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KEYWORDS: Shuddha vrana, Vrana ropana, Chandanaadi taila, hydroxy proline.

EXPERIMENTAL STUDY ON THE ROPANA EFFECT OF CHANDANADI TAILA IN SHUDDHA VRANA



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**ABSTRACT**

Vrana and its management are the fundamental practice of shalya tantra. Ropana means the factors which actively promote or quicken the healing process. Acharya Susruta in his treatise Susruta Samhita mentions about 60 upakramas for the treatment of vrana. One among them is ropana taila. This is an experimental study on Wistar albino rats to find out the ropana effect of Chandanadi taila in Shuddha vrana (tidy wounds) with excision wound model. There were 3 groups- control, standard and test, each group containing 6 rats. In control group no intervention was there. In standard group Povidone iodine ointment and in test group Chandanaadi taila was given as external application. Study period was 24 days. Observational parameters were wound contraction, period of epithelialisation, histopathology and hydroxy proline estimation, assessed on 24th day. Epithelialisation and Hydroxyproline parameters were statistically higher in trial group compared to standard. Histopathology showed higher density of collagen and normal cytoarchitecture compared to control and standard groups. Wound contraction rate was similar in standard and test groups. A pilot clinical observational study on 6 patients of shuddha vrana showed significant improvement in wound contraction, epithelialisation and reduction in pain and tenderness.

INTRODUCTION

Elaborate description of Vrana (wound or ulcer) is available in the Brihatrayees Caraka Samhita, Susruta Samhita and Ashtanga Sangraha. These primary literary sources of Ayurveda describe Vrana, their systematic classification and etiopathogenesis along with their management including various drug preparations. Among the three primary sources, the Susruta Samhita specialises in shalya tantra (ayurvedic surgery), and therefore provides more extensive details on vrana and its management. Vrana means break in the surface of body.¹ Further, vrana can be shuddha (clean) or dushta (unclean/complicated)².

Active promotion of quick healing in clean wounds was extensively practiced and described by Susruta by the name of ropana karma. Ropana karma should be done only in shuddha vrana³. Ropana karma comprises 7 topical measures namely kashaya (irrigation with decoctions), varti (impregnated cloth or cotton), kalka (fresh medicinal pastes), sarpi (ghee based topicals), taila (oil based topicals), rasakriya (cooked medicinal pastes) and avachoorana (dusting with medicinal powders) to actively promote the healing process in shuddha vrana (clean wounds)⁴.

Wound is a break in the integrity of the skin or tissue often which

may be associated with disruption of the structure and function⁵. The body's natural capacities to heal wounds though get activated immediately after the trauma and continue in a sequential manner till the formation of a healthy scar. This normal response gets disrupted in different conditions like slough, infection, blood supply to the area, presence of foreign bodies, unhygienic treatments, and general factors like nutritional conditions, presence of systemic diseases etc.

Active promotion of healing in clean wounds is a continuing challenge in current surgical practice. Presently medical practitioners are using a number of wound dressings to heal such wounds, namely, absorptive, alginates, collagen, anti microbial, composites, foams, wound filters, composites, contact layers, wound filters, hydrogels. However, none of these measures have evolved into standard, universal wound management practices. This is possibly because there is not enough evidence that they actively promote or quicken the healing process; they create the optimum environment so that healing can take place⁶.

Studies on the phenomenon of ropana can contribute significantly to the challenge of healing clean wounds. Previous works have shown promising results.

An experimental study was conducted to access the vrana ropana property of Vranarakshasa taila and Lajjalu moola taila. The study was conducted on albino rats by inducing two wound models (incision and excision). Both the trial drugs were found to be effective in bringing about wound healing action, when compared with control group. Both the trial drugs are having similar wound healing action but statistically Lajjalu moola taila was found to be more effective than Vranarakshasa taila⁷.

Another experimental study was conducted to access the vrana ropana property of Sandhyaraga (Mirabilis jalapa Linn.) in comparison with Madhusnuhi (Smilax china Linn.). The method selected for the study was excisional wounding. The study was done in 7 groups, with one control group, 3 groups receiving madhusnuhi choorna externally, internally and combined, and last 3 groups receiving sandhyaraga choorna externally, internally and combined. 18 days study was conducted. The percentage of wound contraction and period of epithelialization were assessed. It revealed that all drug treated groups showed better results than control group⁸.

This experimental study is one such effort, with additional objective parameters of hydroxy proline estimation and histopathology study. It seeks to evaluate the effect of a Ropana Taila (Chandanadi Taila) described in Susruta Samhita on shuddha vrana (clean excision wounds) induced in Wistar albino rats, compared to Standard (Povidone Iodine) & Control (no intervention). A pilot

observational clinical study was added subsequently in six patients of shuddha vrana, duration of 7 days with pre test and post test photographic observation and subjective criteria.

OBJECTIVES OF THE STUDY

- 1) To study the literature on vrana, ropana and wound healing in detail.
- 2) To study the Ropana effect of Chandanadi Taila in Shuddha Vrana in experimental model of Wistar albino rats with control and standard groups.
- 3) To observe clinical effects by means of a pilot observational clinical trial on 6 patients of vrana, 7 days duration, with pre-test and post-test photographic observation and subjective criteria.

Materials and methods:-

This is an experimental study on Wistar albino rats.

Source of data:-

Experimental animals:

Wistar albino rats were selected from animal house of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The experimental protocol was approved by the institutional animal ethical committee.

Rats were fed with normal rat diet and water ad libitum throughout the study. They were acclimatized in the laboratory condition for two weeks prior to experimentation. The housing provided had controlled lighting of 12:12h light and dark cycle. Temperature of 25°C and relative humidity of approximately 50%. As bedding material filter papers were provided. The cage cleaning, and changing the filters paper has done daily.

Materials required:-

1. 18 Wistar albino rats (250g-500g)
2. Povidone iodine ointment
3. Chandanaadi Taila
4. Albino rat cages
5. Scissors
6. Betadine solution
7. Mosquito forceps
8. Artery forceps
9. Blunt forceps
10. Surgical cotton
11. Surgical gauze
12. Surgical gloves
13. Scalpels, etc.

Route of Administration: External Application

Duration of study: 24 days

Animal grouping:-

Group	No. Animals	Treatment provided
Control	6	No intervention
Standard	6	Povidone iodine ointment
Test (Chandanadi Taila)	6	Chandanadi Taila

Statistical analysis:-

The data obtained was analyzed by employing one way ANOVA followed by Dunnet's multiple t-test as post hoc test. Graph pad Inst 3 would be used for this purpose. A p value of less than 0.05 was considered to indicate statistical significance.

Inclusion criteria:-

Healthy 18 albino rats of either sex with average weight of 250-500gm had selected randomly for the study.

Exclusion criteria:-

- Which does not fulfill the above criteria.
- Infected rats.

Parameters	Control	Standard
Pre-healed rats	00(0%)	02(5%)
Respiratory depression	00(0%)	00(0%)

Those 18 Wistar albino rats which are under other experiments had excluded.

Experimental protocol:-

Detailed study plan:

Excision wound:

The animals were anesthetized by using ketamine [50 mg/kg, IP] and xylazine [3.2 mg/kg, IM]. An impression has made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved one day prior to the experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm². Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The animals were then grouped and treated as follows:

Group I: No intervention,

Group II: Povidone iodine ointment,

Group III: Test drug (Chandanadi Taila).

Parameters estimated:-

1. Period of Epithelialization - falling of scar, leaving no raw wound behind has been taken as end point of complete epithelisation and the days required for this were taken as period of epithelisation.
2. Hydroxy proline estimation of the wound bed, after the 24 days of the study.
3. Histopathological study of the wound bed, after 24 days of the study.
4. Wound area contraction - observed on 4th, 8th, 12th, 16th, 20th & 24th day of post wounding

STATISTICAL ANALYSIS AND RESULTS

1. Scar falling day:-

Data: MEAN ± SEM, *P<0.05

The data shows there was decrease in wound healing days observed in standard group and test group when compared to the control group; the observed decrease was found to be statistically non significant.

The data shows there was decrease in wound healing days observed in test group when compared to the standard group, the observed decrease was found to be statistically non significant.

2. Hydroxy proline estimation:-

Data: MEAN ± SEM, **P<0.01

The data showed lower Hydroxy proline estimates in standard group when compared to control group; the difference was found to be statistically very significant.

The data showed lower Hydroxy proline estimates in test group when compared to the control group; the difference was found to be statistically non significant.

The data showed higher Hydroxy proline estimates in test group when compared to the standard group; the difference was found to be statistically non significant.

3. Wound area contraction:-

Data: MEAN ± SEM

The data related to the percentage change in wound area on 4th day can be found in table 1a In control group 5.56% decrease in wound area was observed.

The data shows there was increase in wound contraction observed in standard group when compared to the control group; the observed increase was found to be statistically non significant.

The data shows there was decrease in wound contraction observed in test group when compared to the control group; the observed decrease was found to be statistically non significant. In test group when compared to the standard group, the observed decrease was found to be statistically non significant.

In control group marginal reduction (67.51) in wound area was observed.

The data shows there was increase in wound contraction observed in standard and test groups when compared to the control group; the observed increase was found to be statistically non significant. In test group when compared to the standard group, the observed increase was found to be statistically non significant.

In control group 84.38% reduction in wound area was observed.

The data shows there was increase in wound contraction observed in standard and test group when compared to the control group; the observed increase was found to be statistically non significant.

The data shows there was decrease in wound contraction observed in test group when compared to the standard group; the observed decrease was found to be statistically non significant.

In control group marginal 94.36% reduction in wound area was observed.

The data shows there was increase in wound contraction observed in standard and test group when compared to the control group ; the observed increase was found to be statistically non significant.

The data shows there was decrease in wound contraction observed in test group when compared to the standard group, the observed decrease was found to be statistically non significant.

In control group marginal 99.45% reduction in wound area was observed.

The data shows there was increase in wound contraction observed in standard group when compared to the control group; the observed increase was found to be statistically non significant.

The data shows there was decrease in wound contraction observed in test group when compared to the control group and standard group ; the observed decrease was found to be statistically non significant.

On 24th day all the wounds in all the three category were completely healed.

RESULTS

The experimental study had 4 observational parameters

1. Speed of wound area contraction
2. Period of complete epithelialisation observed by scab falling with no raw area below.
3. Hydroxy proline estimation of the wound bed on 24th day of the study
4. Histopathological study of the wound bed on 24th day of the study

In wound area contraction, the line diagram showed an early steep contraction in test group compared to control group and standard. However there was no statistical difference in test group compared to standard and control.

In period of complete epithelialisation, all the test group rats showed early scab falling compared to control group rats. 4 out of 6 test rats showed early scab falling compared to standard group.

Statistically the test group showed significant results ($p < 0.05$) compared to control group. However, statistically the test group showed non-significant results when compared to standard group.

In Hydroxy proline estimation control group showed higher of Hydroxy proline content than standard and test group, and test group had more Hydroxy proline than standard. Statistically, test group and control had no significant difference, indicating that test group had high hydroxyproline comparable to control. The statistical difference was highly significant amongst control and standard, indicating that hydroxyproline content in standard group was very low.

In Histopathological study on 24th day, the test group showed higher density collagen than standard and control group.

In observational clinical study, overall in 6 patients at the end of 7 days, there was significant wound contraction and epithelialisation. Pain and tenderness were reduced.

CONCLUSION

From experimental study and clinical study following conclusions were obtained:

- In scab falling days Chandanaadi taila is more effective than Povidone iodine
- The estimated Hydroxyproline level was more in Chandanaadi taila group than standard Povidone iodine group. It reveals better wound healing property of Chandanaadi taila
- In histopathology study higher density collagen fibres and better cytoarchitecture are seen in Chandanaadi taila group, again revealing its better wound healing property.
- In wound contraction Chandanaadi taila is equally effective with Povidone iodine in Shuddha vrana.
- Pilot clinical study revealed that Chandanaadi taila showed marked epithelialisation and wound contraction within 7 days.
- No adverse effects were detected in experimental or clinical study.

Overall, experimentally and clinically, Chandanaadi taila is a safe and very effective ropana taila in the context of shuddha vrana, and better compared to standard Povidone iodine ointment.

This study provides adequate objective evidence and empirical clinical tolerance and effectivity to establish that chandanadi taila can be safely used in clinical practice for ropana of shuddha vrana. However, clinical studies with large sample size including objective parameters will add more evidence to the efficacy of Sushruta's unique practice of ropana with Chandanaadi taila, a relatively simple but very effective formulation.

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