Chapter-9

Anti-inflammatory and Anti-pyretic activity of *Eupatorium denophorum* (Family:Asteraceae) leaf Extract

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9.1 Introduction

Inflammation and inflammatory diseases have been recognized for many centuries. The Roman encyclopaedist Celsus described four classic signs of inflammation (rubor, redness; calor, heat; dolour, pain; and tumor, swelling) and described the use of willow leaf extract as an analgesic. Inflammation (or) phlogosis is a psychopharmacological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism the complex events and mediators involved in the inflammatory reactions can induce, maintain or aggravate many diseases.

Inflammation is a fundamental pathophysiological response designed to eliminate any noxious stimulus introduced to the host. Such noxious stimuli includes radiant, chemical, physical, infectious and immune provocation. The inflammation is commonly divided into three phases a) Acute inflammation (characterized by redness, heat, swelling and pain with an accompanying loss of function). b) The immune response (occurs when immunologically competent cells are activated in response to foreign organism or antigenic substances) and c) Chronic inflammation (characterised by persistent pain, swelling and cellular proliferation with an accompanying chronic often major loss of function). One of the most important condition is rheumatoid arthritis, in which chronic inflammation results in pain and destruction of bone and cartilage that can lead to serve disability and in which system changes occur that can result in shortening of life (Katzung, 1998). Inflammation is the phenomenon includes fenestration of microvasculature, leakage of elements of blood into interstitial spaces and migration of leucocytes into inflamed tissue. During this complex response chemical mediators like histamine, 5-hydroxytryptamine, various chemotactic factors, bradykinin, leucotrienes and prostaglandin are liberated locally. Phagocytic cells migrate into the cell surface and cellular lysosomal membranes may be ruptured releasing lytic enzymes. All these events may contribute the inflammatory response.
Some chemical mediators from the cells known to produce inflammation are histamine, lysosomal compounds, prostaglandin, leucotrienes, 5-hydroxytryptamine (serotonin), lymphokines etc.

The greatest disadvantages in the presently available potent synthetic drugs lie in their toxicity and reappearance of symptoms after discontinuation. Therefore, the search for screening and development of drugs for rheumatoid arthritis is an unending problem and there is much hope of finding antirheumatic drugs from indigenous plants.

Aspirin first synthesized in 1853 by Carl Gerhardt, is still one of the most widely used mild analgesic and non steroidal anti-inflammatory drugs (NSAID). It had its medicinal origin in the salicylates and glycosides of willow bark, long used for the treatment of rheumatic diseases, gout and painful conditions of all types. In view of the universal requirement for NSAID, many plants have been utilized for the purpose in traditional medicine and in recent years considerable research effort has been expanded on their investigation. Enzymes have been used to detect anti-inflammatory activity of plants. Flavonoid was found to be a cyclo-oxygenase inhibitor. Lipoxigenase inhibitors are present in the plants of compositae family, used for the treatment of rheumatic disorders. A review of plants exhibiting anti-inflammatory activity reveals that plant species of genera belonging to families have exhibited such potential (Chawla et al., 1987; Handa et al., 1992). Many of these phyto-antiinflammatory agents fulfil the criteria for rational herbal products: quality, safety, efficacy. In addition to the wide range of plants involved there is a similar diversity in the chemical nature of the active constituents. Flavonoids constitute one group widely associated with anti-inflammatory activity (Alcaraz and Jimenez, 1988). Colchicine, an alkaloid of *Colchicum autumnale* is the classical drug for the treatment of acute attack of gout. It may act by reducing the inflammatory response caused by deposits of urate crystals in the joint and by reduction of phagocytosis of the crystals. Its use has been somewhat replaced by allopurinol (inhibition of xanthin oxidase) and by phenylbutazone. The steroids exhibit remarkable anti-inflammatory activity. This category of drugs act by inhibition of phospholipase. Thus preventing the biosynthesis of lipid autocoids that is the
entire cascade of lipid mediators including prostaglandin SRS-A and leukotriens (Lewis and Piper, 1975). In western medicine the enzyme has been introduced for its ability to dissolve fibrin in conditions of inflammatory oedema (Evans, 1997). While performing different phytochemical analysis (chapter-3) the methanol extract of leaves of Eupatorium adenophorum has been found to contain a steroidal, triterpenoid and flavonoid compound. These above compounds are reported to have anti-inflammatory and anti-pyretic (Gupta et al., 1996), antiulcer activities and is helpful in controlling rheumatoid arthritis (Pagel, 1980).

Fever, which means a body temperature above the usual range of normal, may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, malignancy or other diseased states. Most are eventually found to be caused by a hidden infection.

Common causes of fever are:

- Viral and bacterial infections
- Cold and flu-like illness
- Sore throat and strap throat
- Acute bronchitis
- Urinary tract infections
- Upper respiratory infection like tonsillitis, pharyngitis, laryngitis
- Occasionally, more serious problems like pneumonia, appendicitis, tuberculosis and meningitis.
- Collagen vascular disease, rheumatoid disease and auto-immune disease
- AIDS and acute HIV infection
- Regional enteritis
- Ulcerative colitis
- Malignant disorder (cancerous)

The fever associated with infection is thought to result from two actions. The first is the production of prostaglandin in the central nervous system in response to bacterial pyrogens. The second is the effect of interleukin –1 on the hypothalamus. Regulation of body temperature needs a balance between the production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever,
this set point is elevated, and aspirin like drugs promote its return to normal temperature, whereas normal body temperature is only slightly affected. These drugs do not influence body temperature when it is elevated by such factors as exercise or increase in the ambient temperature (Katzung, 1998; Gilman et al., 1996). Search for plant drugs has been initiated to avoid the side effects of synthetic drugs. So many plants were screened for its antipyretic potential and reported.

Search for herbal remedies having potent antipyretic activity received momentum recently as presently available synthetic drugs like paracetamol, nimesulide etc have toxic effects, like liver dysfunction. In 1973, Reverend Edmund Stone, in a letter to the president of the Royal Society, described his success in treating fever with a powdered form of bark of willow. The plants having potential antipyretic activity are Allium sativum, Allium cepa, Fagonia arabica etc. (Roy Chowdhury, 1994). The anti-pyretic activity of matrino, a lupin alkaloid isolated from Sophora subrostrata has been reported (Cho et al., 1986). The plants having potential antipyretic activity are The plants having antipyretic activity are Trigonella foenum-graecum, Dodonaea angustifolia, Salvia africana-lutea and Mallotus peltatus reported (Ahmadiani et al., 2001; Chattopadhyay et al., 2002). The antipyretic activities of Nelumbo nucifera (Sinha et al., 2000) and of jussiaea suffruticosa have also been reported (Murugesan et al., 2000).

The present study was carried out in different experimental animal models using rats to substantiate the anti-inflammatory activity and of to evaluate the antipyretic activity aforementioned plant. Since methanol is the solvent, which brings out most of the components present in any plant material, the present investigation was carried out using a methanol extract of leaves of E. adenophorum.

9.2 Experimental

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

Adult albino rats (Wistar strain) of either sex weighing 180–120g were used. The animals were maintained under suitable nutritional and environmental conditions throughout the experiment. The animals were maintained under standard laboratory condition for an acclimatization period of seven days prior to performing the experiments.

9.2.3 Carrageenin induced rat paw oedema

The rats were divided into four groups and each group consisting of six animals. Oedema was induced by subplanter injection of 0.1 ml 1% freshly prepared suspension of carrageenin (Sigma Chemical Co., USA) into the right hind paw of each rat. The paw volume was measured 0 hr. to 5 hr. after the injection of carrageenin using a plethysmometer (Winter et al., 1962). The methanol extract of *Eupatorium adenophorum* at 100 and 200 mg/kg doses was administered intraperitoneally to two groups of rats. The third control group of rats received 5 ml/kg 2%v/v aqueous tween 80 solution as vehicle control. The fourth standard group received phenylbutazone 100 mg/kg respectively for assessing comparative pharmacological significance. Drug pretreatment was given 30 minutes before the injection of carrageenin.

In all the above cases the degree of oedema formation was estimated by measuring the hind paw volume plethysmographically before and 1hr interval upto 5 hr after carrageenin injection. The oedema rates and inhibition rates were calculated as follow (Lin et al., 1995).

\[
\text{Oedema rate (E) } \% = \frac{V_r}{V_c} \times 100
\]

\[
\text{Inhibition rate (I) } \% = \frac{E_c - E_t}{E_c} \times 100
\]

Where \(V_c\) is the contra–lateral paw volume of the rats (left hind paw without stimulus injection) at t–hour, \(V_r\) is the right hind paw volume of rats (with stimulus injection) at t-hour, \(E_c\) is the oedema rate of the control group and \(E_t\) is the oedema rate of the treated group.
9.2.4 Assessment of Anti-pyretic Activity

Rats of either sex were divided into six groups, comprising six in each group for this experiment. The normal body temperature of each rat was measured rectally at 1 hour interval on a thermometer (Murugesan *et al.*, 2000) and recorded. Fever was induced as per the method (Smith *et al.*, 1938). The rats were trained to remain quiet in restraint cage. After measuring the basal rectal temperature, animals were given a subcutaneous injection of 10 ml/kg of 15% w/v yeast suspended in 0.5% w/v methylcellulose solution. Rats were then returned to their housing cages. After 19 hours of yeast injection, the MEEAL was administered orally at doses of 300 and 400 mg/kg-body wt. to two groups of animals respectively, 2%v/v aqueous tween 80 solution (5ml/kg body wt.) was administered orally to the control group of animals and the remaining group of animals received the standard drug, paracetamol (150 mg/kg body wt.) orally. Rats were restrained from recording of their rectal temperatures at 1 hour just before MEEAL or 2%v/v aqueous tween 80 solution or paracetamol administration and again at 1 hour interval upto 23 hours after yeast injection.

9.3 RESULTS

The anti-inflammatory activity of extract of *E. adenophorum* leaf against carrageenin induced rat hind paw oedema model has been shown in table 24, which showed significant anti-inflammatory activity and the results were comparable to that of phenylbutazone, a prototype non-steroidal anti-inflammatory drug. The extract (200 mg/kg, i.p.) showed maximum inhibition of 37.3% in carrageenin induced rat paw oedema while the standard phenylbutazone (100 mg/kg, i.p.) showed inhibition of 39.7% after 3h of drug treatment.

The anti-pyretic effect of methanol extract of *Eupatorium adenophorum* leaves(MEEAL) on yeast induced pyrexia has been shown in table-25. Treatment with the MEEAL at the doses of 300 and 400 mg/kg body wt. shows the decreased yeast-
provoked elevation of body temperature of rats. The standard drug paracetamol at 150 mg/kg significantly reduced the yeast–provoked elevation of body temperature. The results thus obtained from both the standard drug treated and MEEAL treated groups were compared with the control group and we observed a significant reduction in the yeast elevated rectal temperature.

Table 24. Effect of methanol extract of *Eupatorium adenophorum* Spreng. leaf on Carrageenin induced paw oedema in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>paw oedema rate percentage at different time interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hr.</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>29.2 ± 1.1</td>
</tr>
<tr>
<td>Phenyl butazone</td>
<td>100 mg/kg</td>
<td>23.8 ± 1.4</td>
</tr>
<tr>
<td>MEEAL</td>
<td>100 mg/kg</td>
<td>25.8 ± 1.0</td>
</tr>
<tr>
<td>MEEAL</td>
<td>200 mg/kg</td>
<td>24.9 ± 1.1</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. from six animals in each group.

*p < 0.001, compared with control by student’s *t*–test.

Figures in parenthesis indicates oedema inhibition percentage at different time intervals,

MEEA – Methanol extract of *Eupatorium adenophorum* spreng.

Control –2%v/v aqueous tween 80 solution
Table-25  Effect of MEEAL on yeast induced pyrexia in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Initial Temp. in (°C)</th>
<th>Temp. at 19hr after yeast inj</th>
<th>Temp. (°C) at different hours after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 hr</td>
<td>19 hr</td>
<td>20 hr</td>
</tr>
<tr>
<td>Control</td>
<td>5 ml/kg</td>
<td>37.64±0.1</td>
<td>39.2±0.31</td>
<td>39.3±0.4</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>150 mg/kg</td>
<td>37.2±0.2</td>
<td>39.6±0.21</td>
<td>37.3±0.15*</td>
</tr>
<tr>
<td>MEEAL</td>
<td>300 mg/kg</td>
<td>37.71±0.4</td>
<td>39.35±0.2</td>
<td>38.8±0.1**</td>
</tr>
<tr>
<td>MEEAL</td>
<td>400 mg/kg</td>
<td>37.32±0.3</td>
<td>39.7±0.22</td>
<td>37.9±0.3**</td>
</tr>
</tbody>
</table>

Each value represents mean ± SE of 6 rats.
*p<0.001,**p < 0.01, significantly different compared with control
(Kruskal-Wallis ANOVA test)
Control =2%v/v aqueous tween 80.
MEEAL – Methanol extract of *Eupatorium adenophorum* leaves.

9.5 DISCUSSION

The present study establishes the anti-inflammatory activity of the methanolic extract of *E. adenophorum* leaf in the models used. It is evident that carrageenin induced oedema is commonly used as an experimental animal model of acute inflammation for evaluating the anti-inflammatory effect of natural products (Winter *et al.*, 1962). Carrageenin induced edema is mediated by release of histamine and 5-HT followed by the prostaglandin and kinin and has been frequently used to assess the anti-inflammatory effect of the natural products (Della loggia *et al.*, 1986). The extract probably produced anti-inflammatory activity and anti-5HT activity.

In view of the universal requirement for anti-inflammatory drugs, many plants have been utilized for the purpose in traditional medicine and in recent years considerable research effort has been expended on their investigation. Enzymes have been used to detect anti-inflammatory activity of plants –galangin, a flavonoid of *Alpinia officinarum* (Zinziberaceae) was found to be a cyclo-oxygenase inhibitor. Lipoxigenase inhibitors are
present in *Spilanthes oleracea* (a South America plant of the compositae used for the treatment of rheumatic disorders) and also a Echinacea root, also compositae, which contains a range of compounds including isobutylamides. In a review of plants exhibiting anti-inflammatory activity (Handa *et al*., 1992) cite that species of 96 genera belonging to 56 families are ascribed such activity. A variety of anti-inflammatory drugs of nonsteroidal to steroid nature are available but very few were found to be nontoxic and fit for long term use.

Anti-pyretics are drugs, which reduce elevated body temperature. Regulation of body temperature requires a delicate balance between the production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever, this set point is elevated and drugs like paracetamol do not influence body temperature when it is elevated by factors like exercise or increase in ambient temperature (Goodman, 1996). The present results show that the methanol extract of *Eupatorium adenophorum* leaves (MEEAL) possess a significant antipyretic effect in yeast provoked elevation of body temperature in rats, and its effect is comparable to that of paracetamol (standard drug). Triterpenoids have been reported for its anti-pyretic activity (Trease and Evans, 1985), as the extract having triterpinoids might be showing anti-pyretic activity. In addition to triterpenoids, the plant extract contains steroid like beta-sitosterol might be responsible for such antipyretic activity as well as anti-inflammatory activity. This is to be studied further for the exact mechanism of action.

**9.6 Publication**
