Chapter-8

Analgesic Activity of *Eupatorium adenophorum* (Family: Asteraceae) leaf Extract

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8.1 Introduction

Despite the fact that pain is a universal experience of all mankind and everybody knows what is meant by it. Algesia (pain) is a warning signal and primarily protective in nature, but causes discomfort and are usually evoked by an external or internal noxious stimuli. Analgesia is a state of reduced awareness to pain. Analgesics are agents which relieve pain by acting centrally to elevate pain threshold without disturbing conciousness or altering other sensory modalities. Analgesics decrease pain sensation by increasing the threshold to pain stimuli. Unfortunately, many factors such as sex, circulatory change, skin temperature, sweating, carbon di-oxide tension, anxiety fear, emotion, etc., alter the pain threshold. During pain, arachidonic acid is released from phospholipid fraction of cell membrane. Since there are several types of pain (bright, dull, aching, pricking, cutting, burning etc) and it may arise from different causes (injury, body derangements, or disease). Furthermore, it is now generally agreed that pain involves a large psychic component.

Many drugs used to relieve pain are not analgesics. The general anesthetics obtund pain by producing a hiatus in conciousness, the local anesthetics prevent pain by blocking peripheral nerve fibres, the antispasmodics relieve certain kinds of pain by relaxing smooth muscle and the adrenal corticoids relieve pain associated with rheumatoid arthritis by an anti-inflammatory action.

Following acute tissue damages, as with a cut or knocks to the skin, a variety of cellular components gain access to the extracellular fluid. These include adenosine triphosphate (ATP), 5HT, histamine and bradykinin. Soon after injury macrophages and cells of the immune system invade the damaged area in an attempt to remove cell debris and to prevent or combat any infection by micro-organisms. Activation of the immune cells leads to the activation of phospholipase A2 and the formation of eicosanoids (20-carbon metabolites of arachidonic acid, such as prostaglandin). These in turn are released into the extracellular space and among other things, sensitize nociceptive nerve endings in
the tissue to compounds such as histamine and bradykinin. The resulting stimulation of these afferents gives rise to the perception of pain (Gebhart and McCormack, 1994).

Arachidonic acid is enzymatically converted to prostaglandin (PG) through the cyclooxygenase pathway by the enzyme, cyclo-oxygenase. Inflammation has caused sensitisation of pain receptors to normally painless mechanical or chemical stimuli. Pain that accompanies inflammation and tissue injury probably results from local stimulation of pain fibres and enhanced pain sensitivity (hyperalgesia) in part a consequence of increased excitability of central neurons in spinal cord (Kontinen et al., 1994).

The opium group of narcotic drugs are among the most powerful acting and clinically useful drugs producing depression of the central nervous system. Opium and its preparations exhibit analgesic and narcotic effects which are directly proportional to their morphine content. Traditionally, opium is more frequently employed in the form of tincture for diarrhea and dysenteries. The analgesic action of methanol extract from Teucrium buxifolium has been reported and almost all fraction of the methanol extract of Teurcrium buxifolium L have analgesic activities (Beltran et al., 1998). Catuama is a crude herbal formulation of Brazil, which comprises a mixture of four medicinal plant extracts namely Trichilia catigua (Meliaceae), Paullinia cupana (Sapindaceae), Ptychopetalum olacoids (Olacaceae) and Zinziber officinalis (Zinziberaceae) all inhibited acetic acid induced pain (Zulma et al., 1997).

The aerial parts of the plants have been claimed to be used as antimicrobial, antiseptic, blood coagulant, potentiator of phenobarbitone-induced sleep and as an analgesic (Ansari et al., 1983). The present study was carried out to evaluate the analgesic activity of methanolic extract of Eupatorium adenophorum leaves.

8.2 Experimental

8.2.1. 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
2% v/v aqueous tween 80 solution for the present study. 2% v/v aqueous tween 80 solution was used as control vehicle.

8.2.2. Animals used
Adult albino rats (wister strain) of either sex weighing 180-200g were each and albino mice of either sex weighing 20-25g were used. The care and maintenance of the animals were as per approved guidelines.

8.2.3. Methods
8.2.3.1. Acetic acid induced writhing test
Swiss albino mice of either sex were divided into four groups of six animals each. Groups I-IV were administered with 2% v/v aqueous tween 80 solution (5ml/kg), aspirin 100mg/kg and 200mg/kg, 300mg/kg of methanol extract of *Eupatorium adenophorum* leaf (MEEAL) i.p. respectively 60 minutes before i.p. injection of 0.6% v/v acetic acid solution at a dose of 10ml/kg. Immediately after the administration of acetic acid, the number of writhing or stretches were counted for 15 minutes. A reduction in writhing number was compared with that of the control group. It was considered as evidence for the presence of analgesia, which was expressed as percent inhibition of writhing (Koster and Anderson, 1959). Data were calculated according to the formula:

\[
\frac{(A - B)}{A} \times 100
\]

A= mean number of writhing produced by the control groups.

B= mean number of writhing produced by the test groups.

8.2.3.2. Tail immersion test
Swiss albino mice of either sex were divided into four groups of six animals each. Group-I was given 2% v/v aqueous tween 80 solution (5 ml/kg), group II, III, IV were administered pethidine (5 mg/kg) and MEEAL at the doses of 200 and 300 mg/kg intraperitoneally. The tail (upto 5cm) was then dipped into a pot of water maintained at 55
± 0.5°C. The time taken to withdraw the tail out of water was taken as the reaction time. The reading was taken after 30 minutes of administration of the test drugs (Ghosh, 1984).

**8.2.3.3. Tail flick test**

Wister strain of albino mice of either sex weighing between 150-180g were selected and divided into four groups of six animals each. The tail was placed on the nichrome wire of an analgesiometer (Techno, India) and the time taken by the animal to withdraw its tail from the hot wire was taken as the reaction time. Groups I-IV were administered with Propylene glycol (5ml/kg), and pethidine (5 mg/kg) and the MEEAL at doses of 200 and 300mg/kg were given intraperitoneally. 2%v/v aqueous tween 80 solution (5ml/kg) served as control. Analgesic activity was measured after 30 minutes of administration of test and standard drugs (Ghosh, 1984).

**8.3. Results**

The results of acetic acid-induced writhing test in mice showed in table-21. It indicates that the methanol extract of *Eupatorium adenophorum* leaves significantly increase in the induction time required to produce the writhing movements. The results were comparable to that of aspirin treated control. The results indicated that the methanol extract of leaves at 200 and 300 mg/kg doses displayed 45.97% and 72.87% inhibition of writhing reflexes while the writhing reflexes inhibition in vehicle control group was 0% and with the aspirin treated group it was 73.10% respectively. The numbers of writhing movements were significantly less in the mice treated with the methanol extracts of *Eupatorium adenophorum* leaves when compared to that of 2%v/v aqueous tween 80 solution treated control thereby suggesting its peripheral analgesic effect. The extract showed significant analgesic activity by tail flick and tail immersion tests were shown in Table 22 and 23 respectively.
Table-21: Effect of methanol extract of *Eupatorium adenophorum* leaves (MEEAL) on acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Number of writhing</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 ml/kg</td>
<td>43.50 ± 0.20</td>
<td>__</td>
</tr>
<tr>
<td>Aspirin</td>
<td>100 mg/kg</td>
<td>11.70 ± 0.34*</td>
<td>73.10</td>
</tr>
<tr>
<td>MEEAL</td>
<td>200 mg/kg</td>
<td>23.50 ± 0.40*</td>
<td>45.97</td>
</tr>
<tr>
<td>MEEAL</td>
<td>300 mg/kg</td>
<td>11.80 ± 0.20*</td>
<td>72.87</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. N=6, *p<0.001.Vs control, Student’s t-test

MEEAL- Methanol extract of *Eupatorium adenophorum* Spreng. Leaf

Control- 2%v/v aqueous tween 80 solution.

Table-22: Effect of methanol extract of *Eupatorium adenophorum* leaves by Tail immersion test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Reaction time in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 ml/kg</td>
<td>2.260±0.28</td>
</tr>
<tr>
<td>Pethidine</td>
<td>5mg/kg</td>
<td>4.157±0.37*</td>
</tr>
<tr>
<td>MEEAL</td>
<td>200mg/kg</td>
<td>3.20±0.22*</td>
</tr>
<tr>
<td>MEEAL</td>
<td>300mg/kg</td>
<td>3.40±0.204*</td>
</tr>
</tbody>
</table>

Result expressed as Mean± SEM. N = 6, * p <0.01. Vs control, Student’s t-test

MEEAL-Methanol extract of *Eupatorium adenophorum* Spreng. Leaf

Control-2%v/v aqueous tween 80 solution.
Table-23 : Effect of methanol extract of the *Eupatorium adenophorum* leaves by Tail flick test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Reaction time in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 ml/kg</td>
<td>2.20± 0.204</td>
</tr>
<tr>
<td>Pethidine</td>
<td>5 mg/kg</td>
<td>5.20± 0.390*</td>
</tr>
<tr>
<td>MEEAL</td>
<td>200 mg/kg</td>
<td>3.83± 0.280*</td>
</tr>
<tr>
<td>MEEAL</td>
<td>300 mg/kg</td>
<td>4.36± 0.109*</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SEM. n =6, **P< 0.001 Vs control, Student’s *t*-test
MEEAL - Methanol extract of *Eupatorium adenophorum* Spreng. Leaf
Control-2%v/v aqueous tween 80 solution.

8.4. Discussion

Nociception or algesia is the mechanism whereby noxious peripheral stimuli are transmitted to the central nervous system. Pain is not the same thing as nociception, and includes a strong effective component. Polymodal nociceptors (PMN) are the main peripheral sense organs that respond to noxious stimuli, which include thermal, mechanical and chemical stimuli. PMN are sensitized by prostaglandin, which explains the analgesic effects of aspirin like drugs particularly in the presence of inflammation. NSAIDS act primarily on peripheral pain mechanism, also in CNS to raise pain threshold. Nociceptive fibres terminate in the superficial layers of the dorsal horn forming synaptic connection with transmission neurons running to the thalamus. Transmission in the dorsal horn is subjected to various modulatory influences. Inhibitory mechanisms include the effect of activity in other peripheral sensory neurons and the effect of descending
pathway from the midbrain and brainstem. Acetic acid induced writhing reflex model of analgesic activity the result were comparable to that of aspirin treated group. The number of writhing movements were significantly less in the mice treated with the methanol extracts of *Eupatorium adenophorum* leaves when compared to that of 2%v/v aqueous tween 80 solution treated control, thereby suggesting its peripheral analgesic effect. Opioid cause analgesia partly by activating these descending pathways and partly by inhibiting transmission in the dorsal horn. In Tail flick and Tail immersion tests the reaction time were significantly increased in animal treated with the methanol extracts of *Eupatorium adenophorum* leaves. This effect was comparable to that of pethidine treated control, suggesting central analgesic effect.

8.5. Publication
