of wound healing activity of topical *Ocimum sanctum* Linn in albino rats reported that the topical *Ocimum sanctum* treated wound, which showed greater degree of neovascularization and fibroblast proliferation indicates better granulation tissue formation and collagenization on day 4. Topical *Ocimum sanctum* showed maximum collagenization and minimum with control. Epithelization was early and complete with topical *Ocimum sanctum* on day 7.

#### 4. CONCLUSION

Group treated with Ointment Silver Sulfadiazine + *Ocimum sanctum* showed significant wound healing status, So, the most effective treatment for burn wound healing in this study was Ointment Silver Sulfadiazine + *Ocimum sanctum*.

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