EXPERIMENTAL STUDIES ON WOUND HEALING PROPERTIES OF ANDROGRAPHIS ECHIOIDES (L.) NEES IN WISTAR ALBINO RATS

Mohd Rafiq¹* and Dr. Sadath Ali²

¹Singhania University, Pacheri Bari, Rajasthan – 333515.
²Professor, Glocal University, Mirzapur Pole, Saharanpur, Uttar Pradesh.

ABSTRACT
As we all know in the present situation medicinal plants are very much influenced by the researchers because of its minimal or no side effects. India is sitting on a gold mine of well-recorded and well practiced knowledge of traditional herbal medicine. Andrographis echioides is one of the Indian medicinal plant belongs to the family of Acanthaceae. It is widely distributed in the tropical parts of India and Srilanka. Based on the traditional value of Andrographis echioides still many activities are yet to be proven scientifically. This study was carried out to evaluate the wound healing potential of ethanolic extract of whole plant Andrographis echioides (200mg/kg/day) in Wister albino rat using excision and incision wound model in the form of ointment base. All experiments were conducted according to standard procedures. In excision model, ethanolic extract treated animals exhibit 19.50±0.34% reduction in wound area when compared to control which was 30.12±0.60% and whereas standard exhibit 18.00±0.87%. The extract treated wounds are found to epithelize faster as compared to control. In incision wound model Significant (p<0.001) increase in breaking strength 307.23±1.37 was observed whereas in control group it was 119.33±6.67 and in standard it was 330.41±8.77. The Framycetin sulphate cream (FSC) 1 % w/w was used as standard.

KEYWORDS: Andrographis echioides, Wound healing, Excision wound, Incision wound, Framycetin sulphate cream.
INTRODUCTION
From the past few years, mankind suffered a lot of adverse effects by the use of synthetic drugs. Thus, the time has come, we must pay serious attention towards natural resources which is safe to use.

Wound is nothing but an injury to living tissue caused by various factors like blow, cut or other impacts; whereas wound healing is a process of restoring damaged tissue as closely as possible to its normal state. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue.[1] The entire wound healing process starts at the moment of injury and can continue for months to years. The stages of wound healing are inflammatory phase, proliferation phase, fibroblastic phase and maturation phase.[2]

Nature has endowed India with rich source of medicinal plants. *Andrographis echioides* (L.) Nees is one of the Indian medicinal plant belongs to the family of *Acanthaceae*. It is widely distributed in the tropical parts of India and Srilanka.[3] It is commonly known as “False Water Willow”. Which is traditionally used as anti inflammatory, febrifuge, cooling, alternative for cuts. The extract of the whole plant is also used to cure fever.[4]

It has been known that the process of wound healing can be promoted by several medicinal plant extracts rich in active compounds, such as flavonoids, triterpenes, alkaloids, tannins and other biomolecules.[5] Since *Andrographis echioides* contains flavonoides as a major component.[6] Therefore, in the present work, an attempt has been made to exhibit the efficacy of *Andrographis echioides* against wound healing activity.

MATERIALS AND METHODS

**Collection of Plant Material:** Fresh whole plant material of *Andrographis echioides* was collected from the local fields of Gulbarga. The plant specimen was identified and authenticated by Prof. Dr. Srinath rao, Department of Botany, Gulbarga University, Gulbarga. A voucher specimen is preserved in the herbarium of Department of Botany (Voucher No.5006), Gulbarga University Gulbarga.

**Preparation of ethanol extract**[7]: The dried powder material (2000 Gms) was subjected to soxhlet apparatus for continuous hot extraction. The marc was pressed and ethanol is added.
After extraction the filters were pooled and concentrated under reduced pressure to obtained dried solid mass. The percentage yield was calculated and tabulated. (Table 1).

**Preparation of ointment by fusion method for topical application**

(a) **Preparation of simple ointment**: Wool fat - 2 gm; Hard Paraffin-2 gm; Cetostearyl alcohol -2 gm; White Soft Paraffin-34 gm. Each ingredient was mixed and heated gently with stirring then cooled. The base was then packed in a wide mouth container.

(b) **Preparation of 10% ointment**: 2 gm ethanol extract of *Andrographis echioides* was added slowly to the above melted ingredients and stirred thoroughly until the mass cools down and a homogeneous product is formed. The ointment was then packed in a wide mouth container.[8,9]

**Experimental animals**

Male Wister albino rats of four (4) weeks, weighing between 150 and 200 g were obtained from well maintained central animal house at MRMC Campus, Gulbarga, Karnataka state, India and it was further used for wound healing investigation. The animals were housed in standard environmental conditions of temperature (31 ±1°C), humidity (60± 0.2%) and a 12 h light and 12 h dark cycle. Rats were fed with standard rodent diet and tap water ad libitum. The study was carried out according to the guidelines of laboratory animal care.[10]

**Acute dermal toxicity – fixed dose procedure**[11]

The acute dermal toxicity study was carried out in adult female albino rats by “fix dose” method of OECD Guideline No.434. Extract of the plant *Andrographis echioides* was applied topically at dose level of 2000 mg/kg body weight.

**Grouping of animals**

Animals were divided in to three groups, each group consisting of 6 rats.

- **Group I**: Control group (treated with simple ointment base B.P)
- **Group II**: Received application of standard drug ointment i.e. Framycetin sulphate cream (FSC)_,(1 %w/w)
- **Group III**: Received application of ethanolic extract of *Andrographis echioides* (200 mg/kg/day).
**Wound healing activity:** Excision and incision wound models were used to evaluate the wound-healing activity of ethanolic extract of *Andrographis echioides*.

The study was approved by the Institutional Animal Ethical Committee of HKE College of Pharmacy, Mahadevappa Rampure Road, Gulbarga – 585105 – Karnataka - India, registered under CPCSEA, India.

**Excision wound:** The rats were inflicted with excision wounds under light ether anaesthesia. A circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats\(^{[12]}\). The animals were divided into three groups, each group containing six animals. Group-I was considered as control and received simple ointment base (B.P), Group-II served as reference standard and received framycetin sulfate cream 1% w/w. Group-III received the test formulation. The ointments were applied topically once in a day, till the epithelization was complete starting from the day of experiment. The parameters studied were wound closure and epithelization time. The formulation was applied until complete wound healing. The percentage of wound closure and the period of epithelization were calculated. The period of epithelization was calculated as the number of days required for wound healing. (Results were tabulated in Table 2).

**Incision wound:** In incision wound model, 6 cm long paravertebral incisions were made through full thickness of the skin on either side of the vertebral column of the rat\(^{[13]}\). The wounds were closed with interrupted sutures of 1 cm apart. The animals were divided into three groups, each group containing six animals. The categorization and treatment of experimental animals was similar to that of excision wound model. The ointment containing the test formulation was applied topically once in a day. The sutures were removed on 8th post wound day and the tensile strength of the healed wound was measured on 10th day following continuous water flow technique\(^{[14]}\). (Results were tabulated in Table 3).

**Statistical analysis:** The data is expressed as mean ± SEM and subjected to students‘t’ test and the level of significance was set at \(p < 0.001\).

**RESULTS AND DISCUSSION**

**Table -1: Percentage yield extract of *Andrographis echioides***

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Extract</th>
<th>Nature of extract</th>
<th>Colour</th>
<th>Weight (gm)</th>
<th>% Yield (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Ethanolic extract</td>
<td>Semi solid</td>
<td>Dark Brown</td>
<td>26.56</td>
<td>2.6</td>
</tr>
</tbody>
</table>
Acute toxicity study: Before the study of wound healing activity, Acute toxicity studies of the extract were carried out. The test extracts did not cause any mortality when administered up to a maximum dose of 2000 mg/kg body weight.

Table -2: Effect of ethanol extract of *Andrographis echioides* on excision wound

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Percentage wound contraction on</th>
<th>Epithelization Period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th day</td>
<td>8th day</td>
</tr>
<tr>
<td>Simple ointment base (B.P) (Control)</td>
<td>17.50±0.32</td>
<td>29.34±1.32</td>
</tr>
<tr>
<td>Framycetin sulfate cream 1 % w/w (Reference standard)</td>
<td>35.08±1.62</td>
<td>57.72±1.94</td>
</tr>
<tr>
<td>Test formulation (Ethanolic Extract)</td>
<td>33.00±0.23</td>
<td>55.34±1.10</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M of 6 animals in each group. Numbers in Values are mean ± S.E.M of 6 animals in each group. Numbers in parenthesis indicates percentage of wound contraction. *p < 0.001 vs respective control by students ‘t’ test.

In excision wound model study, the topical application of ethanolic extract of *Andrographis echioides* showed significantly greater wound healing activity when compared to control animals.

Table 3: Effect of ethanolic extract of *Andrographis echioides* on wound healing in incision wound

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Tensile strength in g ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Simple ointment base (BP) control</td>
<td>119.33±6.67</td>
</tr>
<tr>
<td>2</td>
<td>Framycetin sulfate cream 1 % w/w Reference standard</td>
<td>330.41±8.77**</td>
</tr>
<tr>
<td>3</td>
<td>Test formulation</td>
<td>307.23±1.37**</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M of 6 animals in each group. **p < 0.001 vs respective control by student “t” test.

In incision wound model study, significant increase was observed in the skin tensile strength of ethanolic extract of *Andrographis echioides* treated group on 10<sup>th</sup> post wounding day when compared to control.

The results of ethanolic extracts of *Andrographis echioides* on both excision and incision wound model showed significant acceleration in the process of wound healing by decreasing the surface area of the wound and increasing the tensile strength.

Our present study emphasized the present need of medicinal plants against synthetic drugs on wound healing potentials.
CONCLUSION
The wound healing activity of ethanolic extract of *Andrographis echioides* was studied by using excision and incision wound model and the extract showed significant wound healing activity when compared to control and similar to standard FSC (Framycetin sulphate cream). Moreover the extract did not produce any adverse effect and because of this it can be strongly recommend in different wound healing models like burn wound, dead space wound, injury by X-ray radiation and ultraviolet light etc.

ACKNOWLEDGEMENTS
The authors are very thankful to Shaik mohd khasim (Director, Shadan College of Pharmacy, Hyderabad) for their encouragement and providing facilities to carry out this work.

REFERENCES


