Investigation on the wound healing activity of
*Tilvadi ghrita*: a herbal formulation

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*Tilvadi ghrita* (TG) is a ghee-based herbal formulation claimed to promote wound healing in traditional practices. However no systematic studies are reported in modern scientific literature with regard to the verification of the traditional medicinal claims of TG. The present study was undertaken to investigate and rationalize the wound healing activity of TG in experimental rats. *Tilvadi ghrita* increased the tensile strength which is significantly improved over the untreated wounds. Promotion of tensile strength demonstrates wound healing potential of TG in incision wound which may probably be due to promotion of collagenation. This data is further supported by observation of histopathological response in healed tissues which reveal that TG promotes keratinization, epithelization, collagenation and fibrosis. TG also demonstrates its healing potential in excision wound model with significant reduction in wound contraction area with faster healing.

**Keywords:** Tilvadi ghrita, Wound healing activity, Herbal formulation.

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Ayurvedic medicines are largely based upon herbs, either single ingredient or in combination (polyherbal) having specific diagnostic and the therapeutic principles. Panchgavya is a term that refers to the five important products of bovine origin viz. milk, curd, ghee, urine and dung. Ayurvedic texts describe several therapeutic uses of Panchgavya components mostly in combination with herbs. Modern literature documents investigation of few pharmacological actions and clinical uses of Panchgavya formulation. *Tilvadi ghrita* is one such polyherbal formulation containing *Glycyrrhiza glabra* Linn. (25%), *Seasmum indicum* Linn. (25%) and *Ghee* (clarified butter, 50%) as its constituents. It belongs to the Panchgavya class of ayurvedic formulations where ghee is used as base as well as an active ingredient. In the present study *Tilvadi ghrita* is evaluated for its wound healing property employing incision and excision wound models in male Wistar rats. The ancient ayurvedic literature claims the wound healing activity of

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However it is essential to verify its claims using modern day technology and currently accepted scientific norms. The study provides a valuable insight into the claimed pharmacological activity of Tilvadi ghrita (TG).

**Materials and methods**

**Polyherbal Formulation**

*Tilvadi ghrita* (TG) was obtained as a gift sample from Go-Vigyan Anusandhan Kendra, Nagpur. It contains *Glycyrrhiza glabra* (25%), *Sesamum indicum* (25%) and *Ghee* (50%) as its constituents. TG was prepared as per the procedure detailed in ancient Ayurvedic text. The roots of *G. glabra* and seeds of *S. indicum* were crushed to obtain fine powder. The powder obtained from *S. indicum* seeds was suspended into water with stirring to obtain a uniform dispersion. Separately, clarified butter (*ghee*) was heated in a vessel till liquefied and to it both, powdered *G. glabra* and dispersion of *S. indicum* were added and mixed with constant stirring and mild heating to evaporate the water until a liquid to semisolid consistency is achieved. The entire mixture was then allowed to cool to get the ghrita formulation.

**Animals**

Male Wistar rats weighing 200-300 g were selected and individually housed under standard environmental conditions of temperature (23±1°C), 12 hour light and dark cycle and fed with standard pellet diet (Gold Muhor Brand, Lipton India Limited) with water *ad libitum*.

**Treatment**

Animals were inflicted with wounds under light ether anesthesia, semiaseptically. The experimental protocols were approved by the Institute Animal Ethics Committee (IAEC). Animals were divided into three groups consisting of six animals each. A group of six untreated rats were taken as control, (Group I). *Tilvadi ghrita* formulation was applied topically (0.5g) to a separate group (Group II) of six animals, once daily. Another group of six animals (Group III) received application of Framycetine sulphate cream (FSC) 1%w/w (0.5g) which served as positive control. No topical or systemic therapy was given to animals. Rats showing any signs of infection/ deterioration of wounds were totally excluded from study and replaced with new animals. If required the animals were sacrificed with an over dose of ether.

**Excision Wound Model**

A circular wound of about 300mm² area was excised on depilated dorsal thoracic region of excised rats, 5cm away from ear, under light ether anesthesia in semi-aseptic conditions. The animals are housed individually. Wound contraction was monitored by tracing the raw wound area on polythene paper every third day till wounds were completely healed. The wound tracing was retraced on multimeter scale graph paper and expressed as percentage of original size. Percentage or reduction in wound area was taken as measure of wound contraction. The epithelization period was monitored by re-
Incision Wounds Model

Animals were inflicted with two 5 cm long paravertebral incisions through the entire thickness of skin at a distance of about 1.5 cm from midline on each side of depilated back of rat. After mopping the wound intermittent sutures were placed 1 cm apart using surgical nylon thread and curved needle (no.11) and on 10th day tensile strength was measured9.

Histopathological study

Keratinization, epithelization, collagenation, fibrosis and neovascularization were studied from the section of regenerated tissue (10d) and the results studied were numbered from 1 to 5 with 5 standing for maximum similarity and 1 standing for least similarity from normal tissue, comparing the healed tissue in test and untreated wounds.

Statistical analysis

The difference between drug treated group and control group was analysed using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.

Results

In the present study it was observed that the excision wounds healed around day 21-24 due to treatment with TG and FSC as compared to day 30 with untreated control. The study was continued upto day 30 with TG treatment to monitor fall of eschar leaving no raw material behind. The results of excision wound study are shown in Table 1. In incision wound model the mean ± SD of tensile strength for untreated control TG and FSC treatment were 281.30±5.82, 450.36 ± 9.36 and 398.0±6.32 respectively. From the results, it is observed that the wounds treated with the test formulation show increase in tensile strength compared to untreated control group thus promoting

<table>
<thead>
<tr>
<th>Post wounding days</th>
<th>Wound area (mm²)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>378.46 ± 9.26 (0)</td>
<td>330.5± 3.10* (0)</td>
<td>345.05 ± 6.63* (0)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>280.28 ± 8.21 (25.94)</td>
<td>316.0± 3.70* (4.38)</td>
<td>300.05 ± 6.63* (12.94)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>245.66 ± 8.21 (35.08)</td>
<td>272.46± 3.00* (17.56)</td>
<td>243.43 ± 5.41* (29.45)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>209.93 ± 7.79 (44.53)</td>
<td>214.40± 2.8* (35.12)</td>
<td>199.88 ± 2.93* (42.07)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>193.18 ± 6.31 (48.95)</td>
<td>174.15± 6.7* (43.30)</td>
<td>161.91 ± 5.06 (53.07)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>176.66 ± 3.05 (53.40)</td>
<td>130.65± 3.45 (63.67)</td>
<td>120.60 ± 4.47 (65.04)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>151.10 ± 1.54 (60.07)</td>
<td>90.29± 0.80 (72.69)</td>
<td>80.2 ± 0.44 (76.75)</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>121.56 ± 0.41 (67.88)</td>
<td>72.55± 1.20 (88.65)</td>
<td>29.80 ± 0.08 (91.36)</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>101.23 ± 0.15 (73.25)</td>
<td>45.56± 5.24 (93.12)</td>
<td>0.0 (100)</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>60.7 ± 0.08 (83.96)</td>
<td>0.0 (100)</td>
<td>0.0 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD of six animals in each group. Number in parenthesis indicate percentage of wound contraction. All values are significant at p<0.05 as compared to Group I and * indicate not significant.
wound healing. A significant increase in tensile strength (p < 0.05) substantiates the traditional claim of TG.

In the histopathological study, healed tissues were observed for the healing markers like neovascularization, keratinization, collagenation, epithelization, fibrosis. The test formulation TG showed better keratinization, epithelization, collagenation and fibrosis. However neovascularization was not very prominent when compared with untreated control. The results are shown in Table 2 and the photomicrographs in figure 1.

### Discussion

Proper and timely wound healing is a vexing problem faced by all clinicians. In majority of patients normal healing establishes tissue integrity quickly and effectively. However at times this healing is delayed and the ability to accelerate the wound healing becomes a highly desirable objective. Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissues. Wound healing involves a highly dynamic integrated series of cellular, physiological and biochemical processes, which occur in living organism. Repair through regeneration is very common in unicellular and the lower metazoan animal groups while it is highly restricted in the higher animals.

Wound healing involves different phases such as contraction, epithelization, granulation, collagenation. In excision wound study the test formulation Tilvadi ghrita showed better and fast healing compared to untreated control group. The wound contraction ability of TG was not so prominent initially but progressively the contraction ability of TG improved compared to control group. The TG treated group showed much greater contraction of wounds from sixth day onwards than those treated with FSC 1% w/w as the reference standard. The time for wound closure of TG formulation was less than that of untreated group. In incision wound study, there was significant increase in tensile strength of the 10 day old wound due to treatment with TG formulation. Increase in tensile strength is indicative of improved collagenation which significantly contributes to better and effective healing.

Histopathological observations of healed tissue showed incomplete healing with poor keratinization, epithelization,
Fig. 1—Histopathology of 10 d old regenerated tissues (a) Untreated control (b) Tilvadi ghrita treated (c) FSC 1% w/w treated (X 100); K = Keratinization; E = Epithelization; F = Fibrosis.
fibrosis and collagen formation in the untreated rats. The histopathological observation revealed better keratinization in TG treated animals when compared with untreated control. Epithelization improved with TG application when compared with untreated control which may be due to proliferation of epithelial tissue over wound area. It may be concluded that TG promotes keratinization, epithelization and fibrosis comparable with FSC treatment.

Conclusion

Tilvadi ghrita, a herbal formulation demonstrates potent wound healing activity comparable to FSC, and significantly improved than untreated wounds.

Acknowledgment

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References