Wound Healing Activity Of Ethanolic Extract Of Rubia Cordifolia Roots

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ABSTRACT

The present study deals with evaluation wound healing activity of ethanolic extract of Rubia cordifolia roots. The ethanolic extract was prepared by continuous heat extraction subjected to screen for wound healing activity using incision, excision, histopathological and dead space wound model and the study was supported with evaluation of granuloma tissue to estimate hydroxyproline content. Significant increase in wound closure rate, skin breaking strength, granuloma breaking strength was observed. The hydroxyproline content was also increased with decrease in scar area. The initial healing action might be due to increased collagen deposition and better alignment, with the obtained results it can be concluded that Rubia cordifolia extract has significant wound healing activity and initial healing may be due to presence of some terpenoids and antimicrobial agents.

Keywords: Rubia cordifolia, Wound healing, Incision wounds, Excision wounds, Hydroxyproline.

INTRODUCTION

Rubia cordifolia Linn (Rubiaceae) distributed in Africa and Asia extending into Australia. The colouring matter present in the roots of Rubia cordifolia is a mixture of purpurine and munjistan is an orange dye occurs in the form of glycosides and is accompanied by isomerd. The roots also contain small amount of xanthopurpurin or purpuroxanthin and pseudopurpurine (Purpurin-3-Carboxylic acid) [1]. The roots are credited with tonic, astringent, anti-dysentric and deobstruent properties. They are also used in rheumatism and form ingredients of several ayurvedic formulations. Paste has been used for application to ulcer, inflammation and skin troubles [2,3]. The ethanolic extract of aerial part of plants shows hypoglycemic activity in albino rats. The plant also shows positive tests for sterols, terpens and saponins [4,5,6].

MATERIALS AND METHODS

Plant material and Preparation of Herbal Extract

Rubia cordifolia (RC) roots was purchased from the local market and authenticated in Department of Botany, Rashtrasant Tukoji Maharaj Nagpur University Campus, Nagpur. Initially these roots were washed with fresh water to remove adhering dirt and foreign particles. Excess of water shake off and dried at 35–40°C in an oven for 24 hours. The dried roots were subjected to crushed and grinded and the coarsed powder mass of roots weighed and allow to undergo continuous heat extraction.

The shade dried and a powdered root of Rubia cordifolia (1 kg) was extracted exhaustively with 95% ethanol in a soxhlet apparatus by continuous heat extraction. The ethanol extract was concentrated to a small volume and then evaporated to dryness. The percent yield of Rubia cordifolia roots extract is 17.26%. The dry extracts were subjected to various chemical tests to detect presence of different photochemical constituents.

Animals

In the present study male wistar rats (150-200g) were used for the study. They were individually housed and maintained on normal standard diet (Gold Muhor Brand, Lipton India limited) and water ad libitum. Temperature was maintained at 23±1°C with 12hr light and 12hr dark cycle throughout the course of the study.

Wound Healing activity [10]

Animals were wounded under light ether anesthesia, semiaseptically. The animals were assigned into three groups, each group containing six animals. First group was untreated group which was taken as control (Group 1). In second group (Group 2) wounds received topical application of RC extract, while animals in Group 3 (Reference Standard) received treatment of Framycetine Sulphate Cream (FSC) in both excision and incision wound models. No other topical or systemic therapy was given to animals during the course of this study. The experimental protocols were approved by the Institute Animal Ethical Committee.

Excision wounds [11]

Hairs were removed from dorsal thoracic central region of anaesthetized rats. Full thickness from the demarked area was excised to produce wound measuring 23±3mm. Wound was cleaned with cotton swab soaked in alcohol. The test extract and FSC cream was applied on the excised wound once daily for 24 days starting from the first day of wounding. Wound contraction was measured as percent reduction in wound area [12].

Resutured Incision wounds

Animals were anaesthetized and paravertebral incisions (2.5-3.0 cm long) were made through the entire length of skin. After the incision was made, the parted skin was kept together and stitched with nylon thread at 0.5 cm apart with curved needle (No. 11). The sutures were removed on day 8 and healing (tensile) strength was measured on day 10 [13].

Dead space wound model

Under light ether anesthesia, subcutaneous dead space wounds were inflicted in the region of axilla and groin, by making a pouch through a small nick in the skin. Granuloma formation was induced by implanting either sterile cotton pellets or grass piths. Two sterile cotton pellets weighing 10 mg (sterilized by autoclaving) were implanted in axilla by technique of D’Arcy et al as describe by Turner [14], but the granuloma were removed on 10th day. The sutures were mobbed with an alcoholic swab and animals were placed into their individual cages after recovery from anesthesia.

Physical, mechanical and histopathological changes in granuloma tissue were studied in this model. Excision of granuloma from the surrounding tissues were performed on the 10th post wounding day under light ether anesthesia. Cotton pellet granuloma excised from dead space wound were dried overnight at 60°C so
as to obtained a constant dry weight, their weights were noted and expressed as mg/100gm body weight [15]. The excised tissue was cut into two approximately equal halves. One half of the granuloma tissue was used for determination of hydroxyproline content [16]. The other part is kept in 10% formalin solution for histopathological studies to evaluate the effect of extract on collagen formation.

Histopathological study

The histopathology study was done to evaluate the healing promoters like keratinization, epithelization, collagenation, fibrosis and neovascularisation, which were evidenced to promote wound healing in the experimental animals. The section from 10 day old regenerated tissue of incision wound was taken and washed with normal saline solution to study the healing markers. After fixing the section in 10% formalin solution the tissues were dehydrated with 90% ethanol, cut into thin sliced section (7μm thick), stained with haemotoxyline-eosin dye and observed under light microscope for keratinization, epithelization, fibrosis, collagen formation and neovascularization [17]. The interpretation of the results were numbered from 1 to 5 of which 5 stands for maximum similarity and 1 stands for least similarity form normal tissue around the wound area in the RC treated and untreated wounds [18].

RESULTS

The present study deals with evaluation of wound healing activity of ethanolic extract of roots of Rubia cordifolia. The wound healing study was screened by four models like incision, excision, dead space wound model and histopathological study. The acute toxicity study of ethanolic extract of roots of Rubia cordifolia do not show any signs of toxicity up to 3g/kg body weight. Since there was no mortality at higher dose 1/10<sup>th</sup> of maximum dose of extract tested for acute toxicity was screened for evaluation of wound healing activity i.e., 300mg/kg. In the excision wound study the wounds are treated with FSC cream and RC extract shows complete healing in day 24-25. The results of these groups were compared with the healing activity of untreated group which took more than 30 days for wound closure and fall of eschar. The results are shown in Table 1. The comparative percent wound closure activity is shown in Fig. 1.

Figure-1 Comparative wound closure effect of ethanolic extract of Rubia cordifolia roots.

In incision wound study the tensile strength was measured on day 10 of regenerated tissues. The tensile strength of wounds treated with FSC cream and RC extract is 365.41±3.55 and 398.00±6.32 respectively was compared with mean tensile strength ±SEM of untreated group i.e., 281.30±5.82. The comparative tensile strength is shown in Figure 2.

In histopathological study some healing markers were evaluated like keretinization, epithelization, fibrosis, Collagenation and neovascularization. After the histopathological evaluation of slides the RC treated wound shows prominent promotion of keratinization, epithelization, fibrosis and collagen formation, the results are compared with untreated group and wounds treated with FSC cream was consider as reference standard. The comparative promotion of these markers are shown with the help of photomicrographs in Figure 3 (a), (b) and (c). The promotion of healing markers is four times more than that of untreated wound. The results are shown in Table 2.

Table 1

<table>
<thead>
<tr>
<th>Post wounding days</th>
<th>Wounding area (mm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Group 1*</th>
<th>Group 2*</th>
<th>Group 3*</th>
<th>F values</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>111.86±5.07</td>
<td>333.28±2.88</td>
<td>369.50±4.38</td>
<td>F(2,15) = 6.664</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>272.51±5.06</td>
<td>304.22±4.05</td>
<td>308.61±1.68</td>
<td>F(2,15) = 25.940</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>225.29±4.66</td>
<td>261.12±3.31</td>
<td>245.99±2.04</td>
<td>F(2,15) = 24.937</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>184.11±4.28</td>
<td>225.36±5.63</td>
<td>217.23±4.62</td>
<td>F(2,15) = 24.532</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>160.72±4.49</td>
<td>171.94±5.56</td>
<td>157.87±5.94</td>
<td>F(2,15) = 3.492</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>135.81±3.76</td>
<td>73.71±2.25</td>
<td>129.09±3.79</td>
<td>F(2,15) = 103.47</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>106.72±5.47</td>
<td>171.94±5.56</td>
<td>157.87±5.94</td>
<td>F(2,15) = 161.53</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>114.4±4.51</td>
<td>106.59±4.42</td>
<td>144.96±2.24</td>
<td>F(2,15) = 207.29</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>97.26±3.58</td>
<td>97.54±3.58</td>
<td>135.81±3.79</td>
<td>F(2,15) = 570.40</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>66.9±2.57</td>
<td>106.59±4.42</td>
<td>144.96±2.24</td>
<td>F(2,15) = 671.29</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM of animals in each group. Number in parenthesis indicates percent of wound contraction. All the values are significant at p<0.001 as compared to Group 1. * n = 6, ^ topical application
Table 2: Histopathological examination of wounds treated with ethanolic extract of Rubia cordifolia roots at the end of 10 Days *.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>F values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratinization</td>
<td>0.48±0.03</td>
<td>4.00±0.09</td>
<td>3.66±1.22</td>
<td>F(2,15) = 457.31</td>
</tr>
<tr>
<td>Epithelization</td>
<td>1.23±0.55</td>
<td>4.18±0.79</td>
<td>4.16±0.44</td>
<td>F(2,15) = 731.29</td>
</tr>
<tr>
<td>Fibrotaxis</td>
<td>2.01±0.07</td>
<td>4.35±0.07</td>
<td>4.00±0.05</td>
<td>F(2,15) = 333.31</td>
</tr>
<tr>
<td>Collagenization</td>
<td>1.85±0.42</td>
<td>4.65±0.04</td>
<td>4.34±0.05</td>
<td>F(2,15) = 107.41</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>0.50±0.06</td>
<td>3.71±0.05</td>
<td>4.26±0.12</td>
<td>F(2,15) = 540.42</td>
</tr>
</tbody>
</table>

Values are reading of mean ± SEM of six animals. Value 5 refers to maximum similarity and 0 refers to least similarity of wound from normal tissue. All the values are significant at p<0.001.

Table 3: Effect of orally administered ethanolic extract of Rubia cordifolia roots on dead space wounds in rats *.

<table>
<thead>
<tr>
<th>Parameter Studied</th>
<th>Granuloma Breaking Strength (g)</th>
<th>Dry Granuloma weight (mg % of B.W.)</th>
<th>Hydroxyproline content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>278.89±2.603</td>
<td>43.08±2.381</td>
<td>6.89±0.4604</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>336.69±3.152</td>
<td>65.88±1.921</td>
<td>9.97±6.4572</td>
</tr>
<tr>
<td>F values</td>
<td>1.466</td>
<td>1.567</td>
<td>1.014</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. * P<0.001 vs. control; Student’s t-test. N = 6, * topical application.

DISCUSSION

In excision wound healing study from the observed values it were assumed that RC extract shows better and faster healing as compared to untreated group. During the initiation of the study day “0” the wound closure with RC extract was very slow as compared to FSC treated wounds. As the study progress the wound healing efficiency increases shows complete healing on day 24-25 which is same for Group 3 (wounds treated with FSC cream). But the untreated group took more time to complete wound closure. The study was carried out till fall of eschar leaving no scar behind. This shows healing potential of RC extract with better and faster wound closure.

It has been reported that terpenoids posses an ability to increase the collagen content, which is one of the factor for promotion of wound healing [19]. As the title plant is rich in terpenoids it may be responsible for wound healing activity. The experimental plant also shows antimicrobial activity as reported earlier these are the factors count for wound healing activity of Rubia cordifolia. Thus it may be concluded that the root of Rubia cordifolia shows significant wound healing activity. Further studies are in progress to isolate the bioactive component of plant extract.

REFERENCES


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