EVALUATION OF THE WOUND HEALING EFFECT OF HERBAL OINTMENT FORMULATED WITH SALVIA SPLENDENS (SCARLET SAGE)

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ABSTRACT

The evaluation of wound healing effect of herbal ointment formulated with Salvia splendens (Scarlet Sage) embedded in the ointment bases having strength of 5%, 10% and 15% which have been evaluated in vivo using the excision wound healing model, incision wound healing model and various biochemical parameters like Collagen, Mucopolysaccharides, DNA and Proteins. The evaluation of wound healing is compared with that of Nitrofurazone ointment (0.2% w/w). Salvia splendens was extracted using methanol and the extract formulated as herbal ointments. The herbal ointments were used to treat wounds inflicted on experimental Albino Mice. The wound healing effect of the formulations were compared to that of a standard antibiotic Nitrofurazone. In all cases, there was a progressive decrease in wound area with time, indicating the efficacy of the formulations in healing the induced wounds. By the 18th day, the ointment containing 15% w/w of Salvia splendens in ointment base showed 100% healing. The wound areas in the animals treated with the standard Antibiotics, Nitrofurazone showed a 100% healing by the 18th day, indicating that the plant extract, at that given concentration, had a better wound healing property than the standard antibiotic. The granulation tissue weight and hydroxyproline content in the dead space wounds were also increased significantly, in the herbal ointment formulated with Salvia splendens in the treated animals as compared with the standard antibiotic Nitrofurazone treated animals.

Keywords: Wounds, Healing, Herbal ointment, Salvia splendens, Nitrofurazone (0.2% w/w)

INTRODUCTION

Wound healing, or wound repair, is an intricate process in which the skin (or another organ-tissue) repairs itself after injury.1 In normal skin, the epidermis (outermost layer) and dermis (inner or deeper layer) exists in a steady-state equilibrium, forming a protective barrier against the external environment. Wounds are unavoidable events of life wounds may arise due to any agent that induces stress & injury and their healing has been one of the well-known problems. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and function. Treatment is therefore aimed at minimizing the undesired consequences. Management of under healing of wounds is a complicated and expensive program and research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Some of these drugs have been screened scientifically for evaluation of their wound healing activity in different pharmacological models, but the potential of many of the traditionally used herbal agents remains unexplored. In few cases, active chemical constituents were identified. Hence, there is dearth of rational pro-healing agents for the wound management programme, which can hasten the healing process.

Salvia Splendens (Scarlet Sage or Red Salvia) family Lamiaceae, is one of the Ornamental plant, having medicinal value which has not been fully studied scientifically. The plant is found in Brazil and in India. Traditionally, the leaves are used for the treatment of wounds, ulcer and also applied for itchy skin. The roots are used as cold and cough. The seeds of the plant is useful in emetic, dysentry, Haemorroids and colic disorders. It has been reported that the leaves contain essential oil are Salviorin A, α-pinene, β-pinene, linalyl, thujone, camphor, bornoil and bornyl acetate. A large number of mono- and sesquiterpenoids and small amount of triterpenoids and steroids.

Even though, traditionally, leaves of Salvia splendens (Scarlet Sage) were extensively used for the treatment of variety of wounds; however, no scientific data in its support is available. The present study was undertaken to ascertain the effect of hydroalcoholic extract of Salvia splendens (Scarlet Sage) leaves on experimentally induced wounds in rats, 3,4,5,6,7,8,9,10,11,12,13,14,15,16,17

MATERIALS AND METHODS

Collection, Authentication and Preparation of plant sample

The leaves of Salvia splendens ( Lamiaceae) was collected from Mecon Nursery, Lac Research Center, Namkum, Hazaribag Nursery of Hazaribagh and Ranchi Districts. The plant were authenticated by National Herbarium, Botanical Survey of India, Shibpur, Botanical Garden, Kolkata. The voucher specimen was submitted in the Department of Pharmaceutical Sciences, B.I.T., Mesra.(Herbarium no. CNH-I-1(44)/2006/Tech.II)

The leaves of Salvia splendens were dried in shade for about a week followed by drying at 30°C ~ 40°C in oven for a day. The leaves were then grind to coarse powder in an iron Mortar and Pestle. The powdered materials was passed from sieve no. 20 . Finally, the powder is used for extraction.

Preparation of extract

Dried leaves of Salvia splendens ( about 60 grams) will be extracted with 300 ml Methanol (80%) in a soxhlet apparatus for 72 hrs. After extraction, the solvent will be filtered and then evaporated under reduced pressure.

Total yield = 0.341 g

Ointment preparation for topical application

An alcohol free extract of Salvia splendens leaf extract was used for the preparation of the ointment for topical application. A 10% (w/w) and 15% (w/w) of extract ointment was formulated using soft white paraffin base.
Chemicals
All chemicals and reagents used were of analytical grade.

Experimental animals
Albino rats (Rattus norvegicus) of 140±20g body weight and were used in study. Animals were procured from Laboratory Animal house (Reg no. 621/02/ac/PCPSEA) of Birla Institute of Technology, Mesra. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-27°C and humidity 60-65%) with 12:12 light: dark cycles. Food was provided in the form of dry pellets and water ad libitum. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal Ethics Committee.

Model design
The design of wound healing activity was performed by two models:

1) Excision Wound Model
2) Incision Wound Model

1) Excision wound model19-22
The back of each rat was shaved under Pentobarbitone (4mg/kg) anesthesia and prepared for operation. Thereafter open circular wound of 500mm² area was produced in each rat by excising the skin. For this purpose a marker was used to mark the area to be excised. The wounded animals were kept separately. Rats wound were left undressed to the open environment, this model was used to monitor wound contraction and epithelisation time. The standard drug (0.2% w/w nitrofurazone ointment), simple ointment; methanolic extract ointment 10%w/w and 15%w/w of leaves of Salvia splendens were applied everyday till the wound was completely healed.(Table no.1, Fig. 2)

Measurement of wound area:23
The progressive changes in wound area were measured planimetrically by tracing the wound margin on a graph paper every alternate day. The changes in healing of wound i.e the measurement of wound on graph paper was expressed as unit (mm²). Wound contraction was expressed as percentage reduction of original wound size.

\[
\text{Healed Area} \times 100
\]

\[
\% \text{Wound Contraction} = \frac{\text{Total Area}}{\text{Healed Area}}
\]

Method
30 albino rats were used for excision wound model and the ointment is applied topically and animal were divided into following groups:

Group- I : No ointment was applied and served as control.
Group- II : Ointment base was applied and served as vehicle control.
Group- III : 10%w/w ointment was applied once daily.
Group- IV : 15%w/w ointment was applied once daily.
Group- V : Nitrofurazone ointment (0.2%w/w) was applied once daily

Six animals were taken in each group. All the above mentioned treatments were started from the day of operation and continued till the 20th day of healing. On 2nd, 4th, 8th, 10th, 12th, 14th, 16th, 18th and 20th days the wound area of each rat was traced on a graph paper and measured with the help of planimeter.25

Biochemical analysis of wound tissue24,25,26
The animals were anaesthetise on 4th, 8th and 12th day after treatment. Then, the granulation tissue were removed from each wound and tissue was divided into two parts for following analysis:

- Estimation of mucoplysacharides (Table 2) and collagen (Table 3)
- Estimation of DNA (Table 4)

2) Incision wound model27,28
A 6 cm long abdominal incision was made in shaved area of anesthetized rat and closed with interrupted sutures at a distance of 1cm. Thereafter they were kept individually in different cages.

Method
30 albino rats were used and an incision wound was made. These animals were divided into following groups:

Group- I : No ointment was applied locally and served as controls.
Group- II : Ointment base was applied once daily vehicle control.
Group- III : 10%w/w ointment was applied once daily.
Group- IV : 15%w/w ointment was applied once daily.
Group- V : Nitrofurazone (0.2%w/w) ointment was applied once daily

Six animals were taken in each group. On the 10th day the animals were sacrificed and there tensile strength was measured as follows: After sacrificing the animals after anaesthesia, sutures were gently pulled out. Both wound areas from each animal were removed carefully. Wound stripes of equal size (width) were then cut using a knife in which two blades were fixed at a fixed distance. Both ends of each strip were fixed with the help of a pair of steel clips. One clip allowed hanging on a stand and a polyethylene bottle was then allowed to fill with water gradually till the wound strip was broken at the site of wound. The amount of water required to break the wound was noted and expressed as tensile strength of wound in gms. (Table 5, Fig. 1)

Fig. 1: View of incision wound model
Table 1: Evaluation of *Salvia splendens* leaves extract and Nitrofurazone ointment on

<table>
<thead>
<tr>
<th>Post wounding days</th>
<th>Wound area (mm²) (mean±SE) and percentage of wound contraction</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple ointment</td>
<td>Nitrofurazone ointment</td>
<td>Salvia extract 10%/w ointment</td>
<td>Salvia extract 15%/w ointment</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>526±3.1</td>
<td>512±2.7</td>
<td>531±2.9</td>
<td>522±4.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>438±2.2 (16.7%)</td>
<td>414±14.1 (19.1%)</td>
<td>426±1.7 (19.80%)</td>
<td>407±2.2 (22.00%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>392±3.4 (25.5%)</td>
<td>306±2.6** (40.20%)</td>
<td>352±1.6 (33.70%)</td>
<td>331±5.1* (36.60%)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>314±4.2 (35.2%)</td>
<td>233±2.8** (54.50%)</td>
<td>293±3.3* (44.80%)</td>
<td>246±2.8* (52.9%)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>306±3.9 (41.8%)</td>
<td>109±1.6** (63.10%)</td>
<td>228±4.3* (57.1%)</td>
<td>176±5.2** (66.3%)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>299±0.8 (45.1%)</td>
<td>108±2.2 ** (78.90%)</td>
<td>165±1.7** (68.9%)</td>
<td>117±5.6** (77.60%)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>268±2.7 (49.0%)</td>
<td>64±1.8** (87.50%)</td>
<td>128±3.4** (75.9%)</td>
<td>73±1.8** (86.00%)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>242±1.6 (54.0%)</td>
<td>30±2.2** (94.10%)</td>
<td>82±1.4** (84.50%)</td>
<td>34±2.1** (93.50%)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>218±0.8 (58.5%)</td>
<td>8±0.2 ** (98.40%)</td>
<td>36±1.1** (93.20%)</td>
<td>11±0.1** (97.90%)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>196±2.4 (62.7%)</td>
<td>0±0.0** (100%)</td>
<td>12±0.8** (97.7%)</td>
<td>0±0.00** (100%)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>18±7.3.6 (64.4%)</td>
<td>0±0.0* (100%)</td>
<td>0±0.0** (100%)</td>
<td>0±0.00** (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2: Excision wound model--- A. Day 0 (Control); B. Day 0 (Treated); C. Methanolic extract (after 18 days)

Table 2: Effect of *Salvia* extract (10% and 15%/w/w) ointment and Nitrofurazone (0.2%/w/w) on mucopolysaccharide content of granulation tissue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mucopolysaccharide content in granulation tissue on post wounding day (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th</td>
</tr>
<tr>
<td>Vehicle (simple ointment)</td>
<td>0.9±±0.11</td>
</tr>
<tr>
<td>Nitrofurazone (0.2%/w/w ointment)</td>
<td>1.8±±0.02*</td>
</tr>
<tr>
<td>Salvia extract (10%/w ointment)</td>
<td>1.21±±0.02*</td>
</tr>
<tr>
<td>Salvia extract (15%/w ointment)</td>
<td>1.43±±0.01*</td>
</tr>
</tbody>
</table>

Result were statistically significant compared with the corresponding control values; (simple ointment) and P-values were calculated by student’s t-test (n=6); *P<0.05, **P<0.1, ***P<0.01, ****P<0.001

Table 3: Effect of *Salvia* extract (10% and 15%/w/w) ointment and Nitrofurazone (0.2%/w/w) ointment on collagen content of granulation tissue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Collagen content in granulation tissue on post wounding day (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th</td>
</tr>
<tr>
<td>Vehicle (ointment base)</td>
<td>2.1±±0.24</td>
</tr>
<tr>
<td>Nitrofurazone (0.2%/w/w ointment)</td>
<td>3.57±±0.11****</td>
</tr>
<tr>
<td>Salvia extract (10%/w/w ointment)</td>
<td>2.23±±0.18</td>
</tr>
<tr>
<td>Salvia extract (15%/w/w ointment)</td>
<td>3.0±±0.05***</td>
</tr>
</tbody>
</table>

Result were statistically significant compared with the corresponding control values (simple ointment) and P-values were calculated by student’s t-test (n=6) *P<0.05, **P<0.02, ***P<0.01,** **P<0.001

Table 4: Effect of *Salvia* extract (10% and 15%/w/w) and Nitrofurazone (0.2%/w/w) ointment on DNA content of granulation tissue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DNA content in granulation tissue on post wounding day (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th</td>
</tr>
<tr>
<td>Vehicle (ointment base)</td>
<td>5.15±±0.13</td>
</tr>
<tr>
<td>Nitrofurazone (0.2%/w/w ointment)</td>
<td>9.03±±0.12**</td>
</tr>
<tr>
<td>Salvia extract (10%/w/w ointment)</td>
<td>6.25±±0.02**</td>
</tr>
<tr>
<td>Salvia extract (15%/w/w ointment)</td>
<td>7.18±±0.04**</td>
</tr>
</tbody>
</table>

Result were statistically significant compared with the corresponding control (simple ointment) and P-values were calculated by student's t-test (n=6) *P<0.05, **P<0.001.
Table 5: Effect of Salvia extract (10% and 15% w/w) ointment and Nitrofurazone (0.2% w/w) ointment on Tensile Strength

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tensile strength after 10 days of incision wound (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (simple ointment base)</td>
<td>260.16±10.11</td>
</tr>
<tr>
<td>Nitrofurazone (0.2% w/w) ointment</td>
<td>399.16±6.45*</td>
</tr>
<tr>
<td>Salvia extract (10% w/w) ointment</td>
<td>331.16±7.14*</td>
</tr>
<tr>
<td>Salvia extract (15% w/w) ointment</td>
<td>366.83±3.41*</td>
</tr>
</tbody>
</table>

Results were statistically significant compared with the corresponding control (simple ointment) and P-values were calculated by student’s t-test (n=6) *P<0.001

RESULTS

Wound healing by excision wound method in rats

Result were statistically significant compared with the corresponding control values (simple ointment) and P-values were calculated by student’s t-test (n=6) *P<0.001

Effect of topical application of leaves of Salvia extract (10% and 15% w/w) on Excision and Incision wound model

For excision wound model the effect of topical treatment of extract at 10% and 15% w/w ointment showed, the significant (P<0.01) increase in the contraction rate of animals treated as compared with control on all days of the treatment (Table 1, Graph 1), whereas the tensile strength of incised wound on 10th day of wounding were...
treated with extract (10% and 15% w/w) was significant at (P<0.001) as compared with control (Table 5, Graph 2).

Biochemical studies

Effect of topical application of extract (10% and 15% w/w) on Muco polysaccharide content of granulation tissue.

The topical treatment group of 10% and 15% w/w was significant at P<0.001, the amount of hexosamine content of granulation tissue on 4th day increases as compared to control.

Effect of topical application of extract (10% and 15% w/w) on DNA content of granulation tissue.

Effect of topical treatment of extract 10% and 15% w/w on DNA content of the granulation tissue was highly significant (P<0.001) on the 4th day other than 8th and 12th day and as compared with control (Table no. 4, Graph 3)

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23. Mazumdar (Professor, Birla Institute of Technology, Mesra, Ranchi) for their encouragement and providing facilities to carry out this work.