EVALUATION OF WOUND HEALING ACTIVITY OF DIFFERENT CRUDE EXTRACTS OF ANOGEISSUS ACUMINATA AND GYMNOSPORIA EMERGINATA

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ABSTRACT
India has a rich tradition of plant based knowledge on health care. A large number of plants are used by folklore traditions in India for treatment of cuts, wounds and burns. Anogeissus acuminata (Family: Combretaceae) is popular all over the world for its medicinal uses in skin diseases like eczema, dermatitis, skin ulcers etc. and Gymnosporia emerginata (Family: Cleastraceae) is used for rheumatism, ulcer etc. In India, it is used traditionally for various types of skin diseases. Hence the present study was aimed to evaluate its scientific validity. The Methanolic extract of same were investigated for the evaluation of its healing efficiency on excision wound model in mice. However, there is no scientific data to support the wound healing activity of Anogeissus acuminata and Gymnosporia emerginata on excision wound model. Hence, we have conducted the present study to explore the wound healing activity. It would be an economical option for treatment of wounds.

Keywords: Anogeissus acuminata, Gymnosporia emerginata, wound healing, excision wound.

INTRODUCTION
Wound healing is a dynamic process of tissues restoration and re-establishing the injured skin and underlying tissues. It involves a systematic progression of events i.e. inflammation, angiogenesis, proliferation and collagen synthesis for final healing.

To restore the integrity and to avoid severe damage to the body, rapid wound healing is required. The present system of treatment by use of cortisone and other anti inflammatory drugs may impair the healing process. Alternate method of treatment by using medicinal plants has been focused by many workers who have found the therapeutic benefits of traditional system of medicine in wound repair1, 2, and 3.

Wounds are physical injuries that result in an opening or breaking of the skin. Wound healing is a complex process that results in the concentration and closure of the wound and restoration of a functional barrier1. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue2. Repair of injured tissues includes inflammation, proliferation, and migration of different cell types3. Inflammation, which constitutes a part of the acute response, results in a coordinate influx of neutrophils at the wound site4. In spite of tremendous advances in the pharmaceutical drug industry, the availability of drugs capable of stimulating the process of wound repair is still limited. The search for natural remedies for healing has drawn attention to herbals.

In wound healing mechanism following the migration of platelets, the first response cells, neutrophils and macrophages migrate to the wound. Numerous enzymes and cytokines are secreted by macrophages and neutrophils. Among these TNF-α is the one which stimulates the angiogenesis, helps to build up the tissue granulation bed and thus has significant potential to improve the healing process5. Plants may exert their affect by modulating the cytokine(s) secretion during different conditions. TNF-α is a major cytokine secreted by macrophages and neutrophils during the inflammation phase5.
EXPERIMENTAL

Healthy Wistar albino rats (12 weeks old) of either sex weighing between 150-180 g, obtained from the animal house, National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. Rats were maintained under controlled condition of light (14h) and temperature (24±2°C). Food pellets and water was provided ad libitum. Rats were divided into 5 groups of 6 rats each for the wounding experiment. The experiments were approved by the “Institutional Animal Ethics Committee (IACE)’’.

Induced wounds

The rats were divided into three groups of 6 each. The rats were inflicted with excision wounds as described by Morton and Malone\(^6\). An area of 2×2 cm\(^2\) on the lateral side of thigh region, previously cleaned with soapy water, was shaved and disinfected with 70% alcohol. Under local anesthesia, using 1ml of chloroform (2%, 100 mg/5 ml) in depth of muscle, excision wound was created using sterile surgical blade. The entire wound was left open.

Excision wound model\(^7\)

Excision wounds were used for the study of rate of contraction of wound and epithelization. Animals were anaesthetized with slight vapour inhalation of di-ethyl ether and the right side of each rat was shaved. Excision wounds sized 300mm\(^2\) and 2mm depth were made by cutting out layer of skin from the shaven area. The entire wound was left open. The treatment was done topically in all the cases. The ointment was applied at a dose of 1%, 5%, 10%, 30% mg/kg/day for 16days of various concentrations. Wound areas were measured on days 1, 4, 8 and 16 for all groups, using a transparency sheet and a permanent marker.

Statistical analysis

All results are expressed as mean ± SEM. Significance of difference between control and drug treated groups were determined by one way ANOVA followed by Dunnell’s test.

RESULTS AND DISCUSSION

The results of the evaluations carried out on the extracts are listed in Table 1. It shows % Inhibition of excision wound by different plant extracts.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Drug</th>
<th>Route</th>
<th>4(^\text{th})Day (percentage inhibition)</th>
<th>8(^\text{th})Day (percentage inhibition)</th>
<th>12(^\text{th})Day (percentage inhibition)</th>
<th>16(^\text{th})Day (percentage inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Topical</td>
<td>1.75 ± 0.021</td>
<td>1.52± 0.018</td>
<td>1.3± 0.057</td>
<td>1.1 ± 0.018</td>
</tr>
<tr>
<td>2</td>
<td>Standard(1%w/w)</td>
<td>Topical</td>
<td>1.2 ± 0.028*** (31.4%)</td>
<td>1.2 ± 0.035** (5.92%)</td>
<td>0.4 ± 0.051*** (69.2%)</td>
<td>0.1± 0.01*** (90.9%)</td>
</tr>
<tr>
<td></td>
<td>Standard(5%w/w)</td>
<td></td>
<td>1.1 ± 0.078*** (37.4%)</td>
<td>0.81± 0.019*** (46.7%)</td>
<td>0.2 ± 0.03*** (84.6%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Standard(10%w/w)</td>
<td></td>
<td>0.95±0.071*** (45.7%)</td>
<td>0.6± 0.021*** (60.5%)</td>
<td>0.1 ± 0.025*** (92.3%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Standard(30%w/w)</td>
<td></td>
<td>0.9 ± 0.076*** (48.5%)</td>
<td>0.4 ± 0.021*** (73.6%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>MEGE (1%w/w)</td>
<td>Topical</td>
<td>1.68± 0.023 (4%) (4%)</td>
<td>1.42 ± 0.063 (6.5%)</td>
<td>1.25 ± 0.035 (3.8%)</td>
<td>1.05 ± 0.024 (4.5%)</td>
</tr>
<tr>
<td></td>
<td>MEGE(5%w/w)</td>
<td></td>
<td>1.5 ± 0.02 (14.2%)</td>
<td>1.4 ± 0.012 (7.23%)</td>
<td>1.2±0.025 (7.6%)</td>
<td>1.02± 0.014 (7.2%)</td>
</tr>
<tr>
<td></td>
<td>MEGE(10%w/w)</td>
<td></td>
<td>1.5±0.084 (14.2%)</td>
<td>1.4 ± 0.040 (7.8%)</td>
<td>1.1±0.036 (15.3%)</td>
<td>1.0 ± 0.062 (9.09%)</td>
</tr>
<tr>
<td></td>
<td>MEGE(30%w/w)</td>
<td></td>
<td>1.4 ± 0.018 (20%)</td>
<td>1.25 ± 0.024 (17.7%)</td>
<td>1.0 ± 0.036 (23%)</td>
<td>0.95± 0.062 (13.6%)</td>
</tr>
</tbody>
</table>

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Effect of different plant on excision wound parameters are shown in the following figures.

**Fig.-1: Day 4**

**Fig.-2: Day 8**
Fig.-3: Day 12

Fig.-4: Day 16

Wounds are referred to as disruption of normal anatomic structure and function. Skin wounds could happen through several causes like physical injuries resulting in opening and breaking of the skin\(^8\). The most common symptoms of wounds are bleeding, loss of feeling or function below the wound site, heat and redness around the wound, painful or throbbing sensation, swelling of tissue in the area and pus like drainage\(^9\). Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes. The aim in these processes is to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin. Healing process, a natural body reaction to injury, initiates immediately after wounding and occurs in four stages. The first phase is coagulation which controls excessive blood loss from the damaged vessels. The next stage of the healing process is inflammation and debridement of wound followed by re-epithelisation which includes proliferation, migration and differentiation of squamous epithelial cells of the epidermis.

Study on animal models showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced epithelisation. The animals treated with 1\%, 5\%, 10\%, and 30\% and extract showed significant results when compared with different groups and
control. The treated wound after six days itself exhibit marked dryness of wound margins with tissue regeneration.

However, histological evaluation showed that, increased cellular infiltration from haematoxylin and eosin staining in treated cases may be due to chemotactic effect enhanced by the crude extract which might have attracted inflammatory cells towards the wound site. Increased cellular proliferation may be due to the mitogenic activity of the plant extract, which might have significantly contributed to healing process. Early dermal and epidermal regeneration in treated mice also confirmed that the extract had a positive effect towards cellular proliferation, granular tissue formation and epithelisation.

Tannins and anthraquinones are the major phytoconstituents present in this plant which may be responsible for wound healing action. The plant Portulaca oleracea containing the tannins possesses wound healing activity as that of the Anogeissus acuminata. The gel of ethanolic extract of the plant Vernonia scorpioiides possess wound healing action by improving regeneration and organization of the new tissue due to the presence of tannins. The embelin isolated from the ethanol extract of plant Emblica officinalis containing condensed tannins when formulated as a gel possess significant wound healing property as that of ointment prepared by Anogeissus acuminata methanol extract. A number of secondary metabolites/active compounds isolated from plants have been demonstrated in animal models (in vivo) as active principles responsible for facilitating healing of wounds. Some of the most important ones include tannins from Terminalia arjuna, oleanolic acid from Anredra diffusa, polysaccharides from Opuntia ficus-indica, gentiopicroside, sweroside and swertiamarine from Gentiana lutea, shikonin derivatives (deoxyshikonin, acetyl shikonin, 3-hydroxyisovaleryl shikonin and 5,8-O-dimethyl acetyl shikonin) from Onosma argentatum, asiaticoside, Asiatic acid, and madecassic acid from Centalla asiatica quercetin, isorhamnetin and kaempferol from Hippophae rhamnoides curcumin from Curcuma longa.

REFERENCES


[RJC-743/2011]

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