RESEARCH ARTICLE

Evaluation of burn wound healing property of *Hemidesmus indicus* with and without supportive treatment of ointment silver sulfadiazine in rabbits

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ABSTRACT

Background: India has an estimated annual burn incidence of 6–7 million. Nearly 10% of these are life threatening and require hospitalization. *Pseudomonas aeruginosa* is a major cause of infection and death in patients with severe burns. Hence, there is a need to develop drugs which will decrease complications and prevent infections more effectively than the presently used drugs. *Hemidesmus indicus* root extract is traditionally used as antioxidant, antibacterial, and anti-inflammatory which may be responsible for faster wound healing. **Aims and Objectives:** The present study was carried out to evaluate the burn wound healing property of *H. indicus* in rabbits. **Materials and Methods:** *H. indicus*, ointment silver sulfadiazine and 24 rabbits were used in this study. Animals were acclimatized and divided randomly into four groups. After producing partial thickness, burn wound ointment silver sulfadiazine was applied daily on the burn wound and *H. indicus* was administered orally once daily. Healing was assessed by percentage of wound contraction. **Results:** At the end of treatment, control group showed 60.37 \pm 5.44% wound contraction, group treated with ointment silver sulfadiazine showed 60.97 \pm 1.69% wound contraction, and group treated with ointment silver sulfadiazine with *H. indicus* showed 81.75 \pm 6.62% wound contraction. **Conclusions:** The present study concludes that combination of ointment silver sulfadiazine with *H. indicus* was more effective than ointment silver sulfadiazine alone.

KEY WORDS: Burns; Wound Healing; Hemidesmus indicus

INTRODUCTION

Burn can be defined as tissue damage caused by a variety of agents such as heat, chemicals, electricity, sunlight, or nuclear radiation. Every year, about 2 million people receive medical treatment for burn injury.^[1]

Wound is defined as disruption of cellular, anatomical, and functional continuity of a living tissue. It may be produced

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by physical, chemical, thermal, microbial, or immunological insult to the tissue. $^{[2]}$

Pseudomonas aeruginosa is a major cause of infection and death or a contributing factor in the death of patients with severe burns.^[3]

In ancient Indian Medical System, silver has been described as therapeutic agent for many diseases. There is an increasing use of silver as an efficacious antibacterial and antifungal agent in wound care products.^[4] The gold standard in topical burn treatment is silver sulfadiazine, a useful antibacterial agent for burn wound treatment.^[5]

Hemidesmus indicus locally called as Anantamul (Indian Sarsaparilla) root extract is traditionally used as antioxidant, antibacterial, anti-inflammatory,

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immunomodulatory, and antidote in the Indian System of Medicine.^[6]

Hence, 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 effect of *H. indicus* with and without supportive treatment of ointment silver sulfadiazine.

MATERIALS AND METHODS

This was a preclinical study on rabbits conducted in the Department of Pharmacology, J. N. Medical College, Wardha, and Central Research Laboratory and Central Animal House of Datta Meghe Institute of Medical Sciences (Deemed University), Wardha. The duration of the study was 2 years (2012–2014).

Approval from the Institutional Animal Ethics Committee

The research protocol was approved by the Institutional Animal Ethics Committee of Datta Meghe Institute of Medical Sciences (Deemed University), Wardha, Maharashtra, India, on December 28, 2011, vide Ref. no. DMIMSU/IAEC/2011–2012/011.

Procurement of Plant Material

The plant material of *H. indicus* (powder) was obtained from "Rasashala" of Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha.

Procurement of Ointment Silver Sulfadiazine

Ointment silver sulfadiazine was procured from Rexcin Pharmaceuticals Private Limited, Solan, Himachal Pradesh.

Animals

Twenty-four New Zealand white rabbits were used in this study and were obtained from Central Animal House of Datta Meghe Institute of Medical Sciences (Deemed University), Sawangi (Meghe), Wardha.

Inclusion and Exclusion Criteria

Rabbits of both sexes (either male or female) of the age group of 6–8 months and weighing 1.5 kg–2 kg were included in this study. Unhealthy and pregnant rabbits were excluded from the study.

Acclimatization of Animals

Animals were acclimatized for 8 days in the Central Animal House before. 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*.

Grouping of Animals

Twenty-four rabbits with the age group of 6-8 months and weight 1.5-2 kg were divided randomly into four groups of six animals each [Table 1].

Infliction of Burn Wound

The area on the back of the rabbit is to be shaved and animal kept for fasting overnight. The next day the animals are anesthetized 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^2 is heated in the blue portion of the flame for 5 min and then immediately kept on the shaved part for 30 s with minimal pressure.^[7]

Administration and Application of Drugs

Ointment silver sulfadiazine was applied daily on the burn wound. *H. indicus* was administered orally in the form of distilled water suspension in the dose of 500 mg/kg body weight once daily.

Assessment of Healing By Estimation of Wound Contraction

Wound contraction was monitored by measuring the progressive changes by tracing the raw wound area on a transparent paper on 1^{st} , 7^{th} , 14^{th} , 21^{st} , and 28^{th} days of wound production. The tracing was then transferred to 1 mm^2 graph sheet, from which the wound surface area was evaluated.^[8]

Initial wound size -Wound contraction (%) = $\frac{Specific day wound size}{Initial wound size} \times 100$

| Table 1: Grouping of animals | | | | |
|--------------------------------------|-------------------|--|--|--|
| Group | Number of animals | | | |
| Control: Not receiving any treatment | 6 | | | |
| Standard: Treated with SS | 6 | | | |
| Treated with Hi | 6 | | | |
| Treated with SS with Hi | 6 | | | |

Hi: Hemidesmus indicus, SS: Silver sulfadiazine

| Table 2: Percentage of wound contraction | | | | | | | |
|--|---|------------|------------|--------------------------------|------------|--|--|
| Group | Percentage of wound contraction (mean±SD) | | | | | | |
| | 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 | 72.00±3.66 | | |
| Hi | 0±0 | 19.48±8.01 | 36.03±8.14 | 49.35±7.94 | 60.97±1.69 | | |
| SS+Hi | 0±0 | 41.68±5.77 | 55.97±7.35 | 67.62±4.40 | 81.75±6.62 | | |
| Group | Comparison of percentage of wound contraction at 28 th day (by one-way ANOVA and Dunnett's test) | | | | | | |
| | Mean difference | SE | Р | Level of significance | | | |
| | | | | (as compared to control group) | | | |
| Standard | 11.63 | 2.41 | 0.000 | P<0.05 (significant) | | | |
| Hi | 0.60 | 2.41 | 1.000 | P>0.05 (not significant) | | | |
| SS+Hi | 21.38 | 2.41 | 0.000 | P<0.05 (significant) | | | |

SE: Standard error, SD: Standard deviation, Hi: Hemidesmus indicus, SS: Silver sulfadiazine

Statistical Analysis

Results were reported as mean \pm standard deviation. The data were analyzed by Student's *t*-test, one-way ANOVA followed by Dunnett's test. *P* < 0.05 was considered statistically significant.

RESULTS

Wound contraction was measured on 1st, 7th, 14th, 21st, and 28th days of wound production and expressed in the form of percentage of wound contraction [Table 2].

After the end of treatment at day 28, control group showed $60.37 \pm 5.44\%$ wound contraction, standard group treated with ointment silver sulfadiazine showed $72 \pm 3.66\%$ wound contraction, group treated with *H. indicus* showed $60.97 \pm 1.69\%$ wound contraction, and group treated with ointment silver sulfadiazine with *H. indicus* showed $81.75 \pm 6.62\%$ wound contraction.

DISCUSSION

Group treated with ointment silver sulfadiazine and *H. indicus* showed significant percentage of wound contraction (81.75 \pm 6.62%). This may be due to combined effect of silver sulfadiazine and *H. indicus*. Beneficial healing effect may be due to antimicrobial activity and wound healing property of ointment silver sulfadiazine and *H. indicus*.

The study conducted by Hosseinimehr *et al.*^[9] on the 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 and Andrews^[10] on the effect of silver sulfadiazine on histopathologic 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. The study conducted by Ganesan *et al.*^[11] reported in his study that methanolic extract of *H. indicus* showed significant wound healing activity by increasing cellular proliferation, promoting the formation of granulation tissue, and improving the healing index. Free radicals are generated at the site of injury, which impair the healing process. Methanolic extract of *H. indicus* showed that healing property may be due to anti-inflammatory and antioxidants (free radical scavenging) properties.

This is an animal study using herbal formulation for burn wound healing and the results may be beneficial to community. Small sample size was the limitation of this study. This study can be conducted with large sample size for more accuracy and for better results.

CONCLUSIONS

The present study concludes that combination of ointment silver sulfadiazine with *H. indicus* was more effective than ointment silver sulfadiazine alone.

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