Chapter-7

Anti-ulcer Activity of *Eupatorium adenophorum* (Family: Asteraceae) leaf Extract

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7.1 Introduction

Last two decades have witnessed the introduction of new drugs for the treatment of duodenal and gastric ulcers. Recent research during this period has also added considerably to our understanding of mucosal protection mechanisms and their role in the recovery of the upper gastrointestinal tract from acute damage. The nature and action of newer anti-ulcer drugs also mark a departure from the classical approach of seeking the anticholinergic–antisecretory types of drugs for the treatment of ulcers. These drugs have been shown either to directly promote the healing of ulcers or to possess antihistaminic activity, more selective antimuscarinic effects, inhibitory effect on gastric proton pump hydrogen potassium ATPase, cytoprotective or adaptive cytoprotective effects. These advances in the discovery of novel and more effective anti-ulcer drugs have also been accompanied by the introduction of a larger number of newer experimental methods to evaluate the anti-ulcer activity of drugs effective in gastro-duodenal ulcers.

The problems encountered in the experimental evaluation of anti-ulcer drugs result in part from the lack of complete understanding of the physiological and biochemical mechanism involved in the formation of ulcers and in part relate to the fact that the drugs are usually tested for activity on normal or quasinormal preparations yet are ultimately applied to abnormal or pathological human states. Most experimental ulcers are acute, nonpenetrating, rapidly healing and nonscanning lesions, while the clinical ulcer is a chronic and penetrating lesion which heals with a scar. In spite of these limitations it is possible to evaluate the therapeutic agents rapidly and with reasonable predictability for their therapeutic usefulness, using the experimentally induced models of gastric and duodenal ulcers and hyperacidity states. In general the criterion used for following a particular method include that the method produces a consistently high incidence of readily discernible ulcers in a definite area of the gastrointestinal tract and that either the production or healing of these ulcers can be modified by known therapeutic agents or surgical procedures used to treat peptic ulcer diathesis in man. It is presumed that the appearance, complications, development and healing of certain experimentally induced ulcers are similar to human clinical ulcers. The main differences are in the formation and
chronicity of ulcers. Nevertheless certain additional requirements become necessary in the selection of experimental models for screening anti-ulcer compounds. Experimental methods must meet the following criteria proposed by Lee and Bianchi, 1971: a) They should be simple, reproducible and allow for easy quantification of results, b) They should make use of a variety of animal species c) They should induce characteristic ulceration in specific location (stomach and duodenum), d) They should involve different mechanisms by which ulceration is produced and e) The ulcers induced should not spontaneously heal during the observation period.

A number of methods and indices have been used to score the extent of ulceration on arbitrary scales which are highly subjective and hence, must be carried on blindly by two trained observers unaware of the experimental protocols. The major characteristic features of experimentally induced gastric erosions and ulcers include their multiplicity, irregular shape, confluence, usual localisation at the acid producing glandular mucosa, and intramucosal haemorrhage. Acute mucosal lesions may be the morphologic manifestations of a variety of etiologic factors including physical, chemical or psychological stresses. As such, quantitative assessment of these lesions is crucial since their extent may be the indicator of intensity of stress or ulcerogenic potential of pharmacological agents. In general, severity of mucosal involvement has been assessed by counting the number of haemorrhagic spots or by scoring the number, length and width of mucosal lesions induced by restraint stress. Usually circular lesions are observed. Many times linear lesions and petechiae are also seen. In spite of the advancements in the studies on the pathophysiology of gastric ulcer disease and introduction of highly effective H2-blockers and gastric proton pump hydrogen potassium ATPase inhibitors, we have yet to discover an effective anti-ulcer drug which not only heals the gastric ulcers but also effectively prevents their recurrence.

*Gingko biloba* extract, a traditional medicine prepared from Gingko leaves has a complex range of biological activity including potent antioxidant activity (Shetty *et al*., 2000). Several herbal drugs and Ayurvedic preparations including saponin glycosides have been shown to protect against drug induced gastric mucosal injury (Manonmani *et al*., 1995).
In the recent study we have observed that the tribal people of Sikkim use the fresh juice of this plant to recover from gastric ulcer. The present study was, therefore, undertaken to evaluate the antiulcer activity of the extract of this plant.

7.1. Experimental

7.2.1. Plant Material- The dried methanol extract of *Eupatorium adenophorum* leaves as explained in chapter-3 was used in this experiment. The extract was suspended in propylene glycol for the present study. Propylene glycol was used as control vehicle.

7.2.2. Animals used- Male wister rats, weight between (180-200) g were taken for the study. The animals were housed in polypropylene cages with dust free rice husk as bedding materials. 10 hours light : 14 hour dark cycle was maintained throughout the experimental period. All the animals were given synthetic pellet diet for rat procured from Amrut Laboratory Animal Feed, Pune, India. The care and maintenance of the animals were as per the approved guidelines.

7.2.3. Methods

In the pretreatment study, animals were divided into four groups, each comprising of six animals. The control group received the control vehicle (2% w/v propylene glycol in 0.2 ml/kg body wt. dose), other two test groups received the test drugs (methanolic extract of *Eupatorium adenophorum* leaves in doses of 400 mg/kg and 800 mg/kg body wt. respectively) and the rest group received reference standard drug (ranitidine in 50 mg/kg body wt. dose). All the animals were treated orally through hypogastric canula daily for continuous 10 days. After the pretreatment, indomethacin (20 mg/kg, p.o.) was administered to the overnight fasted rats and the animals were sacrificed under deep ether anesthesia after 3 hours. The stomach of each animal was incised along the greater curvature for examination of ulcers (Pillai *et al*., 1985).

**Gastric Ulcer Index**

The gastric ulcer lesions were counted and the mean ulcer index was calculated according to scoring system as described earlier (Pandit *et al*., 2000): I, presence of edema, hyperemia and single submucosal punctiform hemorrhage; II, presence of
submucosal hemorrhagic lesions with small erosions; III, presence of deep ulcers with erosions and invasive lesions. Therefore, the ulcer index was calculated as follows:

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\text{Ulcer Index} = (\text{Number of lesion I}) + (\text{Number of lesion II}) \times 2 + (\text{Number of lesion III}) \times 3
\]

7.3 RESULTS

Both the doses of the methanol extract of *Eupatorium adenophorum* leaf (MEEAL) 400 mg/kg and 800 mg/kg were found to produce significant reduction (30.04% and 52.22%) of indomethacin–induced lesion index as compared to control (Table-20). However a significant reduction of all these responses similar to the standard drug ranitidine was noticed with the plant extract. It can be correlated with the rapid absorption of these drugs through the mucosa.

**Table-20 Effect of Methanol Extract of Eupatorium adenophorum Leaf (MEEAL) on Indomethacin-Induced Gastric Ulcer in Rats.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer Index</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 ml/kg</td>
<td>25.16± 1.51</td>
<td>---------------</td>
</tr>
<tr>
<td>MEEAL</td>
<td>400 mg/kg</td>
<td>17.60 ± 1.09**</td>
<td>30.04</td>
</tr>
<tr>
<td>MEEAL</td>
<td>800 mg/kg</td>
<td>12.02 ± 1.24**</td>
<td>52.22</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>50 mg/kg</td>
<td>8.12 ± 0.57**</td>
<td>67.72</td>
</tr>
</tbody>
</table>

N=6 in each group; **P< 0.001, significantly different compared with control (Kruskal- Wallis ANOVA followed by Dunnet’s test).

MEEAL- Methanol Extract of *Eupatorium adenophorum* Leaf.

7.4 DISCUSSION

The initial experimental data indicated that methanol extract of *Eupatorium adenophorum* Spreng. (Asteraceae) leaf demonstrated significant reduction of gastric ulceration induced by indomethacin. Indomethacin is a prostaglandin biosynthesis...
inhibitor. Prostaglandin are produced in the gastric mucosa and appear to serve a protective role by inhibiting acid secretion and promoting mucus (Tripathi, 1999). It directly inhibited cyclo-oxygenase enzymes and is indirectly responsible for overproduction of leukotrienes and 5- lipoxygenase, which are the prime agents to produce ulceration in gastric tissue (Rainsford, 1987). Protection of indomethacin induced gastric ulcer with the pretreatment of the extract in rat may be either due to its role in 5-lipoxygenase inhibition or leukotriene antagonist activity.

7.5 Publication