EVALUATION OF WOUND HEALING ACTIVITY OF LEAF OF

BARLERIA LUPULINA

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ABSTRACT

Barleria lupulina belongs to the family Acanthaceae and is a large, widespread, polymorphic, pantropical genus of herbs and shrubs comprising some 300 species are used used traditionally for the treatment of variety of diseases including anaemia, toothache, coughs and as a hypoglycemic agent. The present study was aimed at evaluation of wound healing activity of the plant. In the present study the roots of Barleria lupulina were studied for wound healing activity by incorporating the methanolic and the total aqueous extract in simple ointment base B.P. in concentration of 0.5% (w/w), 1% (w/w) and 2% (w/w). Wound healing activity was studied in three types of model in rats viz. excision, incision and estimation of biochemical parameter. In case of the excision wound model wound contraction and period of epithelisation was studied while in incision wound model was evaluated by determining tensile strength and hydroxyproline content in the scab. Treatment of wound with ointment containing 2% (w/w) the methanolic and 2% (w/w) the total aqueous extract exhibited significant (P < 0.001) wound healing activity. The methanolic and total aqueous extracts were analyzed for total phenols content equivalent to Gallic acid. The content of total phenols was 10% (w/w) and 16% (w/w) in methanolic and total aqueous extract respectively.

KEYWORDS: Barleria lupulina, hypoglycemic, Acanthaceae, epithelisation,

INTRODUCTION

Wound is a clinical entity and is as old as mankind, often possesses problem in clinical practice. A lot of research has been envisaged to develop the better healing agents and it has...
been a challenging task to discover healing agents and keep up pace with problems encountered. Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue. Wound healing or wound repair is the body’s natural process of regenerating dermal and epidermal tissue. Wounds can be broadly categorized as having either an acute or a chronic etiology including bites, burns, surgical wound abrasion, laceration or acute inflammatory phase followed by synthesis of collagen and the extracellular macromolecules which are later remolded to form, scar (Deodhar and Rana, 1997). The process of wound healing occurs in four phases: (i) coagulation, which prevents blood loss, (ii) inflammation and debridement of wound, (iii) repair, including cellular proliferation, and (iv) tissue remodeling and collagen deposition (Puratchikody et al., 2006). Wound healing involves continuous cell–cell and cell–matrix interactions that allow the process to proceed in three overlapping phases viz. inflammation cellular proliferation and remodeling. Phase 1 is a coagulation and inflammatory phase (0–3 days) and this involves migration of neutrophils at margin of incision, moving towards the fibrin clot. Phase 2 is a proliferative phase (3–12 days) in which the neutrophils are largely replaced by the macrophages. Granulation tissue progressively invades the incision space and the incisional space is filled with granulation tissue. Collagen fibrils become more abundant and begin to bridge the incision. Phase 3 is a remodeling phase (3–6 months), involving continuous accumulation of collagen and proliferation of fibroblasts. There is marked reduction in leukocyte infiltration and edema. The phase involves synthesis of collagen fibers, leading to increase in tensile strength of the skin (Cotran et al., 1997). Healing requires the collaborative efforts of many different tissues and cell lineages. It involves platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialization. Healing is not complete until the disrupted surfaces are firmly knit by collagen (Govindrajan et al., 2007). Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns (Govindrajan et al., 2007).

Barleria belongs to the family Acanthaceae and is a large, widespread, polymorphic, pantropical genus of herbs and shrubs comprising some 300 species. Its greatest representation is in Africa (particularly the eastern parts) and Asia, with its greatest centre of diversity in tropical East Africa (Balkwill and Balkwill, 1998). Barleria species exhibit several medicinal properties. For instance, leaves of Barleria cristata have been used traditionally for the treatment of variety of diseases including anaemia, toothache, coughs and
as a hypoglycemic agent (Gambhire et al., 2009) and juice of the leaves is used in the treatment of ulcers and fever (Ambasta, 1986). Barleria lupulina Lindl has a strong inhibitory effect against acne-inducing bacteria (Chomnawang et al., 2005). Root decoction or infusions of pounded leaves of Barleria eranthemoides R.Br. is drunk for the treatment of dysentry and taken against infectious diseases (Maregesi et al., 2007). Barleria lupulina Lindl is a small shrub, commonly known as Sornomukhi and distributed in South East Asia. It is well known in Thai folk medicine, as the plant is externally used as an anti-inflammatory against insect bites, snake bites, herpes simplex, herpes zoster and varicella zoster virus lesions and it also has a diuretic effect and anti-amoebic activities (Kanchanapoom et al., 2001; Lans et al., 2001; Sawangjaroen et al., 2006). In preliminary investigations (Suksamrarn, 1986; Byrne et al., 1987; Tuntiwachwuttikul et al., 1998) nine iridoid glucosides have been isolated. Compounds found in the leaves of Barleria lupulina Lindl include barlerin, acetylbarlerin, shanzhiside methyl ester, acetylshanzhiside methyl ester, ipolamiidoside and iridoid glucosides (Lans et al., 2001). Virucidal activity against HSV type 2 strain G, in vitro anti-HSV activity, antimicrobial activity against acne-inducing bacteria, antidiabetic potential, antiulcer activity, isolation of Iridoid Glucosides, the crystal Structure and plant growth-inhibiting properties of 6-O-Acetylshanzhiside Methyl Ester, a neuropharmacological profile, and isolation of nine iridoid glucosides from Barleria lupulina have previously been reported (Chomnawang et al., 2005; Kanchanapoom et al., 2001; Lans et al., 2001; Byrne et al., 1987; Yoosook et al., 1999; Suba et al., 2002; Suba et al., 2004;  

2. MATERIALS AND METHODS

2.1. Materials

The root of Barleria lupulina was procured from local market of Bangalore and was authenticated at Acharya Institute of technology, Bangalore, by carrying out macroscopic and microscopic evaluation. The voucher specimen of the same has been deposited in the department of biotechnology, Acharya Institute of technology, Bangalore.

2.2. Preparation of the root extract

The air-dried crude drug was pulverized to obtain coarse powder. The total aqueous extract was prepared by decoction method with drug:distilled water in ratio of 1:5 (yield: 12.22%, w/w). The powdered drug was defatted by extracting with pet-ether (60–80 °C) followed by extraction of methanol (yield: 09.47%, w/w) with Soxhlet extractor. The extracts thus obtained were concentrated by recovering the solvent by Rotary Flash Evaporator. The
concentrated extract was then evaporated to dryness in vacuum oven at temperature not more than 50 °C. The dried extract was stored at 2–8 °C in refrigerator. The extract was further used for the evaluation of wound healing activity.

2.3. Phytochemical analysis
The methanolic and total aqueous extracts were tested qualitatively for different Phytoconstituents using various chemical tests. The content of total phenols was determined using Folin-Ciocalteu method (Yu et al., 2003) using Gallic acid (Molichem, India) as reference standard.

2.4. Animals
The healthy Wistar albino rats of either sex weighing 150–200 g were housed under standard environmental conditions of temperature and humidity (25±0.50 °C) and 12 h light/dark cycle) were utilized for the studies. The animals were fed with standard pellet diet and water ad libitum. The animal studies were performed in the institute with due permission from Institutional Animal Ethical Committee.

2.5. Acute dermal toxicity
The study was carried out to determine the therapeutic dose of the methanolic and total aqueous extracts. The acute dermal toxicity testing of the methanolic extract and the total aqueous extract was done by applying the Ointments containing methanolic and total aqueous extracts of the highest concentrations of 2% (w/w) on the shaved back of the rats. The OECD guidelines no. 402 (OECD guidelines, 1987) were followed for the study.
2.6. Wound healing activity
The animals were grouped into three major groups viz. control, standard and test with six animals in each group. The control group was treated with simple ointment base B.P. The standard group was treated with Betadine (Win Medicare containing 5% (w/w) Povidone iodine, lot no. PK0247) ointment. The test groups were treated with ointments with different concentrations of extracts viz. 0.5% (w/w), 1% (w/w) and 2% (w/w) incorporated in simple ointment base, in all the three models.

2.6.1. Excision wound model
The rats were anesthetized by administering ketamine (0.5 ml/kg b. w. i.p.). A full thickness of the excision wound of circular area (approx. 500mm²) and 2mm depth was made on the shaved back of the rats 30 min later the administration of ketamine injection. The wounding day was considered as day 0 (Fig. 1). The wounds were treated with topical application of the ointments.

![Fig. 2: A completely healed excision wound after 19 day treatment.](image)

Table 1: Effect of topical application of ointments containing methanolic (meth.) and total aqueous (aq.) extracts of Barleria lupulina roots on wound contraction of excision wound.

<table>
<thead>
<tr>
<th>Group</th>
<th>4th day</th>
<th>8th day</th>
<th>12th day</th>
<th>16th day</th>
<th>Period of epithelization (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.84 ± 2.28</td>
<td>40.89 ± 1.33</td>
<td>57.88 ± 1.47</td>
<td>83.51±0.91</td>
<td>24</td>
</tr>
<tr>
<td>Standard</td>
<td>30.09 ± 1.06</td>
<td>53.48 ± 1.20</td>
<td>78.79 ± 0.98</td>
<td>96.03±0.53</td>
<td>22</td>
</tr>
<tr>
<td>0.5% SO meth. ext</td>
<td>16.21 ± 1.34</td>
<td>42.68 ± 3.56</td>
<td>72.18 ± 1.88</td>
<td>87.07±1.46**</td>
<td>23</td>
</tr>
<tr>
<td>1% SO meth. ext</td>
<td>22.47 ± 0.52</td>
<td>45.32 ± 2.24</td>
<td>76.57 ± 1.46</td>
<td>91.63±0.65**</td>
<td>21</td>
</tr>
<tr>
<td>2% SO meth. ext</td>
<td>23.10 ± 2.15</td>
<td>62.69 ± 3.22</td>
<td>86.61 ± 0.56**</td>
<td>100***</td>
<td>19</td>
</tr>
<tr>
<td>0.5% SO aq. ext</td>
<td>15.23 ± 1.05</td>
<td>40.40 ± 2.42</td>
<td>66.98 ± 1.36*</td>
<td>83.99±0.73*</td>
<td>24</td>
</tr>
<tr>
<td>1% SO aq. ext</td>
<td>16.81 ± 1.59</td>
<td>43.62 ± 3.51*</td>
<td>71.87 ± 0.51*</td>
<td>87.69±0.74**</td>
<td>23</td>
</tr>
<tr>
<td>2% SO aq. ext</td>
<td>20.46 ± 0.59</td>
<td>60.13 ± 0.46*</td>
<td>80.57 ± 0.41**</td>
<td>96.34±0.64**</td>
<td>22</td>
</tr>
</tbody>
</table>

SO, simple ointment base. n = 6 animals in each group, The treated groups are compared by Student t test with the control group, *** P < 0.001, ** P < 0.01, * P < 0.05.
As described above till the wounds were completely healed. The wounds were monitored and the area of wound was measured on 4, 6, 8, 10, 12, 14, 16 post-wounding days and the mean % wound closure is reported in Table 1. The period of epithelization was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound (Nayak et al., 2007). Wound healing rate (Muthusamy et al., 2008):

\[
\text{% of wound closure} = \frac{\text{wound area on day 0} - \text{wound area on day } n}{\text{wound area on day 0}} \times 100
\]

Where \(n\) = number of days 4th, 8th, 12th, and 16th day.

### 2.6.2. Incision wound model

The rats were anesthetized by administering ketamine (0.5 ml/kg b. w. i.p.). Incision wounds of about 6 cm in length and 2 mm in depth were made with sterile scalpel on the shaved back of the rats 30 min later the administration of ketamine injection. The parted skin was kept together and stitched with black silk at 0.5 cm intervals (Fig. 3). Surgical thread (no. 000) and a curved needle (no. 9) were used for stitching. The continuous thread on both wound edges were tightened for good closure of the wounds. The wounds of animals in the different groups were treated with topical application of the Ointments as described above, for the period of 10 days. The wounding day was considered as day 0. When wounds were cured thoroughly, the sutures were removed on the 8th post-wounding day (Fig. 5) and the tensile strength of the skin that is the weight in grams required to break open the wound/skin was measured by tensiometer on the 10th day reported in Table 2 (Nath et al., 2006).

Fig. 3: Incision wound on the day 0.
Fig. 4: A completely healed incision wound after 8 day treatment

Tensile strength was calculated using the following formula (Diwan et al., 2008):

\[
\text{Tensile strength} = \frac{\text{breaking strength (g)}}{\text{cross-sectional area of skin (mm}^2)}
\]

2.6.3. Estimation of biochemical marker

Circular wound with approximate area of 500mm\(^2\) was created using the procedure described in excision wound model. The wounds were treated with topical application of ointments as described above for 10 days. The scab was removed on 11th day and dried in oven at 110 °C. The hydroxyproline content in dried scab was determined by extracting hydroxyproline from scab using concentrated Hydrochloric acid followed by reaction between amino group of hydroxyproline with p-dimethylaminobenzaldehyde to develop red colour. The red colour thus measured on Spectrophotometer at 558nm using the method described by Bergman and Loxley (1963). The results are presented in Table 3.

Fig. 5: Tensiometer: for the measurement of tensile strength of skin.
Table 2: Effect of topical application of ointments containing methanolic (meth.) and total aqueous (aq.) extracts of *Barleria lupulina* root on tensile strength of the skin having incision wound.

<table>
<thead>
<tr>
<th>Group (N= 6)</th>
<th>Tensile strength in gram (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>311.83 ± 3.64</td>
</tr>
<tr>
<td>Standard</td>
<td>624.00 ± 4.91</td>
</tr>
<tr>
<td>0.5% SO meth. ext</td>
<td>629.00 ± 2.63**</td>
</tr>
<tr>
<td>1% SO meth. ext</td>
<td>730.67 ± 3.29**</td>
</tr>
<tr>
<td>2% SO meth. ext</td>
<td>760.83 ± 2.04***</td>
</tr>
<tr>
<td>0.5% SO aq. ext</td>
<td>730.16 ± 2.70**</td>
</tr>
<tr>
<td>1% SO aq. ext</td>
<td>769.67 ± 2.73**</td>
</tr>
<tr>
<td>2% SO aq. ext</td>
<td>859.83 ± 3.19***</td>
</tr>
</tbody>
</table>

SO, simple ointment base. n = 6 animals in each group, The treated groups are compared by Student t-test with the control group, ** P < 0.01. *** P < 0.001.

Table 3: Effect of topical application of methanolic (meth.) and aqueous (aq.) extracts of *Barleria lupulina* on hydroxyproline content in the scab of excision wound.

<table>
<thead>
<tr>
<th>Group (N= 6)</th>
<th>Hydroxyproline (g/500 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.20 ± 0.56</td>
</tr>
<tr>
<td>Standard</td>
<td>15.67 ± 0.63</td>
</tr>
<tr>
<td>2% SO meth. ext</td>
<td>22.65 ± 0.34***</td>
</tr>
<tr>
<td>2% SO aq. ext</td>
<td>18.99 ± 0.38**</td>
</tr>
</tbody>
</table>

SO, simple ointment base. n = 6 animals in each group, The treated groups are compared by Student t test with the control group, ** P < 0.01. *** P < 0.001.

2.7. Statistical analysis

Results obtained from the three wound models have been expressed as mean±SEM and were compared with the corresponding control group (simple ointment B.P.) by applying ANOVA test (Mukherjee et al., 2000).

3. RESULTS

3.1. PHYTOCHEMICAL ANALYSIS

Qualitative phytochemical analysis revealed presence of tannins especially hydrolysable tannins and alkaloids. The presence of alkaloid was confirmed by performing TLC and spraying with Dragendorff’s reagent. The presence of hydrolysable tannins was confirmed by performing co-TLC of the hydrolyzed extracts on Silica Gel GF 254 as stationary phase; ethyl acetate: toluene: methanol: formic acid (3:3:0.2:0.8) as mobile phase and Gallic acid as
the reference standard. The TLC analysis revealed the presence of spot corresponding to Gallic acid and the coinciding UV spectra. The quantitative estimation of total phenols equivalent to Gallic acid by Folin-Ciocalteu method was found to be 10% (w/w) and 16% (w/w) in methanolic and total aqueous extract respectively.

3.2. Excision wound study
The results of wound healing activity by excision wound model are presented in Table 1 Fig. 2. The values presented in the table represent percentage wound healing at 4, 8, 12, 16 days for control (simple ointment B.P. treated group.), standard (povidone iodine treated group) and the test groups viz. the methanolic extract (0.5%, w/w; 1%, w/w and 2%, w/w) and the total aqueous extract (0.5%, w/w; 1%, w/w and 2%, w/w). It is observed that wound contracting ability of animals treated with ointment containing 2% (w/w) methanolic extract was found to be significantly higher \((P < 0.001)\) on days 8, 12 and 16 as compared to the control group. The epithelization period was also found to be the least that is 19 days in case of animals treated with ointment containing 2% (w/w) methanolic extract.

3.3. Incision wound study
The effect of wound healing activity in this model was evaluated by determining the tensile strength of the incision wound of different groups viz. control treated with Simple ointment base B.P., standard group treated with drug povidone iodine and the test group treated with the extracts at different concentrations. The results are presented as mean weight in gram+SEM required breaking open the resutured wound Fig. 4 (Table 2). The animals treated with ointment containing 2% (w/w) methanolic extract and 2% (w/w) total aqueous extract indicated significantly high \((P < 0.001)\) tensile strength as compared to the control group.

3.4. Biochemical marker estimation
The earlier results indicate that the animals treated with ointment containing 2% (w/w) methanolic and 2% (w/w) total aqueous extract have good wound healing activity, hence, the biochemical marker such as hydroxyproline content in the scab of excision wound created in the animals treated with stated extracts was determined on the 11th day and presented in Table 3. The animals treated with ointment containing 2% (w/w) methanolic extract indicated significantly high \((P < 0.001)\) levels of hydroxyproline \((22.659 \mu g/500mg)\) as compared to control \((9.203 \mu g/500mg)\).
4. CONCLUSION
The basic principle of optimal wound healing is to minimize tissue damage and provide an adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part. The result of excision wound model indicates that in the first 4 days there is no significant increase in the wound contraction in all the groups as compared to the control group. The results of the 8th day indicate that there is significant increase ($P < 0.001$) in the percentage wound contraction in the group treated with standard drug that is povidone iodine, 2% (w/w) methanolic and 2% (w/w) total aqueous extract, revealing that the extract has ability to induce cellular proliferation. Hydroxyproline is one of the biomarkers indicating wound healing process, as the content of the same is increased on 10th day. The increased hydroxyproline content in the scab of the animals treated with 2% (w/w) methanolic and total aqueous extracts support the wound healing process. Out of these two extracts, the methanolic extract in the concentration of 2% (w/w) is found to be the most effective. The increase in tensile strength of wounded skin indicates the promotion of collagen fibers. Highest tensile strength of the wounded skin was observed in the animals treated with 2% (w/w) methanolic and the total aqueous extracts. The increased tensile strength reveals that the disrupted surfaces are firmly knit by collagen. The wound healing studies on roots of *Barleria lupulina* indicate that the phenols constituents/tannins play an important role in wound healing process (Muthusamy et al., 2008).

REFERENCES


