Chapter-11

Wound Healing Effect of *Eupatorium adenophorum* Spreng. (Asteraceae) leaf extract.

11.1 Introduction

11.2 Experimental

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11.4 Discussion

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11.1 Introduction

Wound healing is a normal biological manifestation. The coagulum formed immediately after injury consists of fibrin network and enmeshed blood elements. But lipid peroxidation, a widely studied phenomenon of free radical damage, has been reported to be involved in a variety of pathological conditions including inflammation that promotes the production of free radicals by phagocytes which damage to healthy cells and tissues around. This damage is effectively countered by the presence of various antioxidant system in certain plant drugs like *Centella asiatica* (Rosen et al, 1967) which have been reported to elevate antioxidant levels and thus act as effective wound healing agents.

A wound is a disrupted state of tissue caused by physical, chemical, microbial or immunological insult, ultimately healing either by regeneration or fibroplasia. Healing progresses through three general stages 1) Inflammatory 2) proliferation and repair 3) Remodeling stage. During inflammatory stage, as a result of injury, the blood circulation in the local area is reduced which leads to local hypoxia, acidosis and low pH. Following the inflammatory response to injury or wounds, a tremendous proliferation of cells takes place which is actually responsible for the process of repair. The cellular responses include blood supply, surface covering and reproduction of collagen (a macromolecule produced by fibroblasts, chondroblasts and osteoblasts) which ultimately helps to bind the wound margin and development of permanent functional tensile strength. Elemental manganese, Zinc, ferric and copper content in a good percentage have also been reported (Kaibaja and smith, 1988). The role of metal in biological system is well known. Zinc plays a vital role in the diet as well as being a component in enzyme systems. The presence of high Zinc content of the plant prompted us to investigate the wound healing activity of the crude extract of leaves. Indigenous
medicinal plants having the attribute of wound healing property have been successfully employed in folklore medicine. Several such observations have been recorded in ancient literature. When the juice of fresh leaves applied to cuts and wounds remain protected against infection (Rai et al., 1994). The aerial parts of the plants have been claimed to be used as antimicrobial, antiseptic, blood coagulant and potentiator of phenobarbiton induced sleep (Ansari et al., 1983). The antibacterial activity of this plant leaf extract against different microorganisms has already been observed. Based on its use in wound healing in traditional practices the present study was undertaken to evaluate its wound healing activity and hereby to substantiate this claim, which is being reported hereunder.

11.2 Experimental

11.2.1. Plant material

The dried methanol extract of Eupatorium adenophorum leaves as explained in chapter-3 was used in this experiment.

11.2.2. Animals used

Male wister rats, weight between 150-200 g were taken for the study. The animals were housed in polypropylene cages with dust free rice husk as bedding materials. 10 hour light : 14 hour dark cycle was maintained throughout the experimental period. All the animals were given synthetic pellet diet for rat. The care and maintenance of the animals were as per the approved guidelines.

11.2.3. Excision Wound Model Method

Animals were divided into three groups providing six animals in each group. The animals anaesthetized with injecting thiopentone sodium at a dose of 50 mg/kg intraperitoneally. Wide area of the dorsum of each rat was depilated with a pair of scissors and scalpel blade. The depilated area was cleaned with 70%v/v ethyl alcohol for maintenance of aseptic condition. Two full thickness 8 mm diameter punch wound were inflicted on the either side of the midline of depilated dorsum of each rat with the Acupunch instrument. The bleeding was soaked with sterile absorbable cotton and the
wound was left undressed to the open environment. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment (Udupa et al., 1994). Then the drugs, i.e. the reference standard Betadine ointment (5% w/w povidone iodine ointment), simple ointment B.P. and 5% w/w Eupatorium adenophorum methanol extract ointment were applied till the wound was completely healed (Chatterjee et al., 1993). The model was used to monitor wound contraction size. The wound contraction size (mm²) was graphically recorded on each alternate day. Mean values of wound contraction size of day 4, 8, 12 and 16 were estimated for analysis of data.

11.3 Results

The progress of wound healing in excision wound method of the Eupatorium adenophorum leaf extract ointment (5% w/w) treated groups, simple ointment (control) treated group and Betadine ointment (standard drug) treated group of animals have been shown in Table –32. Result has been expressed as mean ± SE and compared with the corresponding control (simple ointment) values, p-values were calculated by student t-test by comparing with control (Woodson, 1987). The leaf extract ointment caused significant contraction of wound comparing with simple ointment (control). The results were comparable with that of standard drug Betadine ointment.

Table-27  Effects of methanol extract of Eupatorium adenophorum leaf (MEEAL) on wound healing by excision wound model method.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4 days</th>
<th>8 days</th>
<th>12 days</th>
<th>16 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple ointment</td>
<td>23.14± 0.523</td>
<td>14.56 ± 0.275</td>
<td>7.50 ± 0.752</td>
<td>5.08 ± 0.249</td>
</tr>
<tr>
<td>Betadine ointment</td>
<td>18.16± 0.942**</td>
<td>4.66± 0.886**</td>
<td>Healed</td>
<td>Healed</td>
</tr>
<tr>
<td>MEEAL Ointment</td>
<td>19.22± 0.852**</td>
<td>9.88 ± 0.159**</td>
<td>3.98 ± 0.738*</td>
<td>Healed</td>
</tr>
</tbody>
</table>

Results were compared with the corresponding control values and p-values were calculated by student’s t-test. *P< 0.01, **p< 0.001.
11.4 Discussion

Wound healing involves different phases such as contraction, epithelialisation, granulation, collagenation, etc. The wound healing of the aerial parts of *L. lavandulaefolia* has been reported (Mahato and Pal, 1986) and also a glycosidal mixture extract of *Centella asiatica* has been reported to be responsible for enhanced repair only in incised wounds (Rosen *et al.*, 1967). In this investigation the methanol extract of Eupatorium adenophorum leaves has been shown inhibition of the lipid peroxidation during proliferative and repairment phase of wound healing.

The wound healing property of *Eupatorium adenophorum* leaf extract may probably be due to the presence of terpenoids in the essential oil, present therein. However, the isolation of the exact component is under way in our laboratory. These above findings indicate the wound healing potential of the *E. adenophorum* leaf extract and thus substantiate its use in folklore medicine in that it contains a growth promoting factors which enhances the healing process.

11.5 Publication