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Screening of Burn Wound Healing Property of *Ocimum sanctum* by Grading of Epithelial Regeneration 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, antimicrobial, anti-inflammatory and wound healing properties. The present study was carried out to evaluate the epithelial regeneration 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 significant epithelial generation (p-value 0.02, p<0.05 Significant).

Keywords: Burn, *Ocimum sanctum*, Silver sulfadiazine, Epithelial regeneration

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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 [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 antimicrobial, anti-inflammatory and wound healing properties [5]. *Ocimum sanctum* treated wound showed greater degree of neovascularization and fibroblast proliferation indicates better granulation tissue formation and collagenization and epithelization was early and complete with topical *Ocimum sanctum* [6].

Pseudomonas aeruginosa is a major cause of infection and death or a contributing factor in the death of patients with severe burns [7].

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 epithelial regeneration 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 Mahatma Gandhi 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

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].

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 Ocimum sanctum (Os)	06
4.	Test 2: Treated with Silver Sulfadiazine + Ocimum sanctum (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 [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.

Estimation of Healing by grading of Epithelial Regeneration: Grading of epithelial regeneration was done by using criteria showed in [Table 2].

Table : 2 – Criteria for Grading of Epithelial Regeneration

Sr. No.	PARAMETER	GRADING
1.	Inflammatory Response	Mild Epithelial Regeneration
2.	Granulation Tissue Formation and/or Angiogenesis	Moderate Epithelial Regeneration
3.	Repair of Connective Tissue and Epithelium and/or Remodelling	Prominent Epithelial Regeneration

Statistical Analysis: Results were reported as mean ± S.D and were analyzed by Chi-Square Test. P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Epithelial regeneration was monitored after completion of treatment, graded as mild, moderate and prominent epithelial regeneration [Figure 1], expressed in percentage [Table 3] and was compared to control group by Chi-Square Test [Table 4].

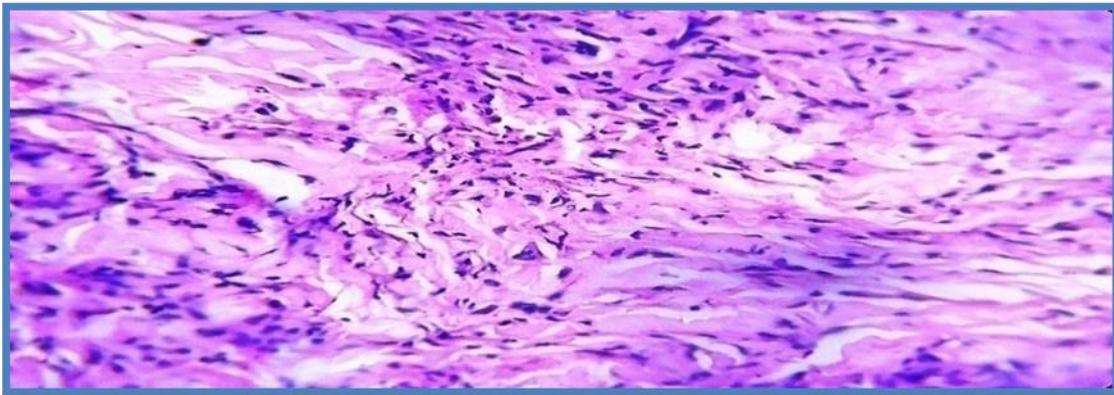
Table : 3 - Grading of Epithelial Regeneration

GROUP	GRADING OF EPITHELIAL REGENERATION		
	Mild	Moderate	Prominent
	NUMBER OF ANIMALS		
Control	04 (66.67%)	02 (33.33%)	00 (0%)
Standard	02 (33.33%)	03 (50.00%)	01 (16.67%)
Os	03 (50.00%)	03 (50.00%)	00 (0%)
SS+Os	00 (0%)	03 (50.00%)	03 (50.00%)

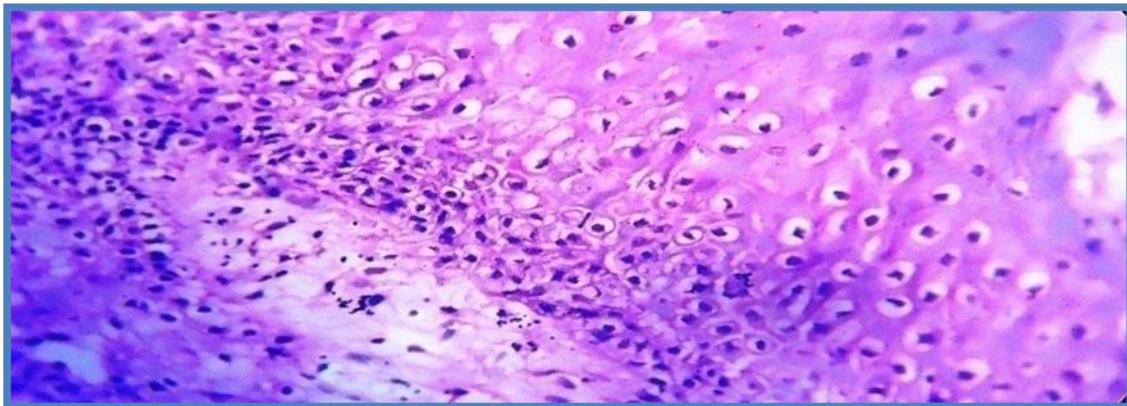
Table : 4 – Comparison of Grading of Epithelial Regeneration

GROUP	COMPARISON OF GRADING OF EPITHELIAL REGENERATION (BY CHI-SQUARE TEST)		
	χ^2 -Value	p-value	Level of Significance*
Standard	1.86	0.39	p>0.05 Not significant
Os	0.34	0.55	p>0.05 Not significant
SS+Os	7.20	0.02	p<0.05 Significant

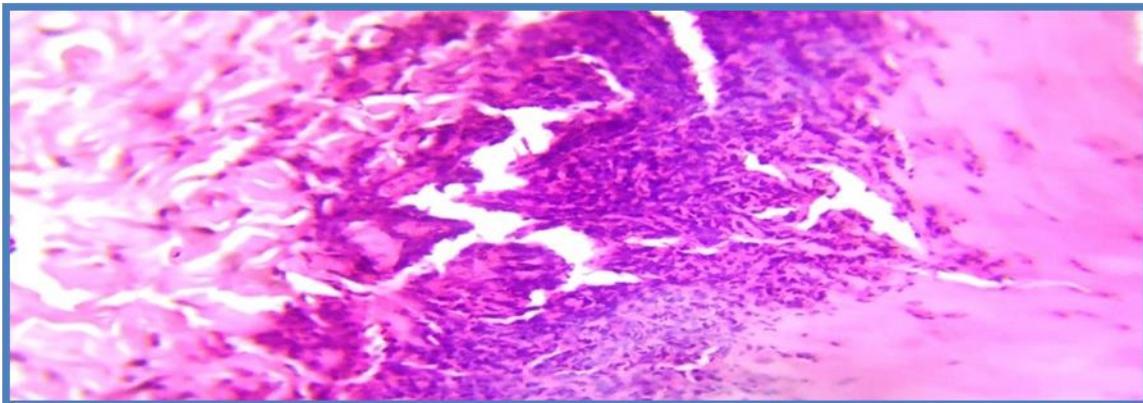
* : as compared to control group.



(a) Microscopic Structure showing Mild Epithelial Regeneration



(b) Microscopic Structure showing Moderate Epithelial Regeneration



(c) Microscopic Structure showing Prominent Epithelial Regeneration

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*.

Study conducted by Hoekstra MJ et al [9] on effect of Silver sulfadiazine on histopathological parameters of burn wound in pig was reported that silver sulfadiazine causes rapid healing through stimulating of reepithelization, formation of granulation tissue and increase in fibroblasts.

Study conducted by Asha B et al [6] 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. Epithelization was early and complete with topical *Ocimum sanctum*.

Study conducted by Gupta VK et al [8] on Evaluation of burn wound healing property of *Ocimum sanctum* by monitoring of period of reepithelization in rabbits concluded that the combination of Ointment Silver sulfadiazine and *Ocimum sanctum* showed minimum reepithelization period.

CONCLUSION

Group treated with Ointment Silver sulfadiazine + *Ocimum sanctum* showed significant epithelial regeneration (p-value= 0.02, p<0.05 Significant), so the most effective treatment was Ointment Silver sulfadiazine + *Ocimum sanctum*.

REFERECES

- [1] Patwardhan B, Vaidya ADB, Chorghade M. Ayurveda and herbal products drug discovery. Curr. Sci.2004;86:789-99.
- [2] Shrivastava P, Durgaprasad S. Burn wound healing property of *Cocos nucifera* : An Appraisal. Indian J. Pharmacol. 2008;40 suppl 4:144-46.
- [3] Kumari P, Yadav P, Verma PR, Kumar S, Arya A. A Review on Wound Healing properties of Indian Medicinal Plants. Ind. J. Fund. Appl Life Sci.2013;3 suppl 1:220-32.
- [4] Atiyeh BS, Costagliola M, Hayek SN, Dibo SA. Effect of silver on burn wound infection control and healing: Review of the literature. Burns.2007;33:139-48.
- [5] Goel A, Kumar S, Singh DK, Bhatia AK. Wound healing potential of *Ocimum sanctum* Linn. with induction of tumor necrosis factor- α . Indian J. Exp. Biol.2010;48:402-6.
- [6] Asha B, Nagabhushan A, Shashikala GH. Study of wound healing activity of topical *Ocimum sanctum* Linn in Albino Rats. J. Chem. Pharm. Res.2011;3 suppl 6:122-26.
- [7] Sutter VL, Hurst V. Sources of *Pseudomonas aeruginosa* infection in Burns: Study of Wound and Rectal Cultures with Phage Typing. Ann. Surg.1966;163 suppl 4:597-602.
- [8] Gupta VK, Pathak SS, Jain MK. Evaluation of burn wound healing property of *ocimum sanctum* by monitoring of period of reepithelization in rabbits. Int. J. Basic Clin. Pharmacol. 2016;5:146-148.
- [9] Hoekstra MJ, L Andrews. Effect of Silver Sulfadiazine on histopathologic parameters of burn wound in pig. Burns. 1993; 29:544-547.