Spider web ointment: A traditional based approach in Cutaneous wound healing

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Spider web is being used in India and other countries to promote wound healing. Its ointment at 2.5 and 5% w/w concentrations was tested for wound healing activity in excision and incision wound models in rats. A significant reduction in the area of excision wound was observed for both the treatments when compared to normal healing and control ointment treatments. The period of epithelization for spider web treatments was much lower than the standard povidone iodine ointment and significant when compared to normal and control ointment treatments. In the linear incision wound model, spider web ointment at 2.5% w/w showed 30.65% increase in the tensile strength compared to normal healing. The histological evaluation revealed that the spider web ointment lowers scores in terms of scab, ulcus, polymorphonuclear cells and high scores of re-epithelization and neovascularization when compared to normal healing group. Both the concentration of spider web ointment (2.5% and 5% w/w) exhibited similar wound healing activity. Therefore, the optimum effective dose of spider web ointment is 2.5% w/w concentration. The wound healing activity may be due to increase in collagen synthesis and probably due to the presence of proteins in the web. The present study provides a scientific base for the traditional use of spider web for wound healing.

Keywords: Spider web, Traditional medicine, Wound healing, Linear incision wound model, Excision wound model


Efforts are being made all over the world to discover agents that can promote healing and thereby reduce the cost of hospitalization and save the patient from amputation or other severe complications. The main objective of wound management is to heal the injury in the shortest possible time with minimal pain and discomfort to the patient. Many diverse and interesting approaches have been applied throughout the medical history. Since ancient times, people used materials in and around them to accelerate wound healing process. Often their use is based on tradition, without any scientific evidence of efficacy and little knowledge about putative active compounds or their mode of actions. Scientific validation of the biological properties of natural products which is identified by personal experience of traditional healers is essential which can provide a cheap method of new drug development.

In this connection, the authors observed that people in and around Bokaro, Jharkhand, India, were applying spider web on small cuts to stop bleeding and healing of wounds. Spider web (Synonym: cobweb) is a device built by a spider out of proteinaceous silk extruded from its spinnerets gland. The spider web is rubbed directly on to the skin wound to promote healing. A survey conducted by the authors confirmed the use and its effectiveness. Literature survey also indicated that in traditional European Medicine, cobwebs are used on wounds and cuts and seem to help healing and reduce bleeding. Spider webs are rich in vitamin K, which can be effective in clotting the blood. Webs were used several hundred years ago as gauze pads to stop an injured person’s bleeding. In spite of its wide use over a long period of time, no systematic approach has been made to study the wound healing activity of spider web. Hence, in the present study an effort was made to systematically study the wound healing effects of spider web using excision and linear incision models. Since spider web is insoluble in aqueous and most of the organic solvents and difficult to suspend in ointment base, an ointment prepared by suspending a suspension of spider web in dimethyl sulfoxide (DMSO) in ointment base was evaluated.

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Materials and methods

Collection of materials and preparation of ointment
Spider web was collected from the inside roofing of houses and separated from spider and other insects. A weighed quantity (2.5 & 5 gm, respectively) of spider web was triturated with DMSO where most of the web dissolved and was suitably suspended in hot ointment base containing white soft paraffin (85%) and liquid paraffin (15%) to make a uniformly suspended ointment. Povidone iodine ointment (5% w/w, B. No. 08k0057) was used as standard (Wokadine: Wockhardt Ltd., Mumbai, India).

Experimental animals
Wistar rats of either sex weighing 150-250 gm were used for the study. The animals obtained from the inbred animal colony of central animal house, Sree Siddaganga College of Pharmacy, Tumkur, were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and 12 hrs light-dark cycles and were acclimatized for 7 days before the study. The animals were randomized into experimental and control groups and housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. Animals were subjected to respective treatments from day zero till the completion of wound healing. Wound area was measured by tracing the wound on a millimeter scale graph paper on pre-determined days (ie. every fourth day) from day 4 - 36 and photographs of wound area were taken. Falling of scab leaving no raw wound behind was taken as end of complete epithelization and the days required for this was taken as period of epithelization. Per cent wound contraction rate was measured as below:

\[
\text{Percent contraction rate} = \frac{\text{Initial wound area} - \text{Wound area on specific day}}{\text{Initial wound area}} \times 100
\]

Linear incision wound model
Two para-vertibral straight incision of 6 cm each were made through the entire thickness of skin on either side at least 1 cm lateral to the vertebral column. Wounds were sutured with 1 cm gap with catgut. The animals were then divided into 5 groups containing 6 animals per group. The formulations were applied topically once in a day for nine days. Sutures of all the animals were removed on 7th post wounding day and the breaking tensile strength was measured on 10th post wounding day by continuous, constant water flow technique using tensiometer (Fig. 2). The percent tensile strength was calculated as per the formula:

\[
\text{Tensile strength} (%) = \frac{\text{TS treated} - \text{TS vehicle}}{\text{TS vehicle}} \times 100
\]

Excision wound model
Excision wound model was employed to study the rate of wound contraction and epithelization. The animals were anaesthetized intramuscularly using ketamine (80 mg/kg, i.p.). The particular skin area was shaved prior to the experiment. An impression was made on the dorsal thoracic region 1cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm² (Fig. 1). Haemostasis was achieved by blotting the wound with cotton swab.

The animals were then grouped into five groups containing six rats per group. The following treatments were made to cover the entire wound area once in a day till the healing completes as given below:

- **Group 1**: Normal healing control (without any treatment)
- **Group 2**: Ointment control (Base + DMSO treatment)
- **Group 3**: Spider web ointment (2.5% w/w)
- **Group 4**: Spider web ointment (5% w/w)
- **Group 5**: Standard Povidone ointment (5% w/w)

Animals were subjected to respective treatments from day zero till the completion of wound healing. Wound area was measured by tracing the wound on a millimeter scale graph paper on pre-determined days (ie. every fourth day) from day 4 - 36 and photographs of wound area were taken. Falling of scab leaving no raw wound behind was taken as end of complete epithelization and the days required for this was taken as period of epithelization. Per cent wound contraction rate was measured as below:

\[
\text{Percent contraction rate} = \frac{\text{Initial wound area} - \text{Wound area on specific day}}{\text{Initial wound area}} \times 100
\]

The cross-sectional full-thickness skin specimens from each group in incision wound model were collected at the end of the experiment to evaluate the histopathological alterations. Samples were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5 μm sections and stained with hematoxylin and eosin (HE) and Masson’s trichrome (MT) stains. The tissues were examined by light microscope at magnification at 100X magnification (Olympus CX41) and graded as mild (+), moderate (++) and severe (+++) for epidermal or dermal re-modeling. Re-epithelization or
Fig. 1—Representative picture of Excision wound in rats at 0 day [A] Initial excision wound; and 20th post wounding day [B] Normal healing control without treatment; [C] Ointment control; [D] Spider web (2.5% w/w); [E] Spider web (5% w/w); [F] Povidone iodine (5%).

Fig. 2—Pictorial representation of linear incision wound model, wound healing in different days and instrument setup to measure tensile strength.
ulcus in epidermis, fibroblast proliferation, mononuclear and/or polymorphonuclear cells, neovascularization and collagen depositions in dermis were analyzed to score the epidermal or dermal re-modeling. Masson’s trichrome stained sections were analyzed for collagen deposition. At the end of the examination, all the wound healing processes were combined and staged for wound healing phases such as inflammation, proliferation and re-modeling in all groups.

Statistical analysis
The statistical analysis was carried out by one-way ANOVA followed by Tukey’s post-test using GraphPad InStat-3, USA. The data were expressed as Mean ± S.E.M.

Results
In excision wound repair model, the results of wound contraction and epithelization period after topical application of the spider web ointment are reported in Table 1. A significant reduction in the wound area was observed by the spider web ointment treatment at 2.5 and 5% w/w concentrations from 4, 8, 12, 16 and 20 post wounding days when compared to normal healing and ointment control groups. The standard povidone iodine ointment (5% w/w) treatment was found to be less effective compared to the spider web treatment. The observed period of epithelization in normal healing and ointment control groups was found to be 32.00 ± 2.02 and 34.60 ± 1.60 days, respectively. Both the concentrations of spider web exhibited 22.00 ± 0.63 and 21.60 ± 0.98 days as period of epithelization, which was found to be much lower than that observed by the standard povidone iodine ointment (26.8 ± 0.97 days). All these values were significant compared to control groups. Spider web ointment (2.5% and 5% w/w) exhibited similar in percentage wound contraction and period of epithelization. Therefore, the optimum effective dose of spider web ointment is 2.5% concentration.

The wound breaking/tensile strength when measured on 10th day of the treatment in the linear Table 1—Effect of spider web ointment on wound contraction and epithelization in excision wound model

<table>
<thead>
<tr>
<th>Post wounding Days</th>
<th>Wound area in mm²</th>
<th>Normal healing</th>
<th>Ointment control</th>
<th>Spider web (2.5%) Ointment</th>
<th>Spider web (5%) Ointment</th>
<th>Povidone iodine (5%) Ointment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>454.60 ± 19.42</td>
<td>450.80 ± 14.77</td>
<td>372.40 ± 44.77</td>
<td>355.40 ± 25.83</td>
<td>467.60 ± 21.35</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>379.00 ± 23.92</td>
<td>406.00 ± 4.49</td>
<td>275.80 ± 30.95</td>
<td>266.40 ± 19.00</td>
<td>445.00 ± 32.68</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>279.40 ± 28.64</td>
<td>275.20 ± 5.18</td>
<td>131.80 ± 16.81</td>
<td>143.20 ± 4.47</td>
<td>343.20 ± 19.24</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>174.60 ± 14.70</td>
<td>190.40 ± 9.45</td>
<td>36.60 ± 5.56</td>
<td>36.60 ± 6.62</td>
<td>207.40 ± 20.20</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>96.20 ± 8.10</td>
<td>115.80 ± 14.11</td>
<td>20.80 ± 4.96</td>
<td>19.60 ± 3.71</td>
<td>107.20 ± 8.54</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>65.20 ± 8.45</td>
<td>80.40 ± 15.11</td>
<td>4.40 ± 1.63</td>
<td>7.20 ± 4.59</td>
<td>55.40 ± 6.10</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>38.80 ± 9.19</td>
<td>44.40 ± 12.16</td>
<td>-</td>
<td>-</td>
<td>21.00 ± 6.13</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>15.20 ± 8.78</td>
<td>18.60 ± 7.92</td>
<td>-</td>
<td>-</td>
<td>1.80 ± 1.20</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>8.00 ± 5.06</td>
<td>14.50 ± 0.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>1.60 ± 1.60</td>
<td>0.40 ± 0.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Period of Epithelization</td>
<td></td>
<td>32.00 ± 2.02</td>
<td>34.60 ± 1.60</td>
<td>22.00 ± 0.63</td>
<td>21.60 ± 0.98</td>
<td>26.80 ± 0.97</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM, n=6. *P<0.05; **P<0.01; ***P<0.001 compared to normal healing group and *P<0.05; **P<0.01; ***P<0.001 compared to ointment control group by One-way ANOVA followed by Tukey’s post-test. Results in the parenthesis indicate percent reduction in wound area.
incision wound model of the normal healing animals was 344.77 ± 27.17 gm and for the ointment control 305.93 ± 21.26 gm (Table 2). These results indicate that the ointment control does not have any effect on wound healing process. However, the spider web ointment at 2.5% w/w concentration showed significant ($P<0.05$) increase of 30.65% in the tensile strength of the linear incision wound (450.44 ± 10.749) compared to the normal healing group. The standard 5% w/w povidone iodine ointment has show better activity than the spider web treatments with 46.02% increase in percent tensile strength (Table 2).

The histopathological effect of spider web ointment on wound healing processes and healing phases in incision wound model on the day 10th of the treatment is shown in Table 3. The spider web ointment at 2.5 and 5% w/w concentrations exhibited low scores in terms of scab, ulcus and polymorphonuclear cells when compared to normal healing group. Similarly, high scores were observed for re-epithelization, fibroblast proliferation, collagen deposition and neovascularization indicating its potent wound healing effect. The standard povidone ointment (5% w/w) also exhibited similar results compared to 2.5% w/w spider web ointment but with slightly higher scores. The spider web and standard povidone iodine ointment applications showed the presence of proliferation and remodeling in the healing phases, however, the normal healing and ointment control treatments showed the inflammatory phase.

**Discussion**

Although healing is a physiological process which does not normally require much help, but still wounds cause discomfort and are susceptible to infection and other complications. Therefore, use of agents expediting healing is indicated. Further, some disorders such as diabetes, immunocompromised conditions, ischemia and conditions like malnourishment, ageing, local infections and local tissue damage due to burn or gun-shot leads to delay in healing or incomplete wound healing is resulted in severe complications. Such conditions specially require the use of agents which can facilitate healing. Wound healing process consists of different phases as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent to each other. In the present study, two models were used to assess the effect of spider web on wound healing based on its traditional use.

In excision wound, animals treated with 2.5 and 5% w/w spider web ointments exhibited significant increase in the rate of wound contraction and period of epithelization. It is known that collagen not only confers strength and integrity to the tissue matrix, but it also plays an important role in homeostasis and epithelization at a later phase of wound healing. Hence, enhanced collagen synthesis by the spider web

### Table 2—Effect of spider web ointment on tensile strength in linear incision wound model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tensile strength (gm)</th>
<th>% Tensile strength compared to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal healing control</td>
</tr>
<tr>
<td>Normal healing control</td>
<td>344.77 ±27.17</td>
<td>--</td>
</tr>
<tr>
<td>Ointment control</td>
<td>305.93 ±21.26</td>
<td>-11.27</td>
</tr>
<tr>
<td>Spider web ointment (2.5% w/w)</td>
<td>450.44 ±10.74</td>
<td><strong>30.65</strong></td>
</tr>
<tr>
<td>Spider web ointment (5% w/w)</td>
<td>382.33 ± 25.68</td>
<td>10.89</td>
</tr>
<tr>
<td>Povidone iodine ointment (5% w/w)</td>
<td>503.43 ± 26.39</td>
<td>46.02</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM, n=6. $^aP<0.05$; $^bP<0.01$ compared to normal healing group and $^cP<0.01$, compared to ointment control group by one-way ANOVA followed by Tukey’s post-test.

### Table 3—Wound healing processes and healing phases of the spider web ointment in incision wound model

<table>
<thead>
<tr>
<th>Group</th>
<th>Wound healing processes</th>
<th>Healing phases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>U</td>
</tr>
<tr>
<td>Normal healing</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Ointment control</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Spider web ointment</td>
<td>-/+</td>
<td>-</td>
</tr>
<tr>
<td>(2.5% w/w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spider web ointment</td>
<td>-/+</td>
<td>-</td>
</tr>
<tr>
<td>(5% w/w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Povidone iodine</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>ointment (5% w/w)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hematoxylin and eosin (HE) and Masson’s trichrome (MT) stained sections were scored as mild (+), moderate (+++) and severe (++++) for epidermal and/or dermal re-modeling. S: scab; U: ulcus; RE: re-epithelization, FP: fibroblast proliferation, CD: collagen depositions, MNC: mononuclear cells, PMN: polymorphonuclear cells, NV: neovascularization, I: inflammation phase, P: proliferation phase, R: remodeling phase.
in rats may contribute significantly to healing and also provide necessary strength to repair the tissue(s).

In incision wound, application of spider web showed greater tensile strength at the concentration of 2.5% w/w, but the activity was lower than the standard povidone iodine ointment. The increase in tensile strength of treated wounds may be due to increase in collagen synthesis, stabilization of fibers and cross-linking of the protein\(^ {14}\). Further, this observation was well correlated with histopathological studies using hematoxylin-eosin (HE) and Masson’s trichome (MT) stains (Fig. 3). HE staining of skin sections revealed the presence of scab, ulcer, polymorphonuclear cells and re-epithelization, whereas MT staining gives an idea about fibroblast, collagen and mononuclear cells content. Both HE and MT staining techniques help to determine the different phases of wound healing such as inflammation, proliferation and remodeling\(^ {9}\). Normal healing and ointment control rats exhibited delayed wound healing as they were in inflammation stage which is the initial stage of healing phase. Treatment of spider web ointment to wounded rats showed increased healing process as they were in remodeling phase (Fig. 3). It is worth pointing out that the wound healing effect is more marked when the rats were applied with 2.5% w/w spider web ointment than that of 5% w/w. Therefore, the optimum effective dose of spider web ointment was found to be 2.5% w/w concentration.

**Conclusion**

In conclusion, the wound healing properties of spider web may be due to increase in fibroblast cells, collagen synthesis and a significant increase in tensile strength, wound contraction rate and epithelization period. The wound healing action may probably be due to the proteins present in the spider web. The present study provides a proof of the traditional use of spider web in India and other countries. There is a need for further studies to identify the active ingredients of spider web and to elucidate its mode of action.

**Acknowledgement**

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**References**


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**Fig. 3—**Histopathological view of wound healing and epidermal/dermal re-modeling. Skin sections showed the hematoxylin and eosin (HE) stained epidermis and dermis in [A], and the dermis stained with Masson’s trichrome (MT) in [B]. The magnification was 100 X. Arrows pointing events during wound healing—S: scab, U: ulcer, RE: re-epithelization, F: fibroblast, C: collagen, MNC: mononuclear cells, PMN: polymorphonuclear cells. (1) Normal healing control; (2) Ointment control; (3) Spider web (2.5% w/w); (4) Spider web (5% w/w); (5) Povidone iodine (5%).


