Chapter VI

ANTH-INFLAMMATORY & ANALGESIC ACTIVITY

INTRODUCTION

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane et al., 1995). It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy et al., 2010).

Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue (Denko, 1992). However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis (Henson et al., 1989).
Types of Inflammation

Acute Inflammation

Acute inflammation may be an initial response of the body to harmful stimuli. An increased movement of plasma and leukocytes, especially granulocytes from the body into the injured tissues is observed. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells within the injure tissues. It starts rapidly (rapid onset) and quickly becomes severe. Signs and symptoms are only present for a few days, but in some cases may persist for a few weeks.

Examples are diseases, conditions and situation which can result in acute inflammation include: acute bronchitis, infected ingrown toenail, sore thorat from a cold or flu, a scratch/cut on the skin, exercise (especially intense training), acute appendities, acute dermatitis, acute tonsillitis, acute infective meningitis, acute sinusitis, or a blow.

Chronic Inflammation

In chronic inflammation, the inflammatory response is out of proportion resulting in damage to the body. The different types of allergies and many autoimmune diseases viz, asthma, rheumatoid arthritis, multiple sclerosis and systemic lupus erythomatosus are a few examples. This means long-term inflammation, which can last for several months and even years. It can result from:
• Failure to eliminate whatever was causing an acute inflammation

• An autoimmune response to a self-antigen- the immune system attacks healthy tissue, mistaking it (them) for harmful pathogens.

• A chronic irritant of low intensity that persists.

Examples of diseases and conditions which chronic inflammation include: asthma, chronic peptic ulcer, tuberculosis, rheumatoid arthritis, chronic periodontitis ulcerative colitis and Crohn’s disease, chronic sinusitis and chronic active hepatitis.

Treatment

Inflammation is currently regularly treated by non-steroidal anti-inflammatory drugs (NSAIDS). The NSAID$s achieve their effect by blocking the activity of COX involved in blocking PG$s secretion resulting in reduced fever and pain of inflammation (Zhang et al., 2005). However, prolonged use of NSAIDS results in side effect viz., a tendency to develop side effects due to inhibition of constitutive COX-1 as well COX-2 induced during inflammation. Specific COX-2 inhibitors viz., rofecoxib and celecoxib have been also used as drugs. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes as they do not block the synthesis of thromboxane A2 by platelets which contain only COX-1 (Kimmel et al., 2005). The attention of pharmacologist throughout the world has been focused on finding out safer and potent anti-inflammatory drug.
Medicinal plants have been source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions. The use of herbal medicines becoming popular due to toxicity and side-effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. India with its biggest repository of medicinal plants in the world may maintain an important position in the production of raw materials either directly for crude drugs or as the bioactive compounds in the formulation of pharmaceuticals and cosmetics etc.

**Animal models**

Inflammation research involves a number of experimental models that can be broadly classified into two types: acute inflammatory models and chronic inflammatory models (Lewis, 1989). Acute models are designed to test drugs modulating erythema, changes in vascular permeability, leukocyte migration, and measurement of local pain, local analgesic action and rat paw edema (Barbosa *et al.*, 2006). Chronic models are designed to find drugs modulating disease process induced by sponge, pellet implants, granuloma pouches and adjuvant induced arthritis (Lewis, 1989).
REVIEW OF LITERATURE

The ethanolic extract of *Acacia farnesiana* leaves were tested for the anti-inflammatory activity by carrageenan induced paw edema for acute inflammation and cotton pellet induced granulation for chronic inflammatory model. The ethanolic extract showed significant anti-inflammatory activity in both the models studied (Hukkeri *et al.*, 2002). Gurulingappa *et al.* (2002) have reported the anti-inflammatory activity of *Aegle marmelos*. Methanol extract of *A.marmelos* showed significant anti-inflammatory activity at a dose of 100 mg/kg.

The methanol extract of *Alstonia macrophylla* leaves were investigated for its anti-inflammatory activity. The extract at a concentration of 200 mg/kg and 400 mg/kg p.o. showed the significant dose dependent anti-inflammatory activity in carrageenan and dextran induced rats hind paw edema as well as in cotton pellet induced granuloma in rats (Arunachalam *et al.*, 2002).

Kaith *et al.* (1996) reported that the petroleum ether, chloroform, alcoholic and aqueous extracts of *Arnebia euchroma* roots were found to exhibit maximal edema inhibition against carrageenan induced rat paw edema at 300 min interval. The ethyl acetate extract of *Atalantia monophylla* showed significant activity at a dose of 100 mg/kg (Gurulingapa *et al.*, 2002).

Studies showed that *Cassia fistula* extract revealed potent anti-inflammatory activity in carrageenan, histamine and dextran induced paw edema in rats (Bhakta *et al.*, 2000). The alcoholic extract *Azadirachta indica* exerted significant anti-inflammatory activity in cotton pellet granuloma assay in rats. The significant and
consistent anti-inflammatory effect of *Bryonia laciniosa* in carrageenan rats at a dose of 200 mg/kg indicates that the plant extracts acts significant activity (Gupta *et al.*, 2003).

The anti-inflammatory effect of the methanol extract of *Drymaria cordata* exhibited significant anti-inflammatory activity in carrageenan, histamine, serotonin, dextran and PGE induced rat hind paw edema (Mukerjee *et al.*, 1998). Ethanol extract of *Echinops echinatus* inhibited the acute inflammation induced in rats by carrageenan, formaldehyde and adjuvant and the chronic arthritis induced by formaldehyde and adjuvant (Singh *et al.*, 1989).

The methanolic extract of *Solanum nigrum* (375 mg/kg b.w.) has showed significant anti-inflammatory and also the methanolic extract decreased the edema induced in hind paw (Ravi *et al.*, 2009). The ethyl acetate extract of *Sarcostemma acidum* showed significant membrane stabilizing action on human red blood cell when compared to standard drug Indomethacin which showed 69.6 % protection of HRBC in hypotonic solution (Gupta Shailesh *et al.*, 2011).

The ethanol root extract of *Aconitum heterophyllum* has potential to inhibit sub-acute inflammation by interruption of the arachidonic acid metabolism (Santhosh Verma *et al.*, 2010). The methanolic extract of *Ricinus communis* exhibited significant anti-inflammatory activity in carrageenan-induced hind paw edema model and also it’s proved significant free radical scavenging activity by inhibiting lipid peroxidation (Heering *et al.*, 1996). *Bacopa monnieri* possesses significant anti-inflammatory activity that may well be relevant to its effectiveness.
in the lealing of various inflammatory conditions in traditional medicine. The anti-inflammatory activity of *Bacopa monnieri* is due to the triterpenoid and bacoside present in the plant (Channa *et al*., 2006).

Ethanol extract *Bombax ceiba* showed significant (p<0.001) anti-inflammatory response followed by aqueous extract (p<0.01) when compared with standard, diclofenac potassium (50 mcg/ml) (Anandarajagopal *et al*., 2013). The extracts of *Urera baccifera*, *Chaptalia nutans*, *Loasa speciosa* and *Loasa triphylla* (500 mg/kg i.p.) showed an anti-inflammatory activity comparable with that of indomethacin. The extracts of *U. baccifera* and *C. nutans*, which showed the greatest anti-inflammatory activity, did not show it when used orally (500 mg/kg p.o.) (Beatriz Badila *et al*., 1999).

**ANALGESIC ACTIVITY**

A single oral dose of ethanol and aqueous extracts of *Emblica officinalis* showed significant reduction in brewer’s yeast induced hyperthrima in rats. Ethanol and aqueous also elicited pronounced inhibitory effect on acetic acid induced writhing response in mice in the analgesic activity (Perianayagam *et al*., 2004). The peripheral analgesic activity was studied on *Hibiscus rosa sinesis* in rats using acetic acid induced writhing response and tail flick method by using Pethedine as standard. The extract showed significant dose-dependent analgesic activity in both the methods (Vivek Tomar *et al*., 2010)

The analgesic activity of alcoholic extract of *Cissus quadrangularis* was studied in mice by Haffner’s Clip and Eddy’s hot plate method. The extract
effective by both oral and intraperitoneal routes significantly increased the reaction time by both methods (Singh et al., 1984). The aqueous extract of *Acacia nilotica* pods decoction produced a significant inhibition (44.16%) of xylene-induced ear swelling in mice as compared with untreated mice. On the other hand, the plant extracts also inhibited rat paw oedema induced by carrageenan and the granuloma formation induced by the cotton pellets in rats in a dose dependant manner. The highest dose of *A. nilotica* extract (100 mg/kg) produced a maximum inhibition of 64.41 and 25.62% respectively for the carrageenan induced paw edema and the cotton pellet induced granuloma in rats (Sokeng et al., 2013).

In analgesic bioassay, oral administration of the ethanol extract of leaves were significantly (p<0.01) reduced the writhing response. The efficacy of leaves extract were almost 35% in *Desmodium pauciflorum*, 56% in *Mangifera indica* and 34% in *Andrographis paniculata* which is found comparable to the effect of standard analgesic drug diclofenac sodium (76%). Leaves extract reduced paw edema in variable percentages but they did not show any significant difference among the leaves (Hassan et al., 2013).

The analgesic bioassay, oral administration of the ethanol leaves extract *Mangifera indica* significantly (P<0.01) reduced the writhing response. The degree of inhibition of leaves extract was 55.8% compared to the effect of standard analgesic drug, Diclofenac Sodium (75.28%). On the other hand, though leaves extract reduce paw edema but they did not show any significant effect (Islam et al., 2010).
Ethanolic extracts of fruits of Coriandrum sativum, leaves of Datura stramonium and Azadirachta indica were subjected to preliminary screening for anti-inflammatory activity in albino rats. All ethanolic extracts exhibited significant anti-inflammatory activity comparable to the standard drug Diclofenac sodium against carrageenan induced rat paw edema method (Gupta Sonika et al., 2010).

MATERIALS AND METHODS

Anti-inflammatory activity of crude extracts of Spathodea campanulata and Delonix elata were screened with the following standard method.

Acute toxicity test

Acute toxicity study was carried out on both plant extracts using Swiss Albino rats. The rats were fasted overnight and the weight of each rat was recorded just before use. Animals were divided randomly into a control and six treatment groups, each group consisting of five rats. Control group received only the vehicle & each treatment group received orally the methanol and aqueous extracts of the studied plants in a dose of 100, 200, 400, 800 and 1200 mg/kg. Animals were kept under close observation for 4 hours after administering the extract (Burger et al., 2005), and then they were observed daily for three days for any change in general behaviour and/or other physical activities.

Anti-inflammatory effect using experimental animals

Chemicals used

Carrageenan and indomethacin were purchased from Sigma Chemical Company, USA, and all the other chemicals and reagents used in the experiments were of analytical grade and were obtained from BDH (England and India),
Antimicrobial and anti-inflammatory activity of *S. campanulata* and *D. elata*

E. Merck (Germany), Sigma Chemical Company (U.S.A.), Sarabhai, M. Chemicals (India), LOBA – Chemie Indo Austranol Co., (India).

**Experimental fauna**

Healthy adult Swiss Albino rats (both sexes) weighing about ~150-180 g were used, which were adapted to metabolic cages for 2 or 3 days maintained under standard conditions (12 h light/12 h dark cycle; 25 ± 3°C; 35–60% humidity), and were fed with a standard rat pellet diet and water *ad libitum* and their mean weight was observed 158 ± 2 g. (Plate 6.1). The study was conducted at RVS Pharmaceutical College, Coimbatore Dt. The animals were used for this experiment with the permission of Institutional Animal Ethical Committee, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore, Tamilnadu, India (R.No.IAEC1012/C/06/CPSEA-Corres-2008-2009).

**Experimental Design**

Rats were divided into two sets and each set contains 4 groups:

**Set I**

**Group I:** Normal control (Carrageenan 1%w/v)

**Group II:** Positive control (indomethacin 10mg/kg, i.p.)

**Group III:** Rats received *D. elata* methanolic extract (200mg/kg of bw)

**Group IV:** Rats received *D. elata* aqueous extract (200mg/kg of bw)

**Set II**

**Group I:** Normal control (Carrageenan 1%w/v)

**Group II:** Positive control (indomethacin 10mg/kg, i.p.)

**Group III:** Rats received *S. campanulata* methanol extract (200mg/kg of
Group IV: Rats received *S.campanulata* aqueous extract (200mg/kg of bw)

The paw-volume measured at 0, 30, 60, 120, 180 min. after carrageenan injection using the plathysmometer. The animals of group III, IV, were pretreated with methanolic extracts and V, VI with aqueous extracts, 60 minutes before the administration of Carrageenan. Acute inflammation was produced by the sub plantar administration of 0.1% carrageenan in normal saline in the left paw or rats. Inhibition of swelling is compared with that of control group (Kulkarni, 2005).

**Oral drugs administration**

Group III & IV of each set rats were assigned to be treated with crude methanol and aqueous extracts (*S.campanulata* and *D.elata*) (200 mg/kg of bw) and the other to an untreated Group I (control group) every day unto the final day of the experiment.

**Carrageenan induced Rat paw edema**

The anti-inflammatory activity of the test compounds were evaluated in Wistar rats employing the method. Animals were fasted overnight and were divided into control, standard and different test groups each consisting of five animals. The different test extracts were administrated to the animals in the test groups at the dose of 200mg/kg by oral route. Animals in the standard group received Indomethacin at the dose of 10 mg/kg, by oral route. Control group animals were received 1% DMSO at the dose of 10ml/kg body weight. Thirty minutes after administration of the respective drugs, all the animals were challenged with 0.1 ml of 1% carrageenan in the sub planter region of left hind paw. Paw volume was
measured by using digital plethysmometer before administration of carrageenan and after 30, 60, 120 & 180 min intervals. The efficacy of different drug was tested on its ability to inhibit paw edema as compared to control group (Kulkarni, 2005). The percentage of inhibition of paw-edema is calculated by

\[
\% \text{ inhibition of paw edema} = \frac{C - T}{C} \times 100
\]

Where,

\(C = \) increase in paw volume of control group
\(T = \) increase in paw volume after administration of extracts

**Analgesic activity**

**Experimental animals**

Healthy adult Swiss Albino rats (both sexes) weighing about \(\sim 150-180 \text{ g}\) were used, which were adapted to metabolic cages for 2 or 3 days maintained under standard conditions (12 h light/12 h dark cycle; 25 ± 3°C; 35–60% humidity), and were fed with a standard rat pellet diet and water *ad libitum* and their mean weight was observed \(158 \pm 2 \text{ g}\).

**Experimental Design**

Rats were divided into two sets and each set contains 4 groups:

**Set I**

- **Group I:** Normal control (Acetic acid 3% v/v)
- **Group II:** Positive control (pentazocine 5mg/kg, i.p.)
Group III: Rats received *D.elata* methanolic extract (200mg/kg of bw)

Group IV: Rats received *D.elata* aqueous extract (200mg/kg of bw)

Set II

Group I: Normal control (Acetic acid 3% v/v)

Group II: Positive control (pentazocine 5mg/kg, i.p.)

Group III: Rats received *S.campanulata* methanol extract (200mg/kg of bw)

Group IV: Rats received *S.campanulata* aqueous extract (200mg/kg of bw).

Acetic acid is administrated in the dose of 30mg/kg or 0.3 ml to the first group (normal control) and number of writhing responses (constriction of abdomen, twisting of trunk and extension of hind limbs) are recorded for a period of 10 mins.

**Oral drug administration**

The animals of group III of each set were pretreated with methanolic extracts and IV with aqueous extracts, 15 minutes before the administration of acetic acid. Reduction in number of writhing is taken as analgesic activity and compared with that of control group (Kulkarni, 2005).

**Writhing Test (Chemical methods)**

Albino rats weighing between 150-180 gm was purchased from RVS Pharmaceutical College, Coimbatore. Animals are divided into 6 groups. Acetic acid is administrated in the dose of 30mg/kg or 0.3 ml to the first group (normal control) and number of writhing responses (constriction of abdomen, twisting of trunk and extension of hind limbs) are recorded for a period of 10 mins. The animals of group III and IV were pretreated with methanol and aqueous extracts, 15
minutes before the administration of Acetic acid. Reduction in number of writhe is taken as analgesic activity and compared with that of control group (Kulkarni, 2005).

**Hot-plate method (Thermal stimulus)**

The rats selected were weighted (150 -180g) and groups into six of five in each and the normal basal reaction time were taken by repeating for 5 times. Group III to & Group IV received methanol and aqueous received extracts respectively at a dose of 200 mg/kg body weight (p.o.). Group II received Pentazocine 5 mg/kg body weight (s.c.) and served as standard.

Group I administrated 1% DMSO in the dose of 10ml/kg body weight (p.o.) served as control. All animals were lowered onto the surface of a hot plate (50 ± 1.00C) enclosed with cylindrical glass and the time for the animal to jump or lick the fore limb was noted as the reaction time (RT). Cut off time in the absence of a response was 15 sec to prevent the animals from being burnt (Sharma and Khanna, 1982). The observations were made before and after administration of respective drugs at 30 min, 60 min, 120 min, and at the end of 180 min (Ghosh, 2005; Vogel, 2008).

**Statistical analysis**

All results are expressed as mean ±S.D. Statistical evaluation was done using one-way analysis of variance (ANOVA), followed by Student’s t- test.
RESULTS

Experimental animals

Thirty Swiss albino rats weighing 150-180 g body weight were used for the study. The animals were divided into six groups of five animals each and treatment schedule were mentioned in materials and methods.

Toxicity studies

No adverse effect or mortality was detected in albino rats up to 1200 mg/kg bw of methanol and aqueous extracts of *Spathodea campanulata* and *Delonix elata* during 24 hr observation period basing on which the respective doses are selected for further study (Table 6.1 & 6.1.a and 6.2 & 6.2.a).

Analgesic activity

While searching the analgesic efficacy of methanol and aqueous extracts by acetic acid induced writhing method, it was found that the standard Pentazocine showed highly significant analgesic activity in acetic acid induced writhing method in Swiss albino rats. Normal control (group I) did not have any significant decrease in average numbers of writhes. Both the plant extracts (*S. campanulata* and *D. elata*) methanolic extracts at a dose of 200 mg/kg showed highly significant activity (P<0.01) as compared to control group. The aqueous extract at 200mg/kg also showed significant activity (P<0.01). There was no significant differences (P<0.05) in the average numbers of writhes with standard as was observed in methanolic extract at 200mg/kg dose level (Tables 6.3 & 6.4 and Fig 6.1 & 6.2).

During the search of analgesic effect of selected extracts of the plant by hot plate method it was observed that Pentazocine showed significant analgesic effect
at 30, 60, 120 and 180 minutes. Peak effect was observed at 120 minute. Normal control (group I) did not have any significant change in basal reaction time. The different dose of methanolic extracts of both the plant extracts showed highly significant effect ($P<0.01$) at 30, 60, 120 and 180 minutes as compared with control group. The aqueous extract 200mg/kg showed a significant activity ($P<0.05$) at 30 minute and highly significant activity ($P<0.01$) at 60, 120 and 180 minutes. The methanolic extract of *D. elata* at a dose of 200mg/kg showed peak effect 13.6 at 120 minute (Tables 6.5 & 6.6 and Fig 6.3 & 6.4).
Table 6.1.

Acute Toxicity study on *Delonix elata*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Death</th>
<th>Dose Difference (mg)</th>
<th>Mean death</th>
<th>Dose Difference X death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Group II</td>
<td>6</td>
<td>0</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>6</td>
<td>0</td>
<td>200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group IV</td>
<td>6</td>
<td>0</td>
<td>400</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group V</td>
<td>6</td>
<td>0</td>
<td>800</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group VI</td>
<td>6</td>
<td>0</td>
<td>1200</td>
<td>-</td>
<td>-</td>
</tr>
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</table>
### Table 6.1.a
Signs and symptoms of *Delonix elata* extract toxicity on rats.

<table>
<thead>
<tr>
<th>Group (Dose)</th>
<th>Signs &amp; symptoms (No. of animals)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Irritability (0)</td>
<td>Tremor (0)</td>
</tr>
<tr>
<td>Group II</td>
<td>Irritability (1)</td>
<td>Tremor (0)</td>
</tr>
<tr>
<td>Group III</td>
<td>Irritability (2)</td>
<td>Tremor (1)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Irritability (3)</td>
<td>Tremor (2)</td>
</tr>
<tr>
<td>Group V</td>
<td>Irritability (4)</td>
<td>Tremor (3)</td>
</tr>
<tr>
<td>Group VI</td>
<td>Irritability (5)</td>
<td>Tremor (4)</td>
</tr>
</tbody>
</table>
Table 6.2

Acute Toxicity study on *Spathodea campanulata*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Death</th>
<th>Dose Difference (mg)</th>
<th>Mean death</th>
<th>Dose Difference X death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>0</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>6</td>
<td>0</td>
<td>200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group IV</td>
<td>6</td>
<td>0</td>
<td>400</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group V</td>
<td>6</td>
<td>0</td>
<td>800</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group VI</td>
<td>6</td>
<td>0</td>
<td>1200</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 6.2.a

Signs and symptoms of *Spathodea campanulata* extract toxicity on rats.

<table>
<thead>
<tr>
<th>Group (Dose)</th>
<th>Signs &amp; symptoms (No of animals)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Irritability (0) Tremor (0) Laboured breathing (0) Staggering (0) Convulsion (0) Death (0)</td>
<td>Normal</td>
</tr>
<tr>
<td>Group II</td>
<td>Irritability (0) Tremor (0) Laboured breathing (0) Staggering (0) Convulsion (0) Death (0)</td>
<td>Good/Normal activities seen</td>
</tr>
<tr>
<td>Group III</td>
<td>Irritability (0) Tremor (0) Laboured breathing (0) Staggering (0) Convulsion (0) Death (0)</td>
<td>Good/Normal activities seen</td>
</tr>
<tr>
<td>Group IV</td>
<td>Irritability (1) Tremor (0) Laboured breathing (1) Staggering (1) Convulsion (0) Death (0)</td>
<td>Good/Normal activities seen</td>
</tr>
<tr>
<td>Group V</td>
<td>Irritability (2) Tremor (2) Laboured breathing (3) Staggering (3) Convulsion (3) Death (0)</td>
<td>Good / Normal activities seen</td>
</tr>
<tr>
<td>Group VI</td>
<td>Irritability (2) Tremor (2) Laboured breathing (3) Staggering (3) Convulsion (3) Death (0)</td>
<td>Poor / Normal activities seen</td>
</tr>
</tbody>
</table>
Antimicrobial and anti-inflammatory activity of *S. campanulata* and *D. elata*

Evaluation of Analgesic activity of methanol and aqueous extracts of *Delonix elata* by Acetic acid induced Writhing method

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Mean No. of Wriths (in 10 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 % v/v</td>
<td>23.53 ± 0.017</td>
</tr>
<tr>
<td>II</td>
<td>5 mg/kg</td>
<td>4.55 ± 0.036**</td>
</tr>
<tr>
<td>III</td>
<td>200 mg/kg</td>
<td>7.26 ± 0.047**</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg</td>
<td>8.18 ± 0.055**</td>
</tr>
</tbody>
</table>

P values: **P<0.01; *P<0.05.
Values are expressed in mean ±SEM, n=5 animals in each group.
One way ANOVA followed by DUNNETT’S, multiple comparison tests

**Group I:** Normal control (Acetic acid)

**Group II:** Standard (Pentazocine) + Acetic acid

**Group III:** Rats received *Delonix elata* Methanol extract (200 mg/kg bw)

**Group IV:** Rats received *Delonix elata* Aqueous extract (200 mg/kg bw)
Fig. 6.1
Evaluation of Analgesic activity of methanol and aqueous extracts of *Delonix elata* by Acetic acid induced Writhing method
Table 6.4
Evaluation of Analgesic activity of methanol and aqueous extracts of Spathodea campanulata Acetic acid induced Writhing method

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Mean No. of Wriths (in 10 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 % v/v</td>
<td>25.24 ± 0.07</td>
</tr>
<tr>
<td>II</td>
<td>5 mg/kg</td>
<td>4.74 ± 0.026**</td>
</tr>
<tr>
<td>III</td>
<td>200 mg/kg</td>
<td>7.32 ± 0.086**</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg</td>
<td>8.47 ± 0.056**</td>
</tr>
</tbody>
</table>

P values: * * P< 0.01; * P <0.05.
Values are expressed in mean ±SEM, n=5 animals in each group.
One way ANOVA followed by DUNNETT’S, multiple comparison tests

**Group I**: Normal control (Acetic acid)

**Group II**: Standard (Pentazocine) + Acetic acid

**Group III**: Rats received Spathodea campanulata Methanol extract (200 mg/kg bw)

**Group IV**: Rats received Spathodea campanulata Aqueous extract (200 mg/kg bw)
Fig. 6.2
Evaluation of Analgesic activity of methanol and aqueous extracts of *Spathodea campanulata* by Acetic acid induced Writhing method
Table 6.5
Evaluation of Analgesic activity of methanol and aqueous extracts of *Delonix elata* by Hot plate method

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Reaction time after administration of drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>I</td>
<td>1 % v/v</td>
<td>5.36±0.55</td>
</tr>
<tr>
<td>II</td>
<td>5 mg/kg</td>
<td>9.3±0.25 *</td>
</tr>
<tr>
<td>III</td>
<td>200 mg/kg</td>
<td>8.16±0.05 *</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg</td>
<td>7.63±0.30 **</td>
</tr>
</tbody>
</table>

P values: * * P<0.01; * P <0.05.
Values are expressed in mean ±SEM, n=5 animals in each group.
One way ANOVA followed by DUNNETT’S, multiple comparison tests

**Group I:** Normal control (Acetic acid)

**Group II:** Standard (Pentazocine) + Acetic acid

**Group III:** Rats received *Delonix elata* Methanol extract (200 mg/kg bw)

**Group IV:** Rats received *Delonix elata* Aqueous extract (200 mg/kg bw)
Fig. 6.3
Evaluation of Analgesic activity of methanol and aqueous extracts of *Delonix elata* by Hot plate method

Antimicrobial and anti-inflammatory activity of *S. campanulata* and *D. elata*

125
Table 6.6
Evaluation of Analgesic activity of methanol and aqueous extracts of *Spathodea campanulata* by Hot plate method

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Reaction time after administration of drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>I</td>
<td>1 % v/v</td>
<td>5.156 ± 0.52</td>
</tr>
<tr>
<td>II</td>
<td>5 mg/kg</td>
<td>9.1 ± 0.18*</td>
</tr>
<tr>
<td>III</td>
<td>200 mg/kg</td>
<td>8.06 ± 0.01*</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg</td>
<td>7.23 ± 0.30**</td>
</tr>
</tbody>
</table>

P values: * * P< 0.01; * P <0.05.
Values are expressed in mean ±SEM, n=5 animals in each group.
One way ANOVA followed by DUNNETT’S, multiple comparison tests

**Group I:** Normal control (Acetic acid)

**Group II:** Standard (Pentazocine) + Acetic acid

**Group III:** Rats received *Delonix elata* Methanol extract (200 mg/kg bw)

**Group IV:** Rats received *Delonix elata* Aqueous extract (200 mg/kg bw)
Fig. 6.4

Evaluation of Analgesic activity of methanol and aqueous extracts of Spathodea campanulata by Hot plate method
**Anti-inflammatory activity**

During the search for anti-inflammatory efficacy of selected extracts of the plant using Carrageenan induced rat paw edema method, it was quite evident that, a gradual increase in paw volume was observed after carrageenan administration and which reached maximum time period and then declined. The standard drug Indomethacin at a dose level of 4mg/kg body weight showed highly significant activity (P<0.01) as compared to control group at 30, 60, 120 and 180 min.

Methanolic extracts of both the plants at a dose of 200mg/kg showed highly significant anti-inflammatory activity (P<0.01) as compared to control group at 30, 60, 120 and 180 min respectively. The aqueous extracts also showed significant activity (P<0.01) at 30, 60, 120 and 180 min (Tables 6.7 & 6.8 and Fig 6.5 & 6.6). The standard drug Indomethacin at a dose of 4mg/kg body weight inhibited the development of edema significantly from 30 min onwards. It showed maximum percentage reduction in paw edema at 180 min. Methanolic extract of *S.campanulata* at the dose of 200mg/kg body weight showed percentage of inhibition of paw edema at 180 min 0.593% (Table 6.8).
Table 6.7
Evaluation of Anti-inflammatory activity of methanol and aqueous extracts of *Delonix elata* (% of paw edema volume)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Paw edema volume in ml as measured by mercury displacement at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>I</td>
<td>0.1 ml</td>
<td>0.529 ± 0.004</td>
</tr>
<tr>
<td>II</td>
<td>10 mg/kg</td>
<td>0.426 ± 0.004</td>
</tr>
<tr>
<td>III</td>
<td>200 mg/kg</td>
<td>0.424 ± 0.003</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg</td>
<td>0.391 ± 0.007</td>
</tr>
</tbody>
</table>

P values: * * P< 0.01; * P < 0.05.
Values are expressed in mean ±SEM, n=5 animals in each group.
One way ANOVA followed by DUNNETT’S, multiple comparison tests

**Group I:** Normal control (Carrageenan)

**Group II:** Standard (Indomethacin) + Carrageenan

**Group III:** Rats received *Delonix elata* Methanol extract (200 mg/kg bw)

**Group IV:** Rats received *Delonix elata* Aqueous extract (200 mg/kg bw)
Fig. 6.5

Evaluation of Anti-inflammatory activity of methanol and aqueous extracts of *Delonix elata* (% of paw-edema volume)

Antimicrobial and anti-inflammatory activity of *S.campanulata* and *D.elata*
### Table 6.8
Evaluation of Anti-inflammatory activity of methanol and aqueous extracts of *Spathodea campanulata*
(% of paw edema volume)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Paw edema volume in ml as measured by mercury displacement at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>I</td>
<td>0.1 ml</td>
<td>0.531 ± 0.004</td>
</tr>
<tr>
<td>II</td>
<td>10 mg/kg</td>
<td>0.427 ± 0.005</td>
</tr>
<tr>
<td>III</td>
<td>200 mg/kg</td>
<td>0.432 ± 0.005</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg</td>
<td>0.399 ± 0.003</td>
</tr>
</tbody>
</table>

P values: **P< 0.01; * P < 0.05.
Values are expressed in mean ±SEM, n=5 animals in each group.
One way ANOVA followed by DUNNETT’S, multiple comparison tests

**Group I:** Normal control (Carrageenan)

**Group II:** Standard (Indomethacin) + Carrageenan

**Group III:** Rats received *Spathodea campanulata* Methanol extract (200 mg/kg bw)

**Group IV:** Rats received *Spathodea campanulata* Aqueous extract (200 mg/kg bw)
Fig. 6.6
Evaluation of Anti-inflammatory activity of methanol and aqueous extracts of *Spathodea campanulata* (% of paw-edema volume)
DISCUSSION

During inflammation, lysosomal hydrolytic enzymes are released into the sites which cause damages of the surrounding organelles and tissues with attendance variety of disorders (Sadique et al., 1989). Various methods were employed to screen and study drugs, chemicals, herbal preparations that exhibit anti-inflammatory properties or potentials. The attention of pharmacologist throughout the world has been focused on finding out safer and potent anti-inflammatory drug. The natural products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to humans and environment.

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 not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility (Winter et al., 1962). Carrageenan induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peek at 3h (Vinegar et al., 1969). It has been reported that the second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin.

Indomethacin is a cyclooxygenase inhibitor, the ethanol extract has activity which is compareable to indomethacin and can be said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan induced paw edema, so inhibition of
carrageenan induced paw edema by the crude extract could also be due to its inhibitory activity on the lipoxygenase enzyme.

Methanolic extract of *Spathodea campanulata* and *Delonix elata* showed a maximum percentage of edema inhibition at 180 min at the dose of 200 mg kg bw. This result indicated that extract with a dose of 200 mg kg body weight showed a maximum anti-inflammatory activity as compared to the reference drug Indomethacin. Intraperitoneal injection of carrageenan leads to inflammation of the peritoneum resulting from carrageenan induced release of interleukin-1 from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory cytokine, induce accumulation of polymorpho nuclear cells by a variety of processes including adhesion and cell mobility (Meade *et al.*, 1986). Leukocyte aggregation is a fundamental event during inflammation. Cell migration occurs as a result of much different process including adhesion and cell mobility.

Since, antinociceptive and/or anti-inflammatory activity of many plants has been attributed to their flavonoids (Pathak *et al.*, 1991; Datta *et al.*, 2004), tannins (Viana *et al.*, 1998), triterpenes (Ahmad *et al.*, 1983; Datta *et al.*, 2004) and coumarins. It is therefore, possible that the antinociceptive and anti-inflammatory effects observed with both plant extracts in the present study may be attributed to the components that are present in abundance in the extracts.

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs (Hasan *et al.*, 2010). The crude extracts of
both the plants showed significant analgesic action compared to the reference drug pentazocine but crude extracts of *D.elata* was found to exhibit higher analgesic activity than *S.campanulata* against acetic acid induced pain in rats at a dose 200 mg/kg b. wt.

Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid (Ahmed *et al*., 2006) via cyclooxygenase (COX), and prostaglandin biosynthesis (Duarte *et al*., 1988). In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products (Derardt *et al*., 1980). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria *et al*., 2008). The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Duarte *et al*., 1988; Ferdous *et al*., 2008).

The significant pain reduction of both the plant extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways. It was found that the observed analgesia was demonstrated by the active constituents, Glutionl, a triterpene (Freire *et al*., 1991, 1993) and Scoparinol, a diterpene (Ahmed *et al*., 2001) isolated from the various plants through a peripherally acting mechanism similar to the non-steroidal anti-inflammatory agents, such as indomethacin and pentazocine. The abdominal writhing induced by acetic acid
was also reported to be less selective (Collier et al., 1968) and proposed to act indirectly by releasing endogenous mediators stimulating neurons that are sensitive to other drugs such as narcotics and centrally acting agents (Toma et al., 2003).

The extracts of the plants and pentazocine also presented a longer latency time than the control group in the hot plate test in a dose related manner. At 120 minutes & 200 mg/kg, administration of the plant extracts the percent inhibition was found 13.6% & 13.1% for *Spathodea campanulata* and *Delonix elata* respectively. The hot plat method is considered to be selective for the drugs acting centrally. The hot plat test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity (Sabina et al., 2009). It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally (Ibironke and Ajiboye, 2007). Therefore, the crude extracts of both the plants must have a central activity. Again, narcotic analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain (Elisabetsky et al., 1995; Pal et al., 1999). The plant extracts of *S. campanulata* and *D. elata* exhibited both types of pain inhibition. The analgesic effect of the plants in both models suggests that they have been acting through central and peripheral mechanism (Sabina et al., 2009).