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Preclinical evaluation of wound contraction property of *ocimum sanctum* with and without supportive treatment of silver sulfadiazine in Rabbits

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Abstract

Burns remain a major public health problem all over the world, especially in developing countries. *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.

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 contraction property of *Ocimum sanctum* in thermal burns with and without supportive treatment of Ointment Silver Sulfadiazine.

After the end of treatment at 28th day, group treated with Ointment Silver sulfadiazine + *Ocimum sanctum* showed 93.17±4.34 percent wound contraction (p-value 0.000, p<0.05 Significant) which was the maximum among all groups.

Keywords: Burn, *Ocimum sanctum*, Silver sulfadiazine, Wound Contraction

Introduction

Ayurveda remains one of the most ancient and yet alive tradition practiced widely in India that has a sound philosophical and experiential basis [1].

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 [2]. Wounds are injuries that results in an opening and break of the skin that causes disturbance in the normal skin anatomy and function. The process of wound healing consists of integrated cellular or biochemical events leading to the building of structural and functional integrity of injured tissues. Plant products are potential agents for wound healing, and largely preferred because of their widespread availability and effectiveness as crude preparations [3].

The gold standard in topical burn treatment is Silver sulfadiazine a useful antibacterial agent for burn wound treatment [4]. *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 [5]. *Pseudomonas aeruginosa* is a major cause of infection and death or a contributing factor in the death of patients with severe burns [6].

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 contraction property of *Ocimum sanctum* with and without supportive treatment of standard drug Ointment Silver sulfadiazine on healing of thermal burn wound in Rabbits.

Materials and Methods

This is a Preclinical experimental study on Rabbits. The Study protocol was approved by Institutional Animal Ethics Committee of the DMIMS (Deemed University) vide Ref. No. DMIMSU/IAEC/2011-12/011 dated 28.12.2011.

Procurement of Plants Material: The Plant material of *Ocimum sanctum* (Leaves Powder) was obtained from M.G. Ayurved College, Salod, Wardha, Maharashtra, India

Procurement of Ointment Silver Sulfadiazine: Ointment Silver sulfadiazine was procured from Rexcin Pharmaceuticals Private Limited, Solan, Himachal Pradesh, India

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Procurement of Anesthetic Agent: Injection Aneket (Ketamine Hydrochloride Injection I.P.) was procured from Neon Laboratories Limited, Thane, Maharashtra State, India.

Animals: 24 Rabbits were obtained from Central Animal House of University. 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.

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 : Not receiving any treatment	06
2.	Standard : Treated with Silver Sulfadiazine	06
3.	Test 1: Treated with <i>Ocimum sanctum</i> (Os)	06
4.	Test 2: Treated with Silver Sulfadiazine + <i>Ocimum sanctum</i> (SS+Os)	06

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*.

Infliction of Burn Wound: The area on the back of the rabbit was to be shaved and animal 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 mm² 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 [7].



Fig 1: Infliction of burn wound

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.

Estimation of Healing by measuring Wound Contraction
Wound contraction was monitored by measuring the progressive changes by tracing the raw wound area on a transparent paper on 1st, 7th, 14th, 21st, and 28th day of wound production. The tracing was then transferred to 1 mm² graph sheet, from which the wound surface area was evaluated [8].

Wound Contraction (%) =

$$\frac{\text{Initial Wound Size} - \text{Specific Day Wound Size}}{\text{Initial Wound Size}} \times 100$$



Fig 2: Measurement of wound contraction on trace paper

Statistical Analysis: Results were reported as mean±S.D and were analyzed by one way ANOVA followed by Dunnett's test. P values <0.05 were considered statistically significant.

Results and Discussion

Wound contraction was measured on 1st, 7th, 14th, 21st and 28th day of wound production and expressed in the form of percentage [Table 2] and was compared to control group by one way ANOVA followed by Dunnett's test [Table 3].

Table 2: Percentage of Wound Contraction

Group	Mean ± Standard Deviation				
	Day 1	Day 7	Day 14	Day 21	Day 28
Control	0±0	20.63±2.67	35.23±2.55	49.53±4.19	60.37±5.44
Standard	0±0	21.17±5.13	38.17±8.58	55.62±4.07	72±3.66
Os	0±0	14.17±4.11	30.62±6.15	50.10±3.39	65.18±2.37
SS+Os	0±0	41.75±9.00	73.37±8.51	87.07±4.81	93.17±4.34

Table 3: Comparison of Wound Contraction (%) At 28th Day

Group	Mean Difference	Standard Error	p-Value	Level of Significance*
Standard	11.63	2.41	0.000	p<0.05 Significant
Os	4.81	2.41	0.234	p>0.05 Not significant
SS+Os	32.80	2.41	0.000	p<0.05 Significant

*: as compared to control group.

After the end of treatment at 28th day, the least effective group was control group because it was not received any treatment. 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 Hossenimehr SJ *et al* [9] 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.

Study conducted by Bairy KL *et al* [10] on Effect of different formulations of Silver sulfadiazine cream on experimentally induced burn wound healing, reported that all four drugs namely Silver Sulfadiazine A, B, C and Silverex cream have significantly favored wound contraction when compared to control animals.

Study conducted by Asha B *et al* [11] on Study of wound healing activity of topical *Ocimum sanctum* Linn in albino rats reported that the topical *Ocimum sanctum* achieved 50% wound contraction by 5.7±0.97 days, and with topical control 12.5±0.29 days. Time taken for 50% contraction of topical

Ocimum sanctum is significantly less compared to topical control, and values are statistically significant.

Study conducted by Goel A *et al.* [5] on Wound healing potential of *Ocimum sanctum* Linn. With induction of tumor necrosis factor- α , concluded that the *Ocimum sanctum* has good healing potential. Feeding of *Ocimum sanctum* extract accelerated in TNF- α production may be one of the mechanisms involved or play their role to accelerate the healing by *Ocimum sanctum* extract.

Conclusion

After the end of treatment at 28th day, Ointment Silver sulfadiazine + *Ocimum sanctum* showed 93.17 \pm 4.34 percent wound contraction (p-value 0.000, $p < 0.05$ Significant), So the most effective group was Ointment Silver sulfadiazine + *Ocimum sanctum*

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