Wound healing activity of Methanolic extract of three Medicinal plants

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Abstract

Hamiltonia suaveolens, Sphaeranthus indicus and Ziziphus jujuba Mill are one of the most important traditional medicinal plants. The primary indigenous use of these plants appears to be of the leaves, flowers and root as a topical treatment for wound healing. The Methanol extract of leaves, flower and root of these plants were used to evaluate the wound-healing activity in rats, using excision and dead space wound models. Animals were randomly divided into six groups of six for each model. Test group animals in each model were treated with the Methanol extract of H. suaveolens, S. indicus and Z. jujuba topically in the form of ointment and the control group animals were maintained with no application. Healing was assessed by the rate of wound contraction, time until complete epithelialization, granulation tissue weight and hydroxyproline content. On day 16, the extract-treated animals exhibited 100% reduction in the wound area when compared with controls which exhibited 63%. The granulation tissue weight and hydroxyproline content in the dead space wounds were also increased significantly in Z. jujuba treated animals compared with controls (P<0.001). Enhanced wound contraction, decreased epithelialization time and increased hydroxyproline content suggest that S. indicus and Z. jujuba root extract may have therapeutic benefits in wound healing.

Keywords: Excision and Dead space wound model, Hydroxyproline, Hamiltonia suaveolens, Sphaeranthus indicus and Ziziphus jujuba.

Introduction

The Indian traditional system of medicine is based on pragmatic facts of the observations and the experience over millennia. More than 1200 diseases are mentioned in different classical texts. Traditional medicine, being a significant element in the cultural patrimony, still remains the main choice for a large majority of people for treating various diseases and ailments. Management in various forms of diseases like Diabetes, Cardiovascular disorders, hepatoprotective, antibacterial, antifungal and the wound healing etc. are made with more than 1000 medicinal plants (89.93%); 58 minerals, metals, or ores (5.24%) and 54 animal and marine products (4.86%)1.

Modern medicine has certain essential polypeptides of the low concentration in the serum of animals, called growth factors2, and controls the cell proliferation. However, a recent study revealed that some of these growth factors may have serious consequences and undesirable, such as cancer3. Classic management of wounds follows different therapeutic steps, starting from the sterile clothing and ending with the rehabilitation of the natural structure and function4. The aim of these remedial measures is not only to speed up the healing process, but also to maintain the quality and aesthetic of healing.

As described in the literature and 70% of wound healing drugs are of plant origin, and 20% of the origin of the metal, and the remaining 10% of the animal products5. These drugs include being effective in different conditions such as wounds and ulcers, sinusitis, abscess, sore syphilis, and larvae in wounds, and wounds sewage, inflammatory changes of wounds, cellulitis, purulative ulcer, cinder diabetes, fistula infrastructure anus. Scientific research from reaching the wound was conducted healing properties of some plants4,5,6. Here an attempt has been made to evaluate three medicinal plants for their wound healing activity.

Materials and Methods

Preparation of extract: The plant materials were collected from North Maharashtra Region except H. suaveolens was collected from Chikhaldara forest, District Amaravati, Maharashtra state, India. The plant materials were shade dried. After complete drying the plant material was crushed and grinded to form coarse powder. One kg of dried powdered plant material was exhaustively extracted in soxhlet apparatus with successive solvents. Methanol extract was selected for further detailed study, on the basis of results obtained from screening of nine selected plants in the laboratory. The solvent extract so obtained was then filtered to remove any suspended impurities. Each extract was separately concentrated under reduced pressure and controlled temperature (55°C to 60°C). All the extracts of plants were preserved in a desiccator. Thus MeOHx of each plants obtained were screened for their wound healing activity in simple ointment form in rat model.
Processing: It has prepared the way IP formulation. And took a 0.3% preservatives (methyl paraben, propyl paraben), and 30% moisturizers (petroleum jelly) and 19.5% emulsifying wax in 500 ml of distilled water and 1.5% factor emulsifying (cetyl alcohol) and 15% glycerine at 45% prepared ointment paraffin Liquid. Were mixed oil and water phases at 700C and then was added 10% methanolic extract of each plant separately. Homogenized and the resulting mixture by hand homogenizer at 3000 rpm to form a creamy and stored in a tight plastic container.

Animal used: Adult Wistar strain rats (Ratus norvegicus) of both sexes, weighing between 180-200gm purchased from Yash Farm, Pune were used for the study. Animal handling and experimental protocol was permitted from the IAEC and the number is IAEC/11/cpsea/MJ/12-13.

Excision wound model: The wound healing evaluation:
Detecting the wound healing activity it was performed according to the excision wound model as illustrated by Morton and Malone and Charde et al. The animals were divided into nine groups of six animals in each.

Group-1: control; Group-2: ointment base, as the placebo; Group-3: 1%w/w Framycetine sulphate, as the standard; Group-4 and 5: 2.5% and 5% MeOHx of H.suavolens; Group-6 and 7: 2.5% and 5% MeOHx of S.indicus; Group-8 and 9: 2.5% and 5% MeOHx of Z.jujuba

All the test samples in the form of ointment were applied topically. Percent wound contraction was calculated as a percentage of the corresponding day 0 (first day) wound area in mm2.

Dead space wound model: Dead Space wounds were inflicted by implanting a polyethylene tube sterilized (300 cu mm) on the dorsal surface of each rat, 1 cm behind the ear. After wounding, on the 10th day, the granulation tissue formed on the implanted polythene tube was completely removed. The weight of the wet granulation tissue was noted and divided in two groups. First group was proceeded for biochemical estimations such as Protein, from the skin wound tissue and DNA and RNA was estimated by diphenylamine and orcinol reagent respectively and Vitamin C, another group of wet granulation tissues were dried at 60°C for 12 hours, and weighed, and the weights were recorded and proceeded for the determination of Hydroxyproline and Glucosamine (Hexosamine).

Statistical Analysis: An additional advantage of the computer system is the easy statistical processing of primary data. Data are expressed as mean ± SD using analysis of one way ANOVA followed by Dunnett’s t-test and Bonferroni’s Multiple Comparison Test by using Graph Pad software. Significance is calculated by comparison between test groups versus control group. The value of p<0.05 were considered significant.

Results and Discussion
Results obtained for the wound healing are shown in Table - 1, wound contraction progressed faster when MeOHx was applied on the wound compared to untreated wounds. In the first two days after wounding, fluid was oozing from the untreated wound (control) and to some extent from standard drug framycetin sulphate ointment treated wounds also. However, in the case of H. suaveolens leaves extract and Z. jujuba root extract treated wounds; the drug adhered on the wound and prevented the discharge from the wound within a few hours after the application.

The wound healing activity of leaves of Hamiltonia suaveolens: In the H. suaveolens treated group of rats, wounds were completely healed in less than 21 days where as in the control group of animals required more than 23 days. Even on the 8th day the wound contraction was 85% in the treated rat whereas it was only 43% in the control stated in Table - 1. Hydroxyproline level was increased at both dose level significantly (p<0.001) as indicated in Table - 2. H. suaveolens produced no change in DNA content in any of the concentrations tested. The protein and RNA content were found to increased significantly with 5% dose of MeOHx, also increased in Vitamin C and Hexosamine (Glucosamine) at 5% dose significantly (p<0.001) are recorded.

The wound healing activity of flowers of S. indicus: Table - 1 summarizes the surface area of the 2.5 and 5% S. indicus treated wound was reduced by 94 and 98% on the day 16th as compared to control (89%) was found to be significant (P<0.05). No change in DNA, Vitamin C and Glucosamine content were found, p>0.05, whereas protein, RNA and hydroxyproline content increase significantly, p<0.001 shown in Table-2. However, our results are in agreement with Sadaf who recently reported ethanolic extract of this plant exhibit 88% wound healing activity in Guinea pigs.

The wound healing activity of root of Ziziphus jujube: In the excision wound model, the percent of wound contraction was measured on every alternate fourth day. Table - 1 reveals that the percentage wound contraction was nearly 100% for MeOHx in 16 days while for the control group of animals it was 23 days (p<0.001). The wound contraction was nearly complete in treatment group of animals (100 ± 0.1) whereas for the control group of animals it was (93.82± 0.40) summarizes in Table - 1. The hydroxyproline content was also increased with decrease in eschar area. All biomolecules are increased by level at both the doses significantly (p<0.001), than other groups of animals. The wound healing may be due to occurrence either of flavonoid/glycoside/tannin or steroidal alkaloid agents in the root of Z. jujuba.
Table 1
Effect of MeOHx of three promising plants on wound contraction in excision wound rat model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>16.17±0.60</td>
<td>10.00±0.44</td>
<td>1.66±0.33</td>
<td>1.66±0.21</td>
<td>20.17±1.17</td>
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<tr>
<td>Placebo</td>
<td></td>
<td>15.00±0.51ns</td>
<td>4.66±0.33^A</td>
<td>1.00±0.00ns</td>
<td>1.70±0.28^A</td>
<td>20.50±1.05</td>
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<tr>
<td>Standard</td>
<td></td>
<td>15.17±0.30ns</td>
<td>7.83±0.65^D</td>
<td>1.66±0.21ns</td>
<td>0.50±0.22^A</td>
<td>18.50±0.83</td>
</tr>
<tr>
<td>H. suavolens I</td>
<td></td>
<td>16.17±0.47ns</td>
<td>6.83±0.60^B</td>
<td>1.16±0.16ns</td>
<td>0.00±0.00^A</td>
<td>16.17±0.75^D</td>
</tr>
<tr>
<td>H. suavolens II</td>
<td></td>
<td>15.50±0.42ns</td>
<td>5.83±0.40^A</td>
<td>0.66±0.21^C</td>
<td>0.00±0.00^A</td>
<td>15.67±1.21^B</td>
</tr>
<tr>
<td>S. indicus I</td>
<td></td>
<td>16.00±0.51ns</td>
<td>7.16±0.54^C</td>
<td>0.66±0.21^C</td>
<td>0.00±0.00^A</td>
<td>14.33±1.03^B</td>
</tr>
<tr>
<td>S. indicus II</td>
<td></td>
<td>14.67±0.33ns</td>
<td>5.50±0.34^A</td>
<td>0.16±0.16^A</td>
<td>0.00±0.00^A</td>
<td>14.03±1.03^B</td>
</tr>
<tr>
<td>Z. jujuba I</td>
<td></td>
<td>15.50±0.34ns</td>
<td>8.00±0.57ns</td>
<td>1.00±0.00ns</td>
<td>0.00±0.00^A</td>
<td>14.07±1.03^B</td>
</tr>
<tr>
<td>Z. jujuba II</td>
<td></td>
<td>16.00±0.51ns</td>
<td>5.66±0.55^A</td>
<td>0.16±0.16^A</td>
<td>0.00±0.00^A</td>
<td>13.00±1.41^B</td>
</tr>
</tbody>
</table>

All values are mean (area in mm2) ± S.D., n=6, I = 2.5% and II = 5%. A = p < 0.0001, B = p < 0.001, C = p < 0.01, D = p < 0.1 and ns = p > 0.05 vs. control.

Table 2
Profile of Bio-molecules involved in healing process in dead space wound model

<table>
<thead>
<tr>
<th>Groups</th>
<th>DNA (µg/ ml)</th>
<th>RNA (µg/ ml)</th>
<th>Protein (µg/ ml)</th>
<th>Hydroxyproline (µg/ ml)</th>
<th>Vit. C (µg/ ml)</th>
<th>Glucosamine (µg/ ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.94±0.33</td>
<td>49.61±0.36</td>
<td>89.48±0.50</td>
<td>44.41±1.11</td>
<td>41.75±0.59</td>
<td>23.23±0.79</td>
</tr>
<tr>
<td>Placebo</td>
<td>18.33±0.16ns</td>
<td>39.34±0.44^A</td>
<td>64.51±0.67^A</td>
<td>43.45±0.54ns</td>
<td>30.71±0.76^A</td>
<td>16.32±0.48^A</td>
</tr>
<tr>
<td>Standard</td>
<td>31.09±0.43^A</td>
<td>58.75±0.47^A</td>
<td>116.20±0.91^A</td>
<td>48.35±0.48ns</td>
<td>60.49±0.82^A</td>
<td>32.59±1.58^A</td>
</tr>
<tr>
<td>H. suavolens I</td>
<td>13.63±0.19^A</td>
<td>83.17±0.50^A</td>
<td>70.46±1.15^A</td>
<td>118.50±5.21^A</td>
<td>32.46±0.93^A</td>
<td>13.46±0.67^A</td>
</tr>
<tr>
<td>H. suavolens II</td>
<td>13.86±0.24^A</td>
<td>94.21±0.44^A</td>
<td>92.83±0.78^ns</td>
<td>132.40±0.50^A</td>
<td>61.37±0.88^A</td>
<td>40.62±0.95^A</td>
</tr>
<tr>
<td>S. indicus I</td>
<td>21.75±0.35^A</td>
<td>81.44±0.42^A</td>
<td>105.00±0.853^A</td>
<td>66.91±0.36^A</td>
<td>30.89±1.17^A</td>
<td>29.68±0.54^B</td>
</tr>
<tr>
<td>S. indicus II</td>
<td>21.61±0.40^A</td>
<td>124.10±0.49^A</td>
<td>144.70±1.40^A</td>
<td>143.30±0.97^A</td>
<td>32.82±0.99^A</td>
<td>29.93±1.39^B</td>
</tr>
<tr>
<td>Z. jujuba I</td>
<td>32.60±0.29^A</td>
<td>58.65±0.45^A</td>
<td>123.50±1.37^A</td>
<td>132.40±0.85^A</td>
<td>65.76±0.86^A</td>
<td>30.39±0.95^A</td>
</tr>
<tr>
<td>Z. jujuba II</td>
<td>41.24±0.27^A</td>
<td>123.50±0.54^A</td>
<td>160.50±1.00^A</td>
<td>194.00±0.80^A</td>
<td>66.43±0.82^A</td>
<td>44.74±0.94^A</td>
</tr>
</tbody>
</table>

All values are mean ± S.D., n=6, I=2.5% and II=5%. A=p<0.0001, B=p<0.001 and ns = p>0.05 vs. control.

Thus, in the present study H. suavolens and S. indicus have high percentage of necrosis as compared with Z. jujuba. Thus, this plant demonstrated outstanding activity as compared to placebo and standard group of animals.

Discussion: Wound healing usually involves inflammatory phase followed by the proliferation of fibroblasts, and the formation of collagen fibers and squeezed dry scar. These stages are concurrent but independent of each other.

In the present study, three different wound models were used. The granulation tissue of the wound is mainly constructed by fibroblast, collagen, edema and small new blood vessels. The undifferentiated mesenchymal cells of wound margin alter...
themselves into fibroblast, which starts move into the wound gap along with the fibrin strands. The collagen composed of amino acid (hydroxyproline) is the chief constituent of extracellular tissue, which gives strength and support. Breakdown of collagen release free hydroxyproline could be used as an index for collagen turnover. The improvement of the wound healing induced by ointment containing extract of H. suavolens, S. indicus, Z. jujuba, framycetin sulphate, placebo and control on rats are shown in Table - 1. Time for wound closure as well as for falling of scab by ointment, placebo and control are comparable and all resulted efficient healing percentage (89-99%) after 15 days of management. The results also indicate that H. suavolens, S. indicus and Z. jujuba have complete epithelization on an average 21.00, 20.00 and 16.00 days respectively when compared to control group of animals (23.00 days).

On the other hand, when % of the wound healing was compared between all test groups versus control, highly significance effect was found only in Z. jujuba treated group of animals. From 4th to 12th days of application significant difference in percentage of healing was observed and maximum difference in percentage of healing were recorded after 8th and 12th days of treatment as 83.66% versus 43.64% and 95.01% versus 80.99% respectively. About 20 members of family Asteraceae followed by 9 members of family Rubiaceae are reported to have wound healing activity in animals. Seven members occurred in Khandesh region i.e. North Maharashtra Region of the family Rhamnaceae. Out of these 3 members are native of this place. No report on any member of Rhamnaceae family appeared in India. About 20 members of family Asteraceae followed by 9 members of family Rubiaceae are reported to have wound healing activity in animals. Seven members occurred in Khandesh region i.e. North Maharashtra Region of the family Rhamnaceae. Out of these 3 members are native of this place.

Conclusion

Therefore, it may be concluded that under current working condition ointment containing extract of Z. jujuba has been found to be highly active healing agent. Hence these results fully justify the ethnobotanical use of the root of Z. jujuba to treat wounds. Therefore, in order to unravel the possible ingredient(s) further isolation study is warranted.

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