Effects of *Equisetum arvense* Ointment on Dermal Wound Healing in Rats

Yusuf Ozay, PhD; Sabri Ozyurt, PhD; Sevda Guzel, MSc; Ali Cimbiz, PhD; Esra G. Olgun, MD; M. Kasim Cayci, PhD

**Abstract:** The aim of this study was to evaluate the effectiveness of *Equisetum arvense* ointment on dermal wound healing in rats. *Methods.* The authors studied a total of 56 wounds in four groups of rats. Each wound measured 15 mm x 15 mm. The first group did not receive treatment while the second group was treated with a 1:1 mixture of Vaseline and lanolin ointment. *Equisetum arvense* ointment doses of 5% and 10% were used in the third and fourth groups. The rats were observed at days 7 and 14 post wounding. The state of the wound healing was evaluated using wound closure ratio and histopathologic studies. *Results.* *Equisetum arvense* 5% and 10% groups and the Vaseline-lanolin group had a statistically significant higher wound closure ratio than the control group (*P* < 0.05). *Equisetum arvense* ointment groups had a 95.26% and 99.96% wound closure ratio (*P* < 0.05) and higher dermal and epidermal regeneration, angiogenesis, and granulation tissue thickness after 14 days as compared to the other groups (*P* < 0.05). *P. Equisetum arvense* ointment exhibits significant wound healing activity in rats. Further clinical and experimental studies are needed to confirm these results.

Wounds are physical injuries that result in an opening or breaking of the skin. Wound healing is a dynamic and complex process that involves a well-coordinated, highly regulated series of events including inflammation, tissue formation, revascularization, and tissue remodeling. During the healing process, various growth factors are secreted to accelerate wound healing. The basic principle of optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition, and a moist wound healing environment to restore the anatomical continuity and function of the affected limb. Many investigators evaluated the wound healing properties of many medicinal herbs, clinically on animal models using excisional, incisional, and dead space models.

The *Equisetum arvense* (EA) plant has been traditionally used in the...
treatment of skin related wounds among animals and for oral infections among humans throughout Turkey. Additionally, EA was traditionally used as a bathing remedy for gynecological diseases, rheumatic diseases, gout, and in the treatment protocol of poorly healing wounds, tumescence, and fractured bones in Europe.7,8

Several studies showed a hypoglycemic antiseptic, antimicrobial, antioxidant, antidepressant, sedative, hepatoprotective, antinociceptive, and anti-inflammatory effects of the EA.9–14 Alternatively, less is known about the role of EA in dermal wounds. Hence, the present study was conducted to investigate the efficacy of topical application of EA ointment according to macroscopic and histological methods in wound healing.

Materials and Methods

Animals. Albino Wistar male rats weighing 180–240 g were obtained from the central animal house of Dumlupinar University for the study. Fifty-six rats were housed individually in cages, maintained under standard conditions (12 h light/12 h dark cycle; 25˚C ± 3˚C) and were fed with standard pellet and water ad libitum. Animal studies were performed after taking approval from Animal Care and Ethics Committee of Medical Faculty of Dumlupinar University and Principles of Laboratory Animal Care guidelines were followed.

Plant material and Equisetum arvense ointment preparation. The plant was collected in Mersin, Turkey during the summer of the 2007. The plant was identified in the Department of Biology in Dumlupinar University Faculty of Art and Science. Air-dried leaves of the plant were powdered. A 1:1 mixture of Vaseline-lanolin was used to formulate EA ointment base. For 10% and 5%, EA powder was mixed with ointment base during an ointment-forming process,15 which consisted of 5 g powder incorporated into 45 g of ointment base (Vaseline-lanolin).

Skin ulcer induction. A deep skin ulcer model was created using rats as follows.15–17 The rats were anesthetized by intraperitoneal injection of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (25 mg/kg) and their back hair was shaved and the application field was outlined with a marking pen just prior to skin removal. A full-thickness skin wound in each rat was prepared by circularly removing skin with 1.5 cm diameter on the dorsum. A total of 56 wounds were studied in four groups (n = 14). The following describes the arrangement of each group. 1) Control group: Nothing was applied to wounds; 2) Vaseline-lanolin (VL) group: Ointment base of Vaseline-lanolin was applied topically to wounds; 3) 5% EA group: 5% EA plus 1:1 mixture of Vaseline-lanolin ointment; 4) 10% EA group: 10% EA plus 1:1 mixture of Vaseline-lanolin ointment. All wounds were cleansed on a daily basis with sterile saline solution. Following cleaning, the ointment base and EA ointments were applied to the wounds. All ointments were applied evenly in sufficient amounts to cover all wound areas.

<table>
<thead>
<tr>
<th>Scores</th>
<th>Epidermal/dermal regeneration</th>
<th>Granulation tissue thickness</th>
<th>Angiogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ±</td>
<td>Little epidermal and dermal organization</td>
<td>Thin granulation layer</td>
<td>Altered angiogenesis (1–2 vessels per site) characterized by a high degree of edema, hemorrhage, and occasional congestion and thrombosis.</td>
</tr>
<tr>
<td>2 ±</td>
<td>Moderate epidermal and dermal organization</td>
<td>Moderate granulation layer</td>
<td>Few newly formed capillary vessels (3–6 per site); moderate degree of edema and hemorrhage. Occasional congestion and intervascular fibrin deposition; absence of thrombosis.</td>
</tr>
<tr>
<td>3 ±</td>
<td>Complete remodeling of epidermis and dermis</td>
<td>Thick granulation layer</td>
<td>Newly formed capillary vessels (7–10 per site); moderate degree of perivascular and interstitial edema and congestion. Absence of thrombosis and hemorrhage.</td>
</tr>
<tr>
<td>4 ±</td>
<td>--</td>
<td>Very thick granulation layer</td>
<td>Newly formed and well-structured capillary vessels (&gt; 10 per site) vertically disposed toward the epithelium and at the wound margins. Slight degree of perivascular edema.</td>
</tr>
</tbody>
</table>


Healing evaluation. At the appropriate time after treatment (7 and 14 days), seven animals from each group were euthanized by ether inhalation. The tissue, including the wound and any surrounding skin and muscle, were excised. Wound healing was evaluated by measuring the reduction of the wound surface area with a stereomicroscope (SZX-ILLD2-200, Olympus, USA) and was photographed (Spot Insight QE, Diagnostic Instruments, USA). The ratio of healing was calculated by the following equation:

\[
\text{Healing ratio (\%) = 100 x (1 - wound area / initial wound area)}
\]

Histological evaluation. After macroscopic evaluation, the excised tissue was fixed with 10% buffered formalin. Each specimen was embedded in a paraffin block and thinly sliced at 2.5 µm, and stained with hematoxylin-eosin. A pathologist who was unaware of the experimental procedure assessed the slides under light microscopy for severity of histopathological changes. Epidermal and dermal regeneration, granulation tissue thickness, and angiogenesis were scored as described by Altavilla et al. According to the criteria (Table 1). Concerning angiogenesis, only mature vessels that contained erythrocytes were counted. To evaluate well formed from poorly formed capillary vessels, the following parameters were considered: presence or absence of edema, congestion, hemorrhage, thrombosis, and intravascular fibrin formation. Paraffin blocks for histological evaluation were prepared from the wounded tissue and histological slides were obtained from the widest area of the wound.

Statistical Analysis

The results were expressed as mean ± SD and statistical differences between several treatments while their respective control was determined by one-way analysis of variance (ANOVA) followed by post-hoc multiple comparison Tukey tests using SPSS 14.0 software. The level of significance was set at \( P < 0.05 \).

Results

Weights and the number of rats in each group are shown in Table 2. Statistical differences were not found between groups initially or during the remainder of the experiment \( (P > 0.05) \). Macroscopic views and wound closure ratios are presented in Figures 1 and 2. On day 7 the wound closure ratio in the control group was 40.84%, 45.14% in VL group, 67.28% in the 5% EA group, and 68.84% in 10% EA group. The EA group and VL group were statistically significant compared to the control group \( (P < 0.05) \). On day 14, wound closure ratios of 77.43%, 87.49%, 95.26%, and 99.96% were observed in
control, VL, 5% EA, and 10% EA groups, respectively. Wound closure ratios were higher in EA groups than control and VL groups \( (P < 0.05) \). Histological results are presented in Table 3 and Figure 3. At the end of the first week, the histological score results were higher, especially in EA groups regarding dermal regeneration and granulation tissue thickness levels \( (P < 0.05) \). By the second week, all histological scores were higher in EA groups compared to the other groups \( (P < 0.05) \).

**Discussion**

A number of studies indicate that plant products are potential agents for wound healing and largely preferred because of their widespread availability, absence of unwanted side effects, and their overall effectiveness. A study expressed the common use of traditional drugs forms demonstrated that 80% of the world population is still dependent on traditional medicinal treatments to cure several skin diseases. In an excisional wound model, significant wound healing activity was observed in the rats treated with ointment of EA. Significant decrease in the period of epithelialization, higher scores of angiogenesis, and wound closure ratio were observed in these groups. In addition, EA ointment increased re-epithelialization and formulated new blood vessels, particularly closer to day 14.

Shukla et al, Moghbel et al, and Pathenoyak and Sunita showed the effects of some herbs (i.e., *Centella asiatica*, *Aloe vera*, *Dendophtthoe falcate*) on excisional wound healing in rats. Our results concur with the literature in terms of wound closure ratio,
which was found to be higher than the studies previously mentioned.

Angiogenesis plays a significant role in wound healing and newly formed blood vessels comprise 60% of the repair tissue. Neovascularization helps hypoxic wounds to attain the normoxic conditions. Re-epithelialization plays a crucial role in cutaneous repair, depending upon the specific type of wound. We observed that the EA ointment increased the angiogenesis phase and re-epithelialization in addition to the overall wound healing process. Namely, the findings obtained from the study assert that the EA ointment yields positive effects on angiogenesis.

Table 2. Weights (in grams) between days 7 and 14*.

<table>
<thead>
<tr>
<th>Groups**</th>
<th>Initially</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 7 (each group)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>205.1 ± 17.5</td>
<td>212 ± 14.8</td>
<td>207.6 ± 16.5</td>
</tr>
<tr>
<td>VL</td>
<td>211 ± 15.8</td>
<td>215.6 ± 15.1</td>
<td>208 ± 17.4</td>
</tr>
<tr>
<td>5% EA</td>
<td>209 ± 15.7</td>
<td>214.9 ± 15.5</td>
<td>199.6 ± 12.3</td>
</tr>
<tr>
<td>10% EA</td>
<td>197.4 ± 10.6</td>
<td>202.7 ± 11</td>
<td>208 ± 16.1</td>
</tr>
</tbody>
</table>

Data are presented mean ± SD.
VL: Vaseline-lanolin
EA: Equisetum arvense
*Each group had 14 rats; 7 were sacrificed in the first week and the others were sacrificed in week 2.
**No statistical difference between groups; P > 0.05 (initial versus day 7 and day 14)

Table 3. Histological scores.

<table>
<thead>
<tr>
<th>Groups**</th>
<th>ER</th>
<th>DR</th>
<th>GTT</th>
<th>Angiogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.0 ± 0.0a</td>
<td>1.14 ± 0.38a</td>
<td>1.0 ± 0.0a</td>
<td>1.0 ± 0.0a</td>
</tr>
<tr>
<td>VL</td>
<td>1.0 ± 0.0a</td>
<td>1.29 ± 0.49a</td>
<td>1.0 ± 0.0a</td>
<td>1.0 ± 0.0a</td>
</tr>
<tr>
<td>5% EA</td>
<td>1.14 ± 0.38a</td>
<td>1.57 ± 0.53a</td>
<td>1.43 ± 0.53a</td>
<td>1.0 ± 0.0a</td>
</tr>
<tr>
<td>10% EA</td>
<td>1.14 ± 0.38a</td>
<td>1.71 ± 0.49a</td>
<td>1.57 ± 0.53a</td>
<td>1.0 ± 0.0a</td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.43 ± 0.54a</td>
<td>1.71 ± 0.49a</td>
<td>1.43 ± 0.53a</td>
<td>1.14 ± 0.38a</td>
</tr>
<tr>
<td>VL</td>
<td>1.57 ± 0.79a</td>
<td>2.29 ± 0.49a</td>
<td>2.29 ± 0.95a</td>
<td>1.86 ± 0.38a</td>
</tr>
<tr>
<td>5% EA</td>
<td>2.0 ± 0.82a</td>
<td>2.43 ± 0.53a</td>
<td>2.71 ± 1.25a</td>
<td>2.57 ± 1.40a</td>
</tr>
<tr>
<td>10% EA</td>
<td>2.86 ± 0.38a</td>
<td>3.0 ± 0.0a</td>
<td>3.86 ± 0.38a</td>
<td>3.86 ± 0.38a</td>
</tr>
</tbody>
</table>

Data are presented mean ± SD.
VL: Vaseline-lanolin
EA: Equisetum arvense
ER: Epidermal regeneration
DR: Dermal regeneration
GTT: Granulation tissue thickness

Data sharing the same letter (a, b, c) were not statistically significant (P > 0.05). Data not sharing the same letter were statistically significant (P < 0.05).

Many studies in literature show that most of the plant extracts used to improve the wound healing process have antimicrobial, antioxidant, and anti-inflammatory effects. At the same time, the EA plant extract had the same effects in previous studies. Additionally, we discovered that this herb contains a critical number of biologically active compounds, therefore, the mechanism of wound healing may not solely be attributed to the antimicrobial, antioxidant, and anti-inflammatory properties of the herb. β-sitosterol, campesterol, and isofucosterol are some of the biologically active compounds of EA. Navarro et al. and Gomez et al. reported that β-sitosterol, campesterol, and isofucosterol have anti-inflammatory effects through mechanisms such as neutrophil migration. Aside from this effect, β-sitosterol increases angiogenesis; therefore, the authors believe that these biologically active compounds might enhance EA’s effects on wound healing. Based on the present results, the authors believe that EA can be used to stimulate wound healing or to effectively treat wounds.

Conclusion

The present study affirms that EA ointment restores impaired wound healing in an animal model. Further studies are needed to demonstrate the possible effects of EA ointment on wound healing in humans.

References

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