In vivo Study on the Efficacy of the Topical Formulations from the Crude Ethanolic Extract of Bidens pilosa L. against Staphylococcus aureus

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Abstract. The efficacy of topically applied ointment and lotion formulations of Bidens pilosa was evaluated against an experimental surgical staphylococcal wound infection in ICR mice. Colonies of Staphylococcus aureus from agar plates were inoculated into a superficial wound, and therapy with the topical preparations was started 24 hours after infection. The control (NSS), standard (Mupirocin), and placebo bases were included in the study to compare the results with the medicated preparations.

Histopathological analysis of the S. aureus infected wounds has shown inflammation after the 1st day and regressed after the 4th day of therapy. After the 5th day, only the Mupirocin and Burburtak ointment groups manifested healing. In non-treated wounds, there was a continuous proliferation of inflammatory cells that lasted up to the 4th day and it only terminated after the 5th day signaling the self-healing process. Therapy with placebo ointment and lotion (polyethylene glycol base) was ineffective, whereas twice daily application of Mupirocin ointment resulted in elimination of the staphylococci. The topical preparations from ethanolic extracts of Bidens pilosa were proven effective but Bidens pilosa ointment showed greater action than Bidens pilosa lotion.

Keywords: Bidens pilosa, Burburtak, in vivo study, Mupirocin

INTRODUCTION

Bidens pilosa Linn. (Asteraceae) commonly known as Burburtak in Tagalog, is one of the many Philippine medicinal plants used as home remedy. It is used for its anti-inflammatory, antiseptic, liver-protective, blood-pressure lowering, and hypoglycemic effects [1]. Phenylpropanoid glucosides, polyacetylenes, a diterpenes, flavonoids, and flavone glycosides have been identified as bioactive components from this plant [2]. These compounds were suggested to be involved in the antioxidant [2], antibacterial...
and antimicrobial activities [3]. Previous preformulation studies also led to the formulation of 15% (Minimum Inhibitory Concentration) ointment and lotion dosage forms utilizing the crude ethanolic extract from the leaves of the plant. This study focused on the evaluation of the efficacy of topically applied ointment and lotion formulations of *Bidens pilosa* against an experimental surgical staphylococcal wound infection in International Cancer Research (ICR) mice.

**EXPERIMENTAL**

**Test Animal.** Thirty female, properly identified, ICR mice, 18-20 g body weight, and approximately 20-30 days old were purchased from the Research Institute for Tropical Medicines (RITM) a week before the actual experiment for acclimatization. The mice were divided into six groups, with five members each.

**Organism.** Colonies of *Staphylococcus aureus* purchased from the Applied Microbiology Laboratory of the Research Center for the Natural Sciences were used as the biological agent. The strain was proven sensitive to Mupirocin (MIC 0.25 mg/L). The culture was maintained on nutrient agar at room temperature.

**Wound preparation and infection.** The technique employed in the study was according to a protocol approved by the Bureau of Animal Industry. After the mice have been anesthetized using Lidocaine administered intraperitoneally, a single 1 cm incision was made using surgical scissors on the dorsal region of the thoracic part of the body. Colonies of *Staphylococcus aureus* were introduced into the wound using a sterile swab. After use, the swabs were scalded.

**Therapy.** The test animals were grouped into six, having five members each, and were assigned to specific preparations: (1) Burburtak 15% ointment, (2) Burburtak 15% lotion, (3) ointment base, (4) lotion base, (5) Mupirocin 2% ointment and (6) Normal Saline Solution (NSS, the control group). Therapy was initiated 24 hours after infection by applying using a sterile cotton swab, approximately 0.2 mL of the topical preparation. It was gently smeared over the wound surface with a gloved finger. Application of the agents was done twice a day, once in the morning and once in the afternoon. The duration of therapy lasted for 5 days. All animals were given the appropriate dose of an analgesic (Paracetamol) after the incision to alleviate pain for the first 24 hours.

**Bacteriological assessment.** Bacteriological assessment started 24 hours after the first treatment. The affected area was observed for the development of the healing process. A member of each group was humanely sacrificed daily by cervical dislocation (Table 1). This was done by an attending veterinarian. The skin samples with incision were fixed with formalin and were subjected to histopathological assessment. The tissues were viewed under electron microscope in low power and high power objective.

**Interpretation of results.** A system of grading infection (Table 2) was employed to descriptively interpret the results obtained from the histopathological testing. This shows the rating of inflamed cells in an infected wound.

<table>
<thead>
<tr>
<th>Group of 5 ICR mice</th>
<th>Day 1 of therapy</th>
<th>Day 2 of therapy</th>
<th>Day 3 of therapy</th>
<th>Day 4 of therapy</th>
<th>Day 5 of therapy</th>
<th>Day 6 of therapy</th>
<th>Day 7 of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound incision and infection with <em>S. aureus</em></td>
<td>Start of therapy</td>
<td>Mouse 1 sacrificed, continued therapy for the rest</td>
<td>Mouse 2 sacrificed, continued therapy for the rest</td>
<td>Mouse 3 sacrificed, continued therapy for the rest</td>
<td>Mouse 4 sacrificed, continued therapy for the rest</td>
<td>Mouse 5 sacrificed</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Grading System of Magnitude of Infection

<table>
<thead>
<tr>
<th>Descriptive Term</th>
<th>Numerical Value</th>
<th>% inflamed cells</th>
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<tbody>
<tr>
<td>Severe</td>
<td>4</td>
<td>65-100</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
<td>35-60</td>
</tr>
<tr>
<td>Mild</td>
<td>2</td>
<td>20-30</td>
</tr>
<tr>
<td>Normal</td>
<td>1</td>
<td>0</td>
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</tbody>
</table>

Table 3. Histopathological Assessment

<table>
<thead>
<tr>
<th>Day of therapy</th>
<th>Control</th>
<th>Burburtak Ointment</th>
<th>Burburtak Lotion</th>
<th>Ointment base</th>
<th>Lotion Base</th>
<th>Bactroban</th>
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<tbody>
<tr>
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<td>3</td>
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<td>1.5</td>
<td>3</td>
<td>2.5</td>
<td>1</td>
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</tbody>
</table>

Table 4. Treated Tissues Viewed under the Electron Microscope after Day 5

Control - NSS

Moderate proliferation of inflammatory cells at the subcutaneous area with necrosis, mild proliferation of fibroblasts.

Bidens pilosa Ointment

Few inflammatory cells and small blood vessels, repaired epidermis is observed.

Bidens pilosa Lotion

Few inflammatory cells in the subcutaneous area are still present, epidermis not completely repaired.
Moderate proliferation of inflammatory cells, few fibroblasts and small blood vessels, granulation and epidermal tissue start to develop.

Moderate proliferation of inflammatory cells at the subcutaneous area only, mild numbers of fibroblasts and small blood vessels, developed granulation tissue, epidermal tissue starts to develop.

No inflammatory cells observed, granulation tissue sloughs off, epidermis repaired.

Figure 1. Graphical Representation of Histopathological Assessment
RESULTS AND DISCUSSION

Table 3 shows that after the fifth day of application, tissue samples treated with Mupirocin 2% ointment and Burburtak 15% ointment were back to normal wherein there were no occurrences of inflammation cells. Burburtak lotion showed very minute number of inflammation cells in the tissues, while several inflamed cells were still evident in the tissues of those individually treated with lotion base, ointment base and NSS. It also shows that the control and the bases reached severe inflammatory responses due to the absence of an active antimicrobial agent in their formulation. Burburtak lotion reached moderate proliferation of inflammatory cells but was able to control the infection by the sixth day. Mupirocin and Burburtak Ointment only had mild proliferation of inflammatory cells. Both agents were able to bring back the state of the tissue to its normal condition after the 5th day. The graphical representation of the histopathological assessment is shown in Figure 1, and the tissues viewed under the electron microscope after Day 5 are shown in Table 4.

CONCLUSION

The topical preparations from the crude ethanolic extract of Bidens pilosa or Burburtak were proven effective against Staphylococcus aureus but Bidens pilosa ointment showed greater activity than Bidens pilosa lotion. Bidens pilosa ointment was found to be as effective as the commercially available 2% Mupirocin ointment against the mentioned microorganism.

ACKNOWLEDGEMENT

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REFERENCES