PHARMACOLOGICAL STUDIES
CHAPTER V

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY
5.1. Introduction:

Inflammation, an evidence of many diseases, is a major concern for physicians throughout the world. Prolonged use of anti inflammatory agents has been associated with gastrointestinal irritation (Indian drugs 42(5) May 2005). In India many ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritis and inflammatory conditions (Rainsford et al., 1980). In this modern era a large Indian population still relies on the traditional system of medicine which is mostly plant based. Hence it is considered to evaluate the traditional use of such a plant Fluggia leucopyrus.

Man depends upon plants for his entire essential requirements like food, clothing and shelter. Plants are also an important source of fine chemicals, which find their application in pharmaceutical industries across the globe (Singh, 1988). Plants have been the traditional source of raw materials and finished medicinals, since many centuries. A rich heritage of knowledge on preventive and curative medicines is available in ancient scholastic works. This is included in the Atharva Veda, Charak Samhita, Sushruta Samhita, etc (WHO Regional Office for the Western Pacific, 1993; Pushpaganda, P., 1995). Since disease, decay and death co-existed with life, the study of disease and its treatment must have also been contemporaneous with the dawn of human intellect (Kirtikar and Basu, 1991). In India, the Science of Ayurveda has provided a system of medical treatment. Most of the remedies for treating illnesses are being taken from plants. During the last few decades, much work has been done in the field of these natural products. India is a vast country, which is often referred to as a subcontinent for Emporium of Medicinal Plants due to the occurrence of several thousand medicinal plants in different bioclimatic zones. Ayurveda and Siddha
systems of medicine, which are the traditional heritage of India include many time
tested medicinal plant drugs for various diseases to which there is no answer in
modern medicine till today. The demands for Ayurvedic drugs or phytomedicines are increasing
day by day globally (Yoganarasimham, 2000).

The development of the science of phyto pharmaceuticals and the hope for
remedies for chronic diseases has generated new enthusiasm among researchers to
develop herbal medicines. Quite a considerable amount of work has been put to study
the potential of herbal medicines. Modern science has accepted the plant kingdom as a
source of new bio dynamic constituents. Scientific investigation regarding some folk
medicines has resulted in bonafide drugs. Natural products have also been
incorporated into many modern formulations. The most important drugs that have
come from plant sources into clinical use are cinchona, opium, ergot, rauwolfia, etc.
All were known to be healers in traditional medicine before their introduction to
modern medicine. As the medicinal value of Indian traditional medicine cannot be
ignored, researchers are gradually becoming interested in identification of active
principles in their extracts with intensive follow-up study of their mechanisms of
action.

5.2. Inflammation:

The word inflammation comes from the Latin word “inflammare” which
means state of being inflame or heat associated with redness and swelling. This is a
complex, integrated host response found only in vertebrates. The inflammatory
response has two facets: i) inflammation and ii) repair. Inflammation serves to
destroy, dilute, or wall off the injurious agent and the tissue cells that may have been
destroyed later. The second factor of the inflammatory response sets into motion. It is a complex series of events, which helps to heal and reconstitute the damaged tissue. 

Repair begins during the active phase of inflammation, but reaches completion usually after the injurious influence has been neutralized. Destroyed cells and tissues are repaired thereby. Both inflammation and repair generally serve useful purpose. Without inflammation, bacterial infections would remain unencountered, wounds would never heal, and injured tissues and organs might be permanently defected. But inflammation may be potentially harmful. Inflammatory reactions underlie the genesis of crippling rheumatoid arthritis, life threatening sensitivity reaction, and some forms of fatal glomerular diseases.

**5.3. Experimental Models used for Testing of Anti-inflammatory Activity:**

(Robert Turner, 1965).

The inflammatory process involves a series of events that can be elicited by numerous stimuli, eg. infectious agents, ischemia, antigen-antibody interactions, chemical, thermal or mechanical injury. The response is accompanied by the clinical signs of erythema, oedema, hyperalgesia and pain inflammatory responses which occur in three distinct phases, each apparently mediated by different mechanisms given as under.

An acute, transient phase characterized by local vasodilation and increased capillary permeability. A Sub-acute phase, characterized by infiltration of leukocytes and phagocytic cells. And a chronic proliferative phase in which tissue degeneration and fibrosis occur. In vivo methods for testing acute and sub-acute inflammation are
5.4. **Paw oedema can be induced by chemical agents:**

- Inflammogens
  - Increase vascular permeability and cause fluid accumulation
    - Eg: histamine, serotonin, dextran, eggwhite
  - Cause fluid accumulation by damaging the tissue
    - Eg. Formalin, kaolin

- Pleurisy tests
- Granuloma pouch technique
- Hyaluronidase Inhibition
  The proliferative phase is measured by methods for testing granuloma formation, such as Cotton wool granuloma, glass rod granuloma, PVC sponge granuloma.
5.5. Experimental Models for Evaluation of Anti-Inflammatory Activity:

The various models available for testing anti-inflammatory activity with reasonable accuracy, minimum time and test compound consumption are mentioned below:

a. Acute models of inflammation: (Nasrin et al., 2005; Iracema et al., 2005)
   - Carrageenan induced oedema model:
   - U.V.light induced erythemea model:

b. Chronic models of inflammation: (Victor et al., 2005; Gupta et al., 2005).
   - Cotton pellet method:
     - Granuloma pouch method: (Perez-Garcia et al., 2005; Isabel et al., 2004).
   - Formaldehyde induced arthritis: (Moura et al., 2005; Eun Mi et al., 2003).
   - Adjuvant induced arthritis: (Lilly et al., 2005; Fan et al., 2005).

5.6. Plants with anti-inflammatory activity:

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Although rheumatism is one of the oldest known diseases of mankind and affects a large population of the world, there have been no substantial progress has been made in achieving a permanent cure. The greatest disadvantages of the presently available potent synthetic drugs lie in their toxicity and reappearance of symptoms after discontinuation of treatment. The research of screening and development of drugs for their anti-inflammatory activity is therefore, an unending problem and there is need of finding out antirheumatic drugs from indigenous plants.
The literature survey reveals that plant species of about 96 genera belonging to 56 families have exhibited anti-inflammatory activity. Some of the plant sources used in traditional systems of medicine with pharmacologically/therapeutically proven anti-inflammatory and antirheumatic claims are mentioned in the table no 1.

Table 1: Some plants with anti-inflammatory activity (The Useful plants of India, 1988; The Medicinal Plants of India, 1987; The Wealth of India, 1952).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Trade names in India</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aconitum napellus</td>
<td>Aconite</td>
<td>Ranunculaceae</td>
</tr>
<tr>
<td>Balanites roxburghii</td>
<td>Gari</td>
<td>Simarubiaceae</td>
</tr>
<tr>
<td>Colchicum autumnale</td>
<td>Colchicum</td>
<td>Liliaceae</td>
</tr>
<tr>
<td>Delonix elata</td>
<td>Vatanarayana</td>
<td>Leguminosae</td>
</tr>
<tr>
<td>Glycyrrhiza glabra</td>
<td>Liquorice</td>
<td>Leguminosae</td>
</tr>
<tr>
<td>Hibiscus rosa sinensis</td>
<td>Jassoon</td>
<td>Malvaceae</td>
</tr>
<tr>
<td>Lawsonia inermis</td>
<td>Hena</td>
<td>Lythraceae</td>
</tr>
<tr>
<td>Mammea longifolia (Vitexin)</td>
<td>Nagkesar</td>
<td>Guttiferae</td>
</tr>
<tr>
<td>Moringa oleifera</td>
<td>Sahinjan</td>
<td>Moringaceae</td>
</tr>
<tr>
<td>Nepeta hindostana (Nepitrin)</td>
<td>Billilotan</td>
<td>Labiatae</td>
</tr>
<tr>
<td>Operculina turethum</td>
<td>Nakpatra</td>
<td>Convolvulaceae</td>
</tr>
<tr>
<td>Pterocarpus santalinus</td>
<td>Raktachandana</td>
<td>Leguminosae</td>
</tr>
<tr>
<td>Randia dumerorum</td>
<td>Mainphal</td>
<td>Rubiaceae</td>
</tr>
<tr>
<td>Salvadoria perecia</td>
<td>Brihatpilu</td>
<td>Salvadoraceae</td>
</tr>
</tbody>
</table>
Analgesics are frequently used in combination, such as the paracetamol and codeine preparations found in many non-prescription pain relievers. They can also be found in combination with vasoconstrictor drugs such as pseudoephedrine for sinus-related preparations, or with antihistamine drugs for allergy sufferers (Melhlisch 2002).

The use of paracetamol, as well as aspirin, ibuprofen, naproxen, and other NSAIDS concurrently with weak to mid-range opiates (up to about the hydrocodone level) has been shown to have beneficial synergistic effects by combating pain at multiple sites of action NSAIDs reduce inflammation which, in some cases, is the cause of the pain itself while opiates dull the perception of pain—thus, in cases of mild to moderate pain caused in part by inflammation, it is generally recommended that the two be prescribed together (Rang et al., 2007).

Recently the Anti microbial activity of this plant was reported by (Bakshu et al.,) but the basis for anti-inflammatory and analgesic activities of this plant extract has not been thoroughly investigated. Taking the above information into account, the present work was carried out to evaluate the anti-inflammatory and analgesic activities of leucopyrosinol and ethylacetate crude extract of leaves parts of Fluggea leucopyrus.

5.7. Materials and Methods:

5.7.1. Chemicals:

Test Compounds: Leucopyrosinol and ethylacetate crude.

Hi-media, Mumbai: Carrageenan.

Ugo Basile, Italy: Plethysmometer.

Qualigens Fine Chemicals, India: Diclofenac.
Nestor Pharma, India: Phenyl butazone.
Analgesiometer (Techno).

5.7.2. Experimental animals:

Wistar rats of either sex (150-200 gm) were used for evaluating anti-inflammatory and analgesic activities. They were housed in polypropylene shoebox type cages with stainless steel grill top and bedded with rice husk. The animals were provided with pelleted diet (Goldmohur, Lipton India) and water ad libitum. They were allowed a one-week acclimatization period before the experimental session. All the experimental protocols were met with the approval of Institutional Animal Ethics Committee.

5.7.3. Anti inflammatory activity of Fluggea leucopyrus.

The albino (Wistar strain) rats (180-200g) of either sex were used for this study. Anti-inflammatory activity was carried out as suggested by (winter et al 1962). The rats were divided into eight groups of six animals each. Group A: Received 0.2 ml of normal saline by oral route which served as normal control. Group B: Received 100 mg/kg body weight of Phenyl butazone by oral route which served as positive control. Group C, D, E: Received leucopyrosinol, at a dose of 100, 150, 200 mg /kg which served as test. Group F, G, H: Received Ethyl acetate (Crude) at a dose of 100, 150, 200 mg/kg which is also served as test. The test extracts and standard reference phenylbutazone were administered orally to rats 1 hour before the injection of 0.1ml of 1% carrageenan suspension in normal saline. A No 26 gauge needle was used to inject 0.1 ml of the carrageenan suspension into the subplantar region of the left hind paw and right hind paw served as control. Immediately thereafter the volume
of injected paw was measured by mercury displacement method using plethysmograph. It was found that the swelling reached a peak between 3 to 5 hours and was stable for several hours. Thereafter for assessing anti-inflammatory activity the oedema volume at predetermined intervals was noted.

The difference in oedema volume between the treated and control were measured and mean volume of oedema was calculated from the data obtained. The percentage reduction in oedema volume was calculated using the formula.

\[ 1 - \frac{Vt}{Vc} \times 100 \]

Where \( Vt \) = paw volume after time \( t \)

\( Vc = \) paw volume of control (carrageenan treated).

5.7.4. Analgesic activity of *Fluggea leucopyrus*.

The analgesic activity of lecopyrosinol and ethyl acetate (crude) of *fluggea leucopyrus* was carried out by thermal method as suggested by D’Amour and Smith in rats. In this study rats were subjected to noxious stimuli by exposing tip of the tail (last 1-2 cm) to radiant heat source by using analgesiometer and the tail withdrawn from the heat source is taken as the cut off time, or end point.

The animals were divided in to eight groups of six animals each.

Group A: Served as normal control which received normal saline. Group B: Received 20mg/kg body weight dose of diclofenac sodium by oral route which served as reference standard. Group C, D, E: Received lecopyrosinol, at a dose of 100, 150, 200 mg/kg which served as test. Group F, G, H: Received ethyl acetate (crude) at a dose of 100, 150, 200 mg/kg which is also served as test. The reaction time was recorded at 0, 15, 30 and 45 minutes.
5.7.5. Statistical Analysis:

The results were expressed as mean ± SEM The significance was evaluated by student t test compared with control and p< 0.001 implied significance (Woodson 1989).

5.7.6. Results:

5.7.7. Anti inflammatory activity:

There was dose dependent significant reduction in carageenan induced rat paw edema at different doses of leucopyrosinol, ethyl acetate and at 100mg/kg phenyl butazone over a period of 4 hour as shown in Table 2.

Significant anti-inflammatory activity was noted with all the three doses of leucopyrosinol and ethyl acetate (crude) at different time intervals (i.e. 1 hour, 2 hour, & 4 hour) and significant percent reduction in oedema volume with all the 3 doses were noted as leucopyrosinol 100 mg/kg (6%, 41.93%, 46.61%, 50.25%) 150 mg/kg (13%, 47.31%, 54.88%, 55.64%), 200 mg/kg (15%, 55.91%, 57.89%, 69.94%), and ethyl acetate (crude) 100 mg/kg (6%, 10.75%, 12.7%, 19.69%) 150 mg/kg (13%, 34.14%, 38.4%, 59.59%), 200 mg/kg (15%, 39.71%, 43.60%, 61.66%) at four time intervals respectively. Leucopyrosinol is highly significant when compared to ethyl acetate extract.
Table 2: Anti-inflammatory activity of leucopyrosinol and ethyl acetate (crude) of *Fluggea leucopyrus*.

ROV- Reduction in Paw edema Volume

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Paw edema Volume in (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0% ROV</td>
</tr>
<tr>
<td>A</td>
<td>Control</td>
<td>0.2 ml</td>
<td>0.45</td>
</tr>
<tr>
<td>B</td>
<td>Phenyl butazone</td>
<td>100</td>
<td>0.54</td>
</tr>
<tr>
<td>C</td>
<td>Leucopyrosinol</td>
<td>100</td>
<td>0.48</td>
</tr>
<tr>
<td>D</td>
<td>Leucopyrosinol</td>
<td>150</td>
<td>0.51</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>200</td>
<td>0.52</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>100</td>
<td>0.48</td>
</tr>
<tr>
<td>G</td>
<td>Ethyl acetate (Crude)</td>
<td>150</td>
<td>0.51</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>200</td>
<td>0.52</td>
</tr>
</tbody>
</table>

n=6

Values are mean ± SEM, Significance at p < 0.001***, p < 0.01** and p < 0.05*. 

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Figure 1: Anti-inflammatory activity of Leucopyrosinol of fluggea leucopyrus.

ANTI INFLAMMATORY ACTIVITY OF LEUCOPYROSINOL

% INHIBITION OF ODEMA VOLUME

TIME IN (HOUR)

std
L P 100mg
L P 150mg
L P 200mg

L P - Leucopyrosinol
Figure 2: Anti inflammatory activity ethyl acetate (crude) of fluggea leucoppyrus.

ANTI INFLAMMATORY ACTIVITY OF ETHYL ACETATE

TIME IN (HOUR)

% INHIBITION OF ODEMA VOLUME

Std
EAE 100mg
EAE 150mg
EAE 200mg

EAE – Ethylacetate extract (crude).
5.7.8. Analgesic activity:

There was dose dependent significant increased in basal reaction time at different doses of leucopyrosinol, ethyl acetate and standard diclofenac sodium at 20 mg/kg over a period of 0, 15, 30, 45 minutes as shown in Table 3. Leucopyrsinol increased the basal reaction time from (4.00±0.26, 4.50±0.34, 4.50±0.02, 4.71±0.30) at 100 mg/kg (4.67±0.215, 5±0.22, 6.3±0.21, 6.83±0.30) at 150 mg/kg and (4.67±0.217, 33±0.218, 17±0.319, 33±0.33) 200 mg/kg where as ethyl acetate showed (4.00±0.26, 4.20±0.002, 4.20±0.16, 4.60±0.30) at 100 mg/kg, and (4.67±0.21, 5.20±0.001, 5.40±0.13, 6.20±0.31) at 150 mg/kg, (4.67±0.21, 6.10±0.001, 6.90±0.20, 8.10±0.24 ) at 200 mg/kg. Leucopyrosinol is highly significant when compared to ethyl acetate extract.
Table 3: Analgesic activity of lecopyrosinol and ethyl acetate (crude) of *fluggea lecopyrus* by tail flick method

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Basal reaction time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>Normal control</td>
<td>0.2 ml</td>
<td>4.00±0.26</td>
</tr>
<tr>
<td>B</td>
<td>Diclofenac Sodium</td>
<td>20 mg/kg</td>
<td>4.33±0.21</td>
</tr>
<tr>
<td>C</td>
<td>Leucopyrosinol</td>
<td>100 mg/kg</td>
<td>4.00±0.26</td>
</tr>
<tr>
<td>D</td>
<td>Leucopyrosinol</td>
<td>150 mg/kg</td>
<td>4.67±0.21</td>
</tr>
<tr>
<td>E</td>
<td>Leucopyrosinol</td>
<td>200 mg/kg</td>
<td>4.67±0.21</td>
</tr>
<tr>
<td>F</td>
<td>Ethyl acetate</td>
<td>100 mg/kg</td>
<td>4.00±0.26</td>
</tr>
<tr>
<td>G</td>
<td>Ethyl acetate</td>
<td>150 mg/kg</td>
<td>4.67±0.21</td>
</tr>
<tr>
<td>H</td>
<td>Ethyl acetate</td>
<td>200 mg/kg</td>
<td>4.67±0.21</td>
</tr>
</tbody>
</table>

n=6

Values are mean ± SEM, Significance at p < 0.001***, p < 0.01** and p < 0.05*. 

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Figure 3: Analgesic activity of leucopyrosinol of Fluggea leucopyrus.
Figure 4: Analgesic activity of crude extract of ethylacetate of *Fluggea leucopyrus*.

EAE – Ethyl acetate extract (crude)
5.8. Discussion:

In the present study, Leucopyrosinol and ethyl acetate were tested for its anti-inflammatory and analgesic activity.

Generally, the inflammatory process involves a series of events that can be elicited by numerous stimuli such as infectious agents, ischaemia, antigen-antibody interaction and thermal or physical injury (Insel, 1990; Osadebe and Okoye, 2003). Inflammation is usually associated with pain as a secondary process resulting from the release of analgesic mediators (Hunskaar and Hole, 1987; Osadebe and Okoye, 2003).

Topical and systematic application of herbal plants is common practice for the treatment of inflammation, particularly, arthritis (ama, vata) which has been followed for many years in the practice of Indian system of medicine ayurveda. Determination of anti inflammatory activity is based on plethysmographic measurement of oedema produce by sub planter injection of carrageen in the hind paw of rat. The increase in oedema in animals treated with standard drug phenyl butazone, methnolic and crude extract of ethyl acetate of Fluggea leucopyrus were compared with increase on oedema of untreated control animals at constant intervals of 1, 2, 4\textsuperscript{th} h, thus percentage inhibition of oedema at known intervals in treated animals was used for the purpose of calculating percent inhibition of oedema of control. The present study revealed that both the extraction of Fluggea leucopyrus showed significant anti inflammatory activity. The maximum activity was observed during 4\textsuperscript{th} h, and the results are significant (p<0.001).

Carrageenan –induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-
inflammatory drugs as it is known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits a high degree of reproducibility. Carrageenan induced edema is a biphasic response. The 1st phase is mediated through the release of histamine, serotonin & kinins, where as the 2nd phase is related to the release of prostaglandin & slow reacting substances. The results of Carrageenan experiment showed maximum activity at 1st and 4th hour after the injection. Both extracts have contained flavonoids; the active principles of these may be responsible for anti inflammatory activity. Flavonoids are reported with anti-inflammatory activity (Charde et al., 2006). The extract on phytochemical studies revealed the presence of the above principles. This confirms their anti-inflammatory activity in animal studies. Thus, in the light of above results, Ayurvedic use of Fluggea leucopyrus is explained. The therapeutic effect of flavonoids on inflammation have previously been reported (Middleton et al., 2000;). The anti-inflammatory effects of flavonoids have been attributed to various mechanisms including inhibition of lipooxygenase and cyclooxygenase activities (Middleton et al., 2000; Singh et al, 1997).

Our results are in accordance with previous studies which have showed the analgesic activity of certain species of plants and their constituents, due to the presence of different principles, such as terpenoids and flavonoids (Jiménez et al. 1986; Alcaráz et al., 1989). In the present study, the phytochemical analysis of both isolated pure compound and ethyl acetate extracts revealed the presence of the flavonoid. Those are known to target prostaglandins, which are involved in the pain perception (Rajnarayan et al., 2001). This confirms their analgesic activity in animal studies.
Leucopyrosinol and ethylacetate exhibited potent analgesic activity at the dose levels of 100, 150 and 200 mg/kg. It is worth noting that these extracts showed significant analgesic activity at dose of 200 mg/kg. The duration as well as the intensity of analgesia induced by Leucopyrosinol and ethylacetate was dose dependent. The analgesic activity showed by leucopyrosinol at 200 mg/kg was almost comparable to that produced by standard drug diclofenac sodium, while at the dose levels of 150 mg/kg and 100 mg/kg. leucopyrosinol showed better analgesic effect than ethyl acetate extract. The duration and intensity of analgesia was also similar to that of the standard. The plant extract showed a rapid onset of analgesic action at all three doses then as compared to diclofenac sodium at 20 mg/kg dose.

5.9. Conclusion:

In conclusion, the results of the present study provide the evidence for the anti-inflammatory and analgesic activity of Leucopyrosinol and ethylacetate crude extract of leaves parts of Fluggea leucopyrus. The flavonoids, present in the extracts may be responsible for their activity. Further studies are underway.
5.10. References:


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