SIMPLE EVALUATION OF THE WOUND HEALING EFFECT OF TOPICAL OINTMENTS MADE FROM SENECIO VERNALIS (ASTERALES: ASTERACEAE) PLANT EXTRACT IN RATS

SIAVASH AHMADI-NOORBKHSH, FARSHID SARRAFZADEH-REZAEI1, MASOUD MAHAM2, AND BAHRAM DALIR-NAGHADEH1

Surgery Resident, Department of Clinical Sciences, 1Department of Clinical Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia 57153-1177, Iran
2Department of Medicinal and Industrial Plants, Institute of Biotechnology, Urmia University, Urmia 57153-165, Iran
s.noorbakhsh@urmia.ac.ir
f.sarrafzadeh@mail.urmia.ac.ir

Received: July 1, 2010         Accepted: December 30, 2010

Abstract

The objective of this study was to evaluate the gross effect of ointments made from Senecio vernalis extract on the healing of cutaneous wounds of rats. Thirty adult female albino rats (average weight of 250±10 g) were randomly divided into six groups. Experimental wounds were made on the dorsal thoracic region of anesthetised rats. Four groups were treated with specific ointment concentrations consisted of 3.75%, 7.5%, 15%, and 30% weight/weight Senecio vernalis ethanol extract in Eucerin™ ointment base; one group was treated by the ointment basis alone (basis) and no treatment was applied to the remaining group (control). Wound healing was assessed by computerised planimetry. Data were analysed statistically using a repeated measures mixed model. Statistical evaluation of mean wound area differentiation between groups, showed no significant changes in overall rate of the wound healing (P>0.05). However, comparisons within each group, revealed that in three groups (3.75%, 7.5%, and 15% extract concentration) a significant wound size reduction happened earlier. These results deny strong hypotheses about the desirable wound healing effects of S. vernalis contrary to other similar plants of the genus Senecio, and anti-ulcer effects of Senecio brasiliensis (a similar species to S. vernalis) in the duodenum and stomach of rats and mice.

Key words: rat, skin, wound healing, plant extract, Senecio vernalis.

Every wound - depending on its location and extent - could be an alarm for the immune system and a challenge for the homeostasis. Wound healing is a complex process, aiming to re-establish the skin’s continuity, thus protecting the body from infections and preventing internal instabilities. In some cases, the speed of the wound closure determines the survival of the patient. In many instances, faster wound healing could have beneficial effects on the welfare of patients as well as the decline of the therapeutic costs. During thousands of years, many preparations have been tested to increase the rate of the wound healing. Nowadays, in spite of many advances in chemical drugs’ pharmacology, the number of these agents are still limited (17) and controversies about their overall outcomes are not uncommon. From the ancient times to now, plants were the most attractive sources to treat the wounds; some to prevent the wound infections, some to relieve the wound pains, and many to intensify the wound closure’s rate. Plants generate plenty amounts of complex materials; just resembling a huge chemical factory. All secrets of this natural factory is set in a seed, which needs no modern technology or expensive materials to form a product. Therefore, attempts to obtain effective medicinal materials from the plant origins, could be a technical and economical idea. Plants of Senecio genus have been widely investigated for their different therapeutic effects (22). The species vernalis of this plant is widely spread in North-Western Iran and domestically named “Pirgiah”, which means “old plant”. It is called “Eastern Groundsel” in English literatures. Senecio plants contain plenty amounts of alkaloids and flavonoids (1, 16, 18, 22), which possess proven effects on the wound healing (22). Unfortunately, there is many poisonous species in this genus; however it has previously been shown that S. vernalis uniquely has no toxic effects (20). Since faster closure of the skin defects is a therapeutic priority, the aim of this preliminary study was to investigate the probable gross effects of the topical administration of the ointments made from
Senecio vernalis extract on the closure of the full thickness skin wounds of rats.

Material and Methods

The study was approved by the Veterinary Faculty Research Council. Thirty adult female albino rats (with average weight of 250 ±10 g) were randomly selected from the animal house of the University. During two weeks of acclimatisation, the rats were randomly housed in single cages. They were fed laboratory animal pellets and provided water ad libitum for the whole period of the study. There were six groups of five animals: no treatment (control), treatment with ointment basis, and treatment with ointments containing 3.75%, 7.5%, 15%, and 30% extract concentrations in Eucerin™ (Beiersdorf, Canada) basis.

To prepare the ointments, Senecio vernalis plant collected in October 2009 from the Urmia city countryside was identified botanically by one of the authors. Aerial parts of the plant were used to prepare the ethanol extract by common extraction method. Eucerin™ was used as the ointment basis. Five different formulations (weight/weight) of ointments (3.75%, 7.5%, 15%, and 30%) were prepared by adding respectively 0.58, 1.16, 2.32, and 4.64 g of the crude extract into 15.4 g of Eucerin™ basis. The ointments were prepared at the first day of the study and were kept refrigerated (5°C) during 15 d of the study period.

To create the experimental wounds, anaesthesia was made using intraperitoneal injection of 90 mg/kg of ketamine (Alfasan, Holland) and 10 mg/kg of xylazine (Alfasan, Holland) (8). Regarding the pilot study, the best place for excisional wounding with minimal chance of contamination or manipulation by the animals was the middle dorsal thoracic region. Since difference in wound healing is due to the cranial-caudal position of the wound site (3), it was strived to make even wounds in all cases. Surgical sites were prepared routinely and excisions were made using a quadrate 1×1 cm grid and delicate surgical blades. The wound photograph was taken by a digital camera. Rats were then randomised to one of the study groups. The wounds were left undressed. An oral dose of 80 mg/kg/d of tramadol (Allapain, Amin Darou, Iran) dissolved in drinking water was used as postoperative anaesthesia for two consecutive days.

Wound healing was assessed from the first day and continued every three days until the complete wound closure. Planimetry was done using proper computer software (Adobe® Acrobat® 8 Professional, Version 8.0.0) with 0.01 mm² accuracy in surface area calculation. The experiment was conducted as a completely randomised design with rats as the experimental unit. The data of outcome variable (wound area) were tested for normal distribution by means of Kolmogorov-Smirnov test. The data were analysed by ANOVA as repeated measurements across the time using PROC MIXED of SAS with start wound area created at first day (before applying the treatments) as a covariate (12). The model included treatments, time period, treatment by time period interaction, and the covariate. Several matrix pertaining to the covariance among the repeated sampling measurements, including UN, Toep, CS, AR, and AR +RE were evaluated. Based on the value for the Akaike’s information criterion (closest to zero), the UN covariance structure was selected as an appropriate model to the data. Least squares means and standard errors for the treatment groups at each time period were obtained by the LSMEANS statement/ PDIFF. The least squares means were compared using Bonferroni test. Significance was set at P ≤ 0.05. The data were reported as least squares means ± SEM.

Results

No clinical signs of toxicity or wound infection were seen during the study period. All wounds were completely healed at the 13th d post-injury. Table 1 represents the mean (± standard error) wound area of rats during the experiment.

<table>
<thead>
<tr>
<th>Days after injury</th>
<th>Control</th>
<th>Basis</th>
<th>3.75% EC</th>
<th>7.5% EC</th>
<th>15% EC</th>
<th>30% EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>72.34 ± 3.27</td>
<td>85.99 ± 10.10</td>
<td>86.96 ± 11.77</td>
<td>96.39 ± 10.36</td>
<td>71.76 ± 9.86</td>
<td>75.48 ± 3.93</td>
</tr>
<tr>
<td>Day 3</td>
<td>66.85 ± 3.51</td>
<td>75.30 ± 9.50</td>
<td>74.33 ± 10.61</td>
<td>90.64 ± 8.63</td>
<td>60.29 ± 7.85</td>
<td>66.25 ± 4.97</td>
</tr>
<tr>
<td>Day 6</td>
<td>39.11 ± 5.23</td>
<td>65.35 ± 6.03</td>
<td>45.34 ± 8.59</td>
<td>37.16 ± 8.01</td>
<td>42.42 ± 8.94</td>
<td>36.90 ± 2.11</td>
</tr>
<tr>
<td>Day 9</td>
<td>16.76 ± 2.89</td>
<td>14.59 ± 7.69</td>
<td>9.56 ± 2.20</td>
<td>14.07 ± 3.60</td>
<td>10.94 ± 2.44</td>
<td>5.73 ± 0.56</td>
</tr>
<tr>
<td>Day 12</td>
<td>1.90 ± 0.38</td>
<td>2.45 ± 3.77</td>
<td>2.20 ± 0.97</td>
<td>2.42 ± 1.13</td>
<td>2.20 ± 0.97</td>
<td>0.47 ± 0.37</td>
</tr>
</tbody>
</table>

EC - extract concentration; different superscript in each row indicates statistically significant differences within the group (P ≤ 0.05).
There was no significant difference of mean wound area between control and treatment groups in any given time point. However, statistical analysis within each group showed dissimilarity in wound healing trend among the groups. Control group had a significant reduction of wound area during day 9 to 12. In basis group, significant wound size reduction was observed earlier (day 6 to 9). Significant wound size reduction in three groups under treatment with 3.75%, 7.5%, and 15% extract concentrations occurred the earliest (days 3-6). The 30% extract concentration group had significant wound size reduction during days 6 to 12.

Discussion

There are high levels of alkaloids, flavonoids, and tannins in genus *Senecio* plants (1, 16, 18, 22). These agents have potential anti-ulcer effects (19, 22). Alkaloids prevent tissue damage by inhibiting phospholipase-A2 enzymatic activity. They enhance epidermal growth factor (EGF) expression (21), which has positive effects on the healing of skin wounds in rats (5, 11) and pigs (2, 13), full thickness dermal wounds of rabbits’ ear (14) and cornea of the primates (4). Chronic application of pyrrolizidine alkaloids obtained from *Senecio brasiliensis*, intensifies EGF expression, which eventually leads to the cicatrization of gastric ulcers (21). Flavonoids are powerful free radical scavengers. Both, flavonoids and tannins speed up the wound healing process by an increase in the amount of the tissue antioxidants and consequently better collagenation (6). It was previously shown that *Senecio brasiliensis* alkaloidal extract, has anti-ulcer effects in the duodenum and stomach of mice and rats (22). Antimicrobial (23), chemical (15), and antiviral effects (7) of different plants of genus *Senecio* have been investigated in other researches. Regarding to the aforementioned literatures, it was expected to observe significant macroscopic effects on the wound healing by applying ointments made from *Senecio vernalis* extract.

In this experiment, wound closure in the control group was assumed as the normal wound healing trend, comparing to the literature (9). In basis group, earlier occurrence of significant wound size reduction could be a result of wound coverage by Eucerin™ and consequent of low oxygen tension in the tissue. Prolonged hypoxia causes the macrophages to secrete cytokines and growth factors, which consequently accelerate angiogenesis. New capillary formation during angiogenesis promotes fibroplasia, which leads to a faster wound closure (9, 10). Ointments containing 3.75%, 7.5%, and 15% extract concentrations had the earliest time of significant wound area reduction comparing to the other groups (Table 1). This may imply some desirable extract related effects on the wound healing. However these effects were not potent enough to cause a significant difference between studied groups. In 30% extract concentration, the condition is rather similar to both basis and control. Its later significant wound size reductions in comparison with other extract groups may implicate detrimental effects of higher concentrations of the plant extract (overdosage). Since drugs with certain effects on some wound healing phases do not necessarily affect other phases (19), it seems that the aforementioned plant extracts have slightly changed the onset time of maximum wound size reduction without any observable alteration in other phases. However this effect was either minor in nature or may be inhibited by some unfavourable plant material(s).

In conclusion, regardless of the strong preliminary hypotheses for this study, different concentrations of *Senecio vernalis* extract ointments showed no significant gross effects on the rate of the excisional wound closure in rats, and aerial part of this plant is not advised to be studied as a wound closure intensifier.

References


