8.1. Introduction

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g. bacterial infection, physical trauma or chemicals) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair (Nathan, 2002) and it is triggered by various mechanical, chemical or immunological stress factors. Inflammation start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger (Huwiler and Pfeilschifter, 2009). Further, it was described as “the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality” or “the reaction to injury of the living microcirculation and related tissues” (Lucas et al., 2006). Although, in ancient times inflammation was recognised as being part of the healing process, up to the end of the 19th century, inflammation was viewed as being an undesirable response that was harmful to the host. Based on visual observation, the ancients characterised inflammation by five cardinal signs, namely redness, swelling, heat, pain and loss of function. The first four of these signs named by Celsus in ancient Rome (30-38 B.C.) and the last by Galen (A.D. 130-200) (Hurley, 1972).

Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury (Vodovotz et al., 2008). The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection (El-Gamal et al., 2010). However, in appropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease (Franklin et al., 2008). Inflammation is a major component of the damage caused by autoimmune diseases and is a fundamental
contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, Alzheimer’s and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever (Kassuya et al., 2009).

Inflammation is mediated by a variety of signalling molecules produced by leucocytes, macrophages and mast cells undergoing various cellular responses such as phagocytic uptake and the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2) and tumour necrosis factor (TNF)-α (Yu et al., 2010) that bring about edema (Kinne et al., 2000).

Inflammatory process has two phases: acute and chronic. Acute and chronic inflammations are known to be complicated processes induced by several different classes of chemical mediators, e.g. prostaglandins, leukotrienes and platelet-activating factor, etc. (Samuelsson et al., 1978). Acute inflammatory response is characterized by an increase in vascular permeability and cellular infiltration leading to oedema as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site for short time (Posadas et al., 2004). Chronic inflammation is the reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents.

8.1.1. Analgesia

Analgesic therapy is domain by two major classes of analgesic drugs; *viz.*, opioids and non steroidal anti-inflammatory drugs (NSAIDs). Both classes of analgesic drugs produce serious side effects, such as gastrointestinal disturbance, renal damage (Dahl and Reader, 2000; Bures et al., 2002).
8.1.2. Inflammatory diseases

Number of degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma, and inflammatory bowel disease are often associated with inflammatory processes (Polya, 2003; Iwalewa et al., 2007). Furthermore, oxidative and inflammatory processes are among the pathological features associated with the central nervous system in Alzheimer’s disease (Howes and Houghton, 2003). There is convincing evidence that cytokines, prostaglandins and nitric oxide play critical roles in the development and perpetuation of inflammation and cartilage and meniscus damage in rheumatoid arthritis and osteoarthritis. There is a growing body of evidences that chronic inflammation is strongly associated with incidence of cancer. For example, colon cancer can arise from inflammatory bowel disease such as chronic ulcerative colitis and Crohn’s disease persistent more than 10 years.

8.1.3. Commercial drugs and their side effects

Steroids, specifically gluco-corticoids and mineral ocorticoids reduce inflammation or swelling by binding to corticoid receptors. These drugs are often referred to as corticosteroids. Long-term use has several side effects e.g. hyperglycemia, insulin resistance, diabetes mellitus, osteoporosis and anxiety effects (Donihi et al., 2006). NSAIDs can be divided into different groups based on their chemical structure, pharmacokinetics and selectivity towards COX-1 or COX-2 (Bancos et al., 2009). There are three major types of NSAIDs action, 1). Anti-inflammatory action for treating several conditions including rheumatoid arthritis, osteoarthritis, musculoskeletal disorders and pericarditis. 2) Analgesia for treating pain of mild to moderate intensity. Their maximum therapeutic efficiency is much lower than that of the opioids, but they do not cause dependence.
3) Antipyretic action, which mediates by the release of endogenous pyrogen from monocytes and macrophages in the presence of infection or inflammation.

8.2. MATERIALS AND METHODS

8.2.2. Preparation of Extract

The lyophilised powder of *Kalinga ornata* 500 mg was mixed with methanol and macerated for 7 days. During maceration the whole content were warmed two times a day at an interval of six hours. At the end of 7th day the extract were filtered through muslin cloth while hot and the extract were concentrated to a semisolid mass and dried in a desiccator. Yield 5mg of extract was used for the below experimental purposes.

8.2.3. Chemical and Drugs

The following standard chemicals and drugs were used for the present investigation Methanol, carrageenan, sodium chloride, Indomethacin and infant (oral) feeding tube were purchased from Sigma Al-rich, Mumbai, India.

8.2.4. Acute toxicity

The acute toxicity test was carried out for MEOH-KO to evaluate any possible toxicity. Swiss mice (n = 6) of either sex were treated with different doses (500, 1000 and 2000mg/kg, p.o.), while the control group received saline (10ml/kg). All the groups were observed for any gross effect for first 4h and then mortality was observed after 24hrs.

8.2.5. Experimental Animals

Swiss albino mice (18-25g) and Wistar albino rats (150-200g) were housed in well ventilated rooms (23±2°C; humidity 65-70% and 10h light/dark cycle) at Central Animal House (RMMCH), Annamalai University. The animals fed with standard pellet diet and water *ad libitum*. All studies were carried out in accordance with
Institutional Animal Ethical Committee, Rajah Sir Muthiah Medical College and hospital (Reg No./160/1999/CPCSEA, Prop. No: 1049), Annamalai University, Tamil Nadu, India.

8.2.6. Experimental Groups

Animals were divided into four groups, consisting of six animals each.

Group1: Normal rats + (1% saline solution of 0.1ml)

Group2: Inflammatory rats + MeOH-KO (200 mg/kg)

Group 3: Inflammatory rats + MeOH-KO (400 mg/kg)

Group 4: Inflammatory rats + Indomethacin (20mg/kg SC)

8.2.7. Carrageenan Induced Paw Oedema

Carrageenan was used to induce paw oedema in Wistar albino rats. For this, the animals were weighed and numbered. A mark was made on both hind paws (right and left) just beyond tibio-dorsal junction, so that every time paw was dipped into the mercury column up to fixed mark to ensure constant paw volume. The initial paw volume (both right and left) was noted for each rat by mercury displacement method. The animals were divided into four groups each comprising at least 6 rats. The 1\textsuperscript{st} group was administered with normal saline, 2\textsuperscript{nd} and 3\textsuperscript{rd} group was treated with test dosages of 200, 400 mg/kg respectively. Fourth group was administered indomethacin subcutaneously. After 30 min, 0.1 ml of 1 \% (w/v) carrageenan was injected in the planter region of the left paw served as reference non inflamed paw for comparison. Then paw volume was noted for both legs of all the group of animal treated at 15, 30, 60 and 120 min after carrageenan challenge. The percent of difference was calculated in the right and left paw volume of each animal of control, test and indomethacin treated group. The mean was compared with percent changes in
paw volume in control and drug treated animals and express as percent oedema inhibition drug.

\[
\frac{V_c - V_t}{V_c} \times 100
\]

Where,

\(V_t\) is the inflammatory increase in paw volume in drug treated rats

\(V_C\) is the inflammatory increase in paw volume in control group of rats.

Percentage inhibition of edema is proportional to anti-inflammatory activities.

8.2.8. Analgesic Activity

8.2.7.1. Acetic Acid Induced Writhing Test

Swiss mice of either sex (n=6) weighing 18–22 g were used. All animals were withdrawn from food 2 hrs before the beginning of experiment and were divided in four groups. Group I was injected with normal saline (10 ml/kg) as control, group II received standard drug diclofenac sodium (10 mg/kg) while the remaining groups III and IV were injected with 200 and 400 mg/kg i.p. of MeOH-KO respectively. After 30 min of saline, diclofenac sodium and plant extract injection, the animals were treated i.p, with 1% acetic acid. The numbers of abdominal constrictions (writhes) were counted after 5 min of acetic acid injection for the period of 10 min (Khan et al., 2010).

8.2.7.2. Hot Plate Test

Mice of either sex (n=6) weighing 18–22 g were acclimatized to laboratory conditions one hour before the start of experiment with food and water available ad libitum. Animals were then subjected to pre-testing on hot plate maintained at 55±0.1°C. Animals having latency time greater than 15sec on hot plate during pre-testing were rejected (latency time) (Brochet et al., 1986). All the animals were
Pharmacological Property

divided into four groups each of six mice. Group I was treated with saline (10ml/kg),
group II was treated with Pentazocine (10mg/kg i.p), Group III and IV were treated
with 200 and 400mg/kg MeOH-KO, i.p. respectively. After 30min of treatment the
animals were placed on hot plate and the latency time (time for which mouse remains
on the hot plate (55±0.1°C) without licking or flicking of hind limb or jumping) was
measured in seconds. The latency time for all groups was recorded at 0, 30, 60, 90 and
120min. Percent analgesia was calculated using the following formula,

\[
\% \text{ Analgesia} = \frac{(\text{Test latency} - \text{control latency})}{(\text{Cut-off time} - \text{control latency})} \times 100
\]

8.2.7.3. Tail Immersion Test

Mice of either sex were divided into four groups each of six animals (18–22g).
Saline (10ml/kg), MeOH-KO at the dose of 200 and 400mg/kg, and Pentazocine
(30mg/kg) were administered intraperitoneally. The animal was kept in vertical
position to hang the tail, which was up to 5cm into a pot of hot water maintained at
55±0.5°C. The time in seconds to withdraw the tail out of water was taken as the
reaction time (Ta). The reading was taken after 0, 30, 60, 90 and 120min of
administration of the test drugs (Singh et al., 1996). The cut-off time, i.e. time of no
response was put at 30s, while Tb was consider the reaction time for control group.

Percentage analgesic activity =Ta – Tb/Tb \times 100

8.2.7.4. Tail-Flick Test

Analgesic response was assessed with a tail-flick apparatus using a method
initially described by D'Amour and Smith (1941). The animals were gently
immobilised using a glove, and the radiant heat was focused on a blackened spot
1–2 cm from the tip of the tail. Beam intensity was adjusted to give a tail flick latency
of 5–8 sec in control animals. Measurement was terminated if the latency exceeded the
end time (15 sec), to avoid tissue damage. In all experiments, mice were tested twice at each time point. The test was performed 30 min before drug administration, which served as the baseline latency, 30, 90 and 150 min after drug administration, Acetylsalicylic acid (300 mg/kg, p.o.) was used as standard (Parimala Devi et al., 2003). The data derived from all groups were standardized by using the following formula for statistical analysis:

\[
\% \text{Antinociceptive activity} \triangleq \frac{100 \times [\delta n - n_i]}{n_i}
\]

Where,

- \(n\) represents tail-flick results at the 30th, 90th and 150th min
- \(n_i\) represents tail-flick results before drug administration.

8.2.7.5. Antipyretic Test

The antipyretic activity was evaluated for MeOH-KO using mice (25–30g) of either sex. The selected animals were healthy and were acclimatized to laboratory conditions before the beginning of experiment. The animals were divided into four groups each of six mice. The normal body temperature of each mouse was recorded using digital thermometer and then pyrexia was induced in all mice by injecting 20% aqueous suspension of Brewer’s yeast (10 ml/kg, s.c.). All groups were fasted overnight but allowed free accesses to drinking water and after 24h rectal temperature of each mouse was recorded.

The induction of pyrexia was confirmed by rise in temperature more than 0.5°C, while animals showed rise in temperature less than 0.5°C were excluded from experiment (Kang et al., 2008). Group I received saline (10 ml/kg) as a negative control, group II received paracetamol (150 mg/kg) as a standard drug while the remaining groups III and IV received 200 and 400 mg/kg i.p. MeOH-KO respectively. After drugs administration, rectal temperature was again recorded periodically at
1, 2, 3, 4 and 5h of drugs administration. The percent reduction in pyrexia was calculated by the following formula.

\[
\text{Percent reduction} = \frac{B - Cn/B - A}{100}
\]

Where,

\(B\) represents temperature after pyrexia induction

\(Cn\) - Temperature after 1, 2, 3, 4 and 5h

\(A\) - Normal body temperature.

8.2.7.6. Statistical Analysis

The results obtained were expressed as mean ± SEM of six animals. For statistical analysis, ANOVA was followed by post hoc Dunnett’s test for multiple comparisons. Effects were considered to be significant at the \(P<0.05\) level.

8.3. RESULTS

8.3.2. Acute toxicity

The methanolic extract of *Kalinga ornata* was found safe at all test doses (500, 1000 and 2000 mg/kg i.p). During 24h assessment time, test animals were found normal.

8.3.3. Anti-inflammatory

In the present study, anti-inflammatory activity of methanolic extract of MeOH-KO was evaluated. Anti-inflammatory activity was tested by different *in vivo* screening models, represents different phases of inflammation. Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation study and is believed to be biphasic. The results obtained from the present study are exhibited in the Table 1. The right paw was always maintained as control in all the groups, which was showing a steady increase with increase of time in all the cases. There was no significant changes in the right paw volume between the
groups (p<0.05). On the other hand the left paw which was treated one showing significant changes in volume between the groups (p<0.05). The left paw of control animals (saline treated) was not showing any decrease in the paw volume compared to their right paw. The maximum activity was observed in the rats treated with the commercial drug indomethacin in 30 and 60min after the induction. *Kalinga ornata* was also showing the maximum activity in both 200mg/kg and 400mg/kg of dose. Among this the maximum was observed in higher dosage (400mg/kg) which was on par with the commercial drug indomethacin.

The percentage of the paw volume of different experimental groups were presented in the Table 1. The maximum paw volume was observed in 30min of treatment, which decreased 60% of the paw volume in the commercial drugs. Among the treated groups, the maximum decrease of 50% was noticed in both the dosages. 200mg/kg of *Kalinga ornata* dosage attained the maximum activity at 30min but 400mg/kg of *Kalinga ornata* attained the peak at 60min, but the same affect persisted even after 120min. Whereas there was a slight decrease in the activity at 120min, in case of 200 mg/kg dose of *Kalinga ornata*. The control group was always showing an increase in the paw volume during every time intervals. Therefore, our results confirmed that the mechanism of the anti-inflammatory effect of *Kalinga ornata* involved in the reduction of prostaglandins through inhibition of cyclooxygenase.

### 8.3.4. ANALGESIC ACTIVITY

#### 8.3.4.1. Acetic acid induced test

The results showed that the pain relief was achieved in a dose dependent manner, at all test doses (200 and 400mg/kg i.p.) as shown in Table. Maximum inhibition (78.90%) was observed at 400mg/kg dose of MeOH-KO. The inhibitory
Pharmacological Property

effect of paracetamol (96.22%) was greater than that of the highest dose of MeOH-KO.

8.3.4.2. Hot plate test

The results of the hot plat test revealed that the latency time was significantly (P<0.05) increased from 17.22% to 68.58% at the dose of 200 to 400mg/kg. The effect was dose dependent and the maximum effect was observed after 60min as shown in Table. The most significant (P<0.01) increase in latency time noticed against 400mg/kg of MeOH-KO was 68.58%. Whereas, the percent inhibition of the standard drug Pentazocine was 76.73%. In the presence of naloxone, the analgesic effect of Pentazocine (30mg/kg) and MeOH-KO (200 and 400mg/kg) was reversed profoundly.

8.3.4.3. Tail immersion test

The analgesic effect of the MeOH-KO was also significant (P<0.05) in tail immersion test and was dose dependent like hot plat test. The reaction time of all doses and Pentazocine is given in Table. The maximum analgesic effect was noticed at 60min after the dose administration. The percent inhibition of pain was 22.29 and 68.58 at 200 and 400mg/kg of MeOH-KO respectively.

8.3.3.4. Antipyretic Test

The MeOH-KO markedly (P<0.01) attenuated hyperthermia induced by yeast. The inhibition was dose dependent and remained significant up to 3h of administration as shown in Table. The maximum antipyretic effect was observed at 400mg/kg i.e. 78.23% while, the paracetamol was 90%.
Table 14. Effect of MeOH-KO on carrageenan induced albino rats in different time intervals

<table>
<thead>
<tr>
<th>Group</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.01±0.006a</td>
<td>0.11±0.03b</td>
<td>0.20±0.08c</td>
<td>0.20±0.08c</td>
<td>0.19±0.07c</td>
</tr>
<tr>
<td>Carrageenan+ 200mg/kg MeOH-KO</td>
<td>0.01±0.006a</td>
<td>0.09±0.03b</td>
<td>0.11±0.06b</td>
<td>0.12±0.06b</td>
<td>0.11±0.06b</td>
</tr>
<tr>
<td>Carrageenan+ 400mg/kg of MeOH-KO</td>
<td>0.01±0.007b</td>
<td>0.06±0.02b</td>
<td>0.11±0.04b</td>
<td>0.12±0.04b</td>
<td>0.10±0.04b</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.01±0.007b</td>
<td>0.05±0.01ab</td>
<td>0.09±0.03b</td>
<td>0.11±0.04b</td>
<td>0.10±0.03b</td>
</tr>
</tbody>
</table>

Values are mean ± SE of six replicates. Values showing different superscript varies significantly at p<0.05.

Table 15. Percentage increase or decrease in left paw volume compared with right of treated albino rats during different time intervals

<table>
<thead>
<tr>
<th>Group</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>+4.9</td>
<td>+4.6</td>
<td>+5.6</td>
</tr>
<tr>
<td>Carrageenan+ 200mg/kg MeOH-KO</td>
<td>0</td>
<td>-26</td>
<td>-52</td>
<td>-52</td>
<td>-49</td>
</tr>
<tr>
<td>Carrageenan+ 400mg/kg MeOH-KO</td>
<td>0</td>
<td>-38</td>
<td>-49</td>
<td>-52</td>
<td>-52</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0</td>
<td>-52</td>
<td>-62</td>
<td>-58</td>
<td>-54</td>
</tr>
</tbody>
</table>

Table 16. Effect of MeOH-KO 200 and 400mg/Kg in Acetic Acid Induced Test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>No. of Writhing in 10min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10ml</td>
<td>64.80±2.68</td>
</tr>
<tr>
<td>MeOH-KO</td>
<td>200 mg/kg</td>
<td>28.00±1.50**</td>
</tr>
<tr>
<td>MeOH-KO</td>
<td>400 mg/kg</td>
<td>20.00±1.14**</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10 mg/kg</td>
<td>10.40±1.36**</td>
</tr>
</tbody>
</table>

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett’s test. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01
### Table 17. Effect of MeOH-KO at 200 and 400 mg/Kg in Hot Plate Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/kg</th>
<th>0min</th>
<th>30min</th>
<th>60min</th>
<th>90min</th>
<th>120min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10ml</td>
<td>9.20 ± 0.02</td>
<td>9.22 ± 0.08</td>
<td>9.16 ± 0.09</td>
<td>9.20 ± 0.03</td>
<td>9.12 ± 0.11</td>
</tr>
<tr>
<td>MeOH-KO</td>
<td>200mg</td>
<td>9.23 ± 0.65</td>
<td>12.85 ± 0.87</td>
<td>14.51** ± 0.54</td>
<td>14.30** ± 0.84</td>
<td>14.09** ± 0.91</td>
</tr>
<tr>
<td>MeOH-KO</td>
<td>400mg</td>
<td>9.24 ± 0.76</td>
<td>19.76* ± 0.22</td>
<td>23.74** ± 0.12</td>
<td>23.10** ± 0.12</td>
<td>22.98** ± 0.69</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>10mg</td>
<td>9.20 ± 0.02</td>
<td>25.34** ± 0.04</td>
<td>25.88*** ± 0.06</td>
<td>25.80*** ± 0.07</td>
<td>25.77*** ± 0.00</td>
</tr>
</tbody>
</table>

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett’s test.

Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01, ***)P<0.001.

### Table 18. Effect Of MeOH-KO at 200 and 400 mg/kg in Tail Immersion Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/kg</th>
<th>0min</th>
<th>30min</th>
<th>60min</th>
<th>90min</th>
<th>120min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10ml</td>
<td>3.22 ± 0.02</td>
<td>3.23 ± 0.04</td>
<td>3.31 ± 0.04</td>
<td>3.28 ± 0.10</td>
<td>3.25 ± 0.12</td>
</tr>
<tr>
<td>MeOH-KO</td>
<td>200mg</td>
<td>3.22 ± 0.28</td>
<td>3.58* ± 0.18</td>
<td>3.95** ± 0.23</td>
<td>3.91** ± 0.48</td>
<td>3.85** ± 0.97</td>
</tr>
<tr>
<td>MeOH-KO</td>
<td>400mg</td>
<td>3.25 ± 0.27</td>
<td>4.82* ± 0.22</td>
<td>5.58** ± 0.72</td>
<td>5.49 ** ± 0.76</td>
<td>5.41** ± 0.27</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>10mg</td>
<td>3.20 ± 0.01</td>
<td>5.60*** ± 0.03</td>
<td>5.85*** ± 0.03</td>
<td>5.79*** ± 0.08</td>
<td>5.71*** ± 0.00</td>
</tr>
</tbody>
</table>

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett’s test.

Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01, ***)P<0.001.
Table 19. Antipyretic Effect of MeOH-KO at 200 and 400 mg/kg I.P. in Yeast Induced Pyrexia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Rectal Temperature (After administration of drug)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>10ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-KO</td>
<td>200</td>
<td>37.0 ± 0.14</td>
</tr>
<tr>
<td>-KO</td>
<td>400</td>
<td>37.0 ± 0.12</td>
</tr>
<tr>
<td>tamol</td>
<td>150</td>
<td>37.0 ± 0.08</td>
</tr>
</tbody>
</table>

Values are triplicate as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnet’s test.

Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01.
8.1. DISCUSSION

Results of the present study showed that the MeOH-KO has marked antipyretic, analgesic and anti-inflammatory effects with a reasonable safety profile. Subcutaneous injection of Brewer’s yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plant materials as well as synthetic drugs for their antipyretic effect (Devi et al., 2003).

Carrageenan-induced paw edema is a well established animal model to assess the anti-inflammatory effect of natural products as well as synthetic chemical compounds. Edema formation due to carrageenan in paw is a biphasic event, during 1–5h; the initial phase (1h or 1.5h) is predominately a non-phagocytic edema followed by a second phase (2–5) h with increased edema formation that remained up to 5h (Mbiantcha et al., 2011).

Acetic acid-induced writhing is a well recommended protocol in evaluating medicinal agents for their analgesic property. The pain induction caused by liberating endogenous substances as well as some other pain mediators such as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis. This pain paradigm is widely used for the assessment of peripheral analgesic activity due to its sensitivity and response to the compounds at a dose which is not effective in other methods. The local peritoneal receptor could be the cause of abdominal writhings (Mbiantcha et al., 2011). Pain sensation in acetic acid induced writhing paradigm is elicited by producing localized inflammatory response due to release of free arachidonic acid from tissue phospholipids via cyclo-oxygenase (COX), and producing prostaglandin specifically PGE2 and PGF2α, the level of lipoxygenase products may also increases in peritoneal fluids. These prostaglandin and lipoxygenase products cause
inflammation and pain by increasing capillary permeability. The substance inhibiting the writhings will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Duarte et al., 1988). Regarding the results of our extract in acetic acid-induced abdominal constriction assay, a prominent inhibition of writhing reflux was observed. These findings strongly recommend that MeOH-KO has peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors which may be the involvement of cyclooxygenase inhibition potential. The profound analgesic activity of MeOH-KO may be due to the interference of their active principles with the release of pain mediators.

Thermal nociception models such as hot plate and the tail immersion tests were used to evaluate central analgesic activity. MeOH-KO showed significant (P<0.01) analgesic effect in both the hot plate and tail immersion tests, implicating both spinal and supraspinal analgesic pathways. In contrast, MeOH-KO showed maximum analgesic effect after 60min of administration. This difference in the maximum analgesic point could be explained by difference in the metabolic rate of each drug or may be the potency of each drug as the analgesic potential of Pentazocine is higher than MeOH-KO (400mg/kg). Moreover, MeOH-KO showed a maximum effect after 60min and remains upto 120min in both thermal tests.

Yeast induced pyrexia its etiology could be the production of prostaglandins (Moltz, 1993). The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia and the inhibition of these mediators is responsible for the antipyretic effect (Rawlins, 1973). The intraperitoneal
administration of MeOH-KO significantly attenuated rectal temperature of yeast induced febrile mice. Thus it can be postulated that MeOH-KO contained pharmacologically active principle(s) that interfere with the release of prostaglandins.

During secondary metabolites screening of the crude extract, important therapeutic principles like alkaloids, saponins, flavonoids, tannins etc. were detected. Therefore, the current findings can be attributed to these groups of chemical compounds. Further study is need on K. ornata to find the exact mechanism of action for its antipyretic, analgesic and anti-inflammatory effects.

In conclusion, the MeOH-KO was proved a natural safe remedy for the treatment of pyrexia, analgesia and inflammation. This study demonstrated scientific rationale for the folk use of the plant as antipyretic, analgesic and anti-inflammatory. Interestingly the Kalinga ornata exhibited both peripheral as well as central analgesic effect which might have been attributed to the presence of such active principles, due to which it has proven folk use in various nervous disorders. Nevertheless, the isolation of active metabolites from the Kalinga ornata will help us further in understanding the mechanism of these activities and identification of lead compounds of clinical utility.