Wound healing activity of the ethanol extract of *Terminalia bellirica* Roxb. fruits

G P Choudhary
School of Pharmacy
Devi Ahilya Vishwavidyalaya
Khandwa Road, Indore-452 017, Madhya Pradesh, India
E-mail: choudhary.gp@gmail.com

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**Abstract**

The wound healing activity of ethanol extract of *Terminalia bellirica* Roxb. fruit was evaluated on excision and incision wound model, in albino rats, in the form of an ointment with two concentrations (2 and 4% w/w ointment) of fruit extract in simple ointment base. Both concentrations of the ethanol extract showed significant response in both the wound types tested when compared with the control group. Nitrofurazone ointment (0.2%w/w) was used as standard drug.

**Keywords:** *Terminalia bellirica*, Belliric Myrobalan, Ethanol extract, Nitrofurazone, Wound healing.

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**Introduction**

Wound healing process involves several steps, including coagulation, formation of granulation tissue, collagenation and acquisition of wound strength. During the formation of new tissue, endothelial cells proliferate and form new blood vessels. In tribal areas different crude drugs preparations are used to treat various skin diseases including wound. *Terminalia bellirica* Roxb. belonging to the family Combretaceae, commonly known as Belliric Myrobalan (Hindi- *Bahera*), is a deciduous tree found throughout the Indian forests and plains. Fruit is astringent, antiseptic, rejuvenative, brain tonic, expectorant and laxative. It is used in coughs, sore throat, dysentery, diarrhoea and liver disorders. It is also useful in leprosy, fever and hair care. In folk medicine it has been used for the treatment of skin diseases as antiseptic and on all types of fresh wound, immediate dusting of *triphala* is done as a wound healer. A survey of literature revealed that no scientific study on the wound healing activity of fruits of this plant has been reported to validate the folklore claims on this property. Therefore, objective of the present study was to evaluate the effect of ethanol extract of *T. bellirica* fruits on different parameters related to wound healing in albino rats.

**Materials and Methods**

**Plant material**

Fruits of *T. bellirica* were obtained from Yucca enterprises, Mumbai and identified at Department of Botany, Holkar Science College, Indore. A voucher specimen has been deposited in our laboratory for further reference.

**Preparation of extract**

Powdered fruits were soxhlet-extracted with 70% ethanol. The ethanolic extract was evaporated in *vacuo* and residue (yield: 33%w/w) was subjected to phytochemical screening. The phytochemical screening revealed the presence of tannins, glycoside and phytosterol.

**Preparation of drug formulation**

The drug formulations with different concentration of the extract were prepared, viz. 2% (w/w) ointment, where 2g extract was incorporated in 100g simple ointment base and 4% (w/w) ointment where, 4g of extract was incorporated in 100g of simple ointment base. Nitrofurazone ointment (0.2 w/w) was used as standard drug for comparing the wound healing potential of the extract in different animal model.

**Animals**

Healthy Wistar rats of either sex weighing 150-200g were used. They were kept in a standard conditions
of temperature (23±1°C), 12h light/dark cycle and fed with rodent diet (Hindustan Lever Ltd., Bangalore) and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee, DAVV, Indore.

Wound healing activity

The wound healing activity was investigated in ether anaesthetized rats in two different wound models (at two different concentrations 2 and 4%w/w).

Incision wound- The animals were divided into four groups of six animals each, the group I control (Simple ointment base B.P.), the group II was reference standard and treated with 0.2%w/w Nitrofurazone ointment. The group III animals were treated with 2%w/w ethanolic extract and the group IV animals were treated with 4%w/w ethanolic extract of fruit. The animals were anaesthetized with anaesthetic ether. Paravertebral incision of 6cm long was made on either side of the vertebral column of the rat. Care was taken to make the incision at least 1cm lateral to vertebral column. The wounds were covered with interrupted sutures of 1cm apart. The animals were caged separately according to groups. The sutures were removed on 8th day after surgery. The tensile strength of the wound was measured on 10th day by tensiometer.7

Excision wound- The animals were divided into four groups of six animals each. The group I was considered as control (treated with simple ointment base B.P.), the group II was reference standard and treated with 0.2%w/w Nitrofurazone ointment. The group III animals were treated with the 2%w/w ethanolic extract ointment and the group IV animals were treated with 4%w/w ethanolic extract ointment of fruit. A circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of animals under light ether anaesthesia. The observation of percentage wound closure was made on 4th, 8th, 12th and 16th post wounding days. Number of days required for falling of the scar without any residual raw wound gave the period of epithelization.8

Table 1: Effect of ethanol extract ointment of fruit of *Terminalia bellirica* on % wound closure of excision wounds

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4th Day</th>
<th>8th Day</th>
<th>12th Day</th>
<th>16th Day</th>
<th>Period of epithelization in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Simple ointment base B.P.)</td>
<td>15.82±0.68</td>
<td>27.21±1.02</td>
<td>48.21±1.80</td>
<td>68.53±2.60</td>
<td>24</td>
</tr>
<tr>
<td>Nitrofurazone-0.2% (Ref.Std.)</td>
<td>35.28±0.15</td>
<td>76.80±0.19</td>
<td>89.81±0.58</td>
<td>97.11±0.48</td>
<td>18</td>
</tr>
<tr>
<td>Ethanolic extract (4%)</td>
<td>34.42±1.01</td>
<td>76.86±1.24</td>
<td>84.32±2.36</td>
<td>92.56±2.10</td>
<td>18*</td>
</tr>
<tr>
<td>Ethanolic extract (2%)</td>
<td>20.16±1.02</td>
<td>35.33±1.82</td>
<td>61.40±2.78</td>
<td>80.12±2.32</td>
<td>20</td>
</tr>
</tbody>
</table>

Values are mean ±SE
*P<0.01 vs control by student's t-test

Statistical analysis- The results are expressed as mean ± SE of six animals in each group. The data were evaluated by Student’s t-test and the values of P<0.01 were considered statistically significant.

Results

The effect of ethanol fruit extract ointment on excision wound model, the wound healing contracting ability in different concentrations was significantly greater than that of the control (i.e. simple ointment treated group). The 4% (w/w) extract ointment treated groups showed significant wound healing from the fourth day onwards, which was comparable to that of the standard drug, i.e. Nitrofurazone ointment treated group of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 4% w/w extract ointment treated group (18±1 days for 100% contraction which was almost similar to that of the Nitrofurazone treated group). The 2%(w/w) extract ointment treated group of animals showed significant wound contraction from the eighth day onwards and achieved 100% with the wound closure time of 20±2 days (Table 1).

The results of tensile strength wound model (tensile strength measurement) is shown in Table 2. The tensile strength of the 4% extract ointment treated group and the Nitrofurazone ointment treated group were comparable to each other. The 2% extract ointment treated group showed a lesser but significant increase in the tensile strength.
compared to the control group. Thus, both concentrations of the fruit extract as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound.

The results of the present study revealed that both concentrations (2 and 4% w/w) of ethanol extract of *T. bellirica* fruit have significant wound healing activity in both incision as well as excision wound models.

### Discussion

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. This is mainly achieved by the synthesis of the connective tissue matrix. Collagen is a major protein of the extracellular matrix and is the major component that ultimately contributes to wound strength. Tannins promote the wound healing through several cellular mechanisms; chelating of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts. T. bellirica fruit contains about 20-40% of tannin, phyllemblin, β-sitosterol, anthraquinones, fixed oil, mannitol, glucose, fructose and rhamnose. Similar findings have been reported with the extracts of the plants containing tannins by earlier workers. The results of this study also revealed that tannins are one of the important phytoconstituents responsible for wound healing mainly due to their astringent and antimicrobial property.

### Conclusion

It can be inferred from this study that the wound healing activity of the fruit of the plant *T. bellirica* is due to its high tannin (20-40%) content, which seems to be responsible for wound contraction and increased rate of epithelization.

### References


12. Rane MM and Mengi SA, Comparative effect of oral administration and topical application of alcoholic extract of *Terminalia arjuna* bark on incision and excision wounds in rats, *Fitoterapia*, 2003, 74, 553-558.