The present study investigates the anti-inflammatory and analgesic properties of ethanol extract of leaves of *Commiphora caudata* (Wight & Arn.) Engl. The anti-inflammatory activity was evaluated by carrageenan induced paw oedema in Wistar albino rats and analgesic effect by acetic acid induced writhing assay in Swiss albino mice. Our findings showed that the oral administration of the extract significantly inhibited carrageenan induced paw oedema and acetic acid induced writhing effects and were comparable to standard drugs, Indomethacin and Aspirin, respectively. The extract also showed significant inhibition of FeCl$_2$-ascorbic acid stimulated lipid peroxidation in rat liver homogenate in vitro. It can therefore, be concluded from the present study that the ethanolic extract of *C. caudata* possesses potent anti-inflammatory and analgesic properties.

**Keywords:** Anti-inflammatory, Analgesic, Antioxidant, *Commiphora caudata*, Lipid peroxidation.

**IPC code; Int. cl.** A61K 36/00, A61K 36/328, A61K 127/00, A61P 29/00, A61P 39/06

**Introduction**

Plants are one of the most important sources of medicines. India is known as the “Emporium of Medicinal plants” due to the availability of several thousands of medicinal plants in the different bioclimatic zones. Chronic inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world’s population. Several modern drugs are used to treat these disorders but, their prolonged use may cause severe adverse side effects, the most common being gastrointestinal bleeding and peptic ulcers. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. The use of natural remedies for the treatment of inflammatory and painful conditions has a long history, starting with Ayurvedic treatment, and extending to the European and other systems of traditional medicines. Plant drugs are known to play a vital role in the management of inflammatory diseases.

*Commiphora caudata* (Wight & Arn.) Engl. is a small tree with a thick trunk and papery bark, growing in the Western Ghat regions of Tamil Nadu, Karnataka and Kerala (Fig. 1). Its local names include *Kilimaram*, *Idingil* and *Kizhuvam*. The leaves and bark have the odour of mangoes, and the oleo-gum resin obtained from the tree is used as incense. The fruit, which is of the size of a pea, is used to make pickles. Leaves of the plant are used in traditional and tribal medicine of Kerala to treat inflammation and pain. A perusal of literature revealed that although *C. caudata* is widely used in traditional medicine as an anti-inflammatory and analgesic agent, these properties have not been scientifically evaluated. Therefore, the present study is an attempt to investigate the anti-inflammatory and analgesic properties of the ethanol extract of *C. caudata* leaves in experimental animals.

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Materials and Methods

Plant Material
The leaves of *C. caudata* (Fig. 2) were collected from the Herbal garden of the Institute during April 2007. This was authenticated by Dr Mathew Dan, plant taxonomist of the Institute and the voucher specimen has been preserved for future reference at the Herbarium of the Institute (TBGT 57020/ dated 11/4/2007).

Preparation of the Plant Extract
The leaves were washed thoroughly with tap water, shade-dried and powdered. The powder (100 g) was extracted with ethanol (1000 ml) overnight, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. The yield (w/w) of the crude extract was found to be 12.06 %. The crude extract was dissolved in 0.5 % Tween-80 to required concentrations and used for the experiments. *C. caudata* leaf ethanolic extract was referred to as CC.

Experimental Animals
Wistar albino male rats (150 g) and Swiss albino mice (25-30 g), were grouped and housed in poly acrylic cages (two animals per cage) and maintained under standard laboratory conditions (temperature 24-28°C, relative humidity 60-70% and 12 h light dark cycles). They were fed commercial rat feed (Lipton India Ltd, Mumbai) and boiled water, *ad libitum*. All experiments involving animals were done according to NIH guidelines, after getting the approval of the Institute’s Animal Ethics Committee (No. 25-1/99AWD176/CPCSEA).

Carrageenan-induced Paw Oedema in Rats
Anti-inflammatory activity of CC was assessed by carrageenan induced paw oedema method\(^1\). Rats were divided into 4 groups (6 animals in each group). Animals of all the groups were injected with 0.1 ml of 1% carrageenan in 0.9% normal saline, under the plantar aponeurosis of the right hind paw. Group I animals (carrageenan control) received p.o., 0.5 % of Tween-80, 30 min prior to carrageenan injection. Group II, the standard reference group was given p.o., an aqueous solution of Indomethacin (5mg/kg), 30 min prior to carrageenan injection. Groups III and IV received p.o., 250 and 500 mg/kg of CC, 30 min prior to carrageenan injection. The paw volume of the rats was measured plethysmographically just before and 3 h after carrageenan injection. The percentage inhibition of oedema was calculated for each group with respect to the vehicle treated control group.

Acetic acid-induced Writhing test in Mice
Analgesia of CC was assessed by the writhing test in mice\(^5\). Mice were divided into 4 groups (6 animals in each group). All the groups received i.p., 0.5% aqueous solution of acetic acid (0.25 ml). Group I, acetic acid control group received a single dose of 0.5% Tween-80 (0.5 ml) p.o., 20 min prior to the administration of acetic acid. Group II, the standard control group received p.o., a single dose of Aspirin (acetyl salicylic acid, 25 mg/kg), 20 min prior to administration of acetic acid. Groups III and IV received p.o., a single dose of 250 and 500 mg/kg CC, respectively, 20 min prior to administration of acetic acid. The number of writhes (full extension of hind limb) per animal was recorded during the 20 min period, beginning 5 min after the injection of acetic acid.

In vitro Anti-lipid Peroxidation Studies
The antioxidant effect of CC was studied in vitro following the modified methods of Yoshiyuki *et al*\(^6\) and Masao *et al*\(^7\). The rat liver tissue (2 g) was sliced and homogenated with 150 mM KCL-Tris-HCl buffer (pH 7.2). The reaction mixture (in triplicate) was composed of 0.25 ml liver homogenate, Tris-HCl buffer (pH 7.2), 0.1 mM ascorbic acid (AA), 4 mM FeCl\(_2\) and 0.05 ml of various concentrations of extract (25, 50, 100 µg/ml). The mixture was incubated at 37°C for 1 h in capped tubes. Then, 0.5 ml of 0.1 N HCl, 0.2 ml of 9.8 % sodium dodecyl sulphate (SDS), 0.9 ml of distilled water and 2 ml of 0.6% thiobarbituric acid (TBA) were added to each tube and the tubes were vigorously shaken. Following this, all the tubes were placed in a boiling water bath at 100°C for 30 min. After cooling, the flocculent precipitate was removed by adding 5 ml of n-butanol, mixed well and centrifuged at 1500 rpm for 20 min.
The absorbance of the supernatant was measured at 532 nm.

**Behavioural and Toxic Effects**

Four groups of 10 mice each were administered p.o., 250, 500, 1000 and 1500 mg/kg of CC. The animals were observed continuously for 1 h for any gross behavioural changes, symptoms of toxicity and mortality, if any and intermittently for the next 6 h and then again, 24 h after dosing with CC.

**Statistical Analysis**

Statistical comparison between control and treated groups were made using analysis of variance, followed by multiple comparisons (significance \( P \leq 0.01 \)).

**Results**

**Anti-inflammatory Activity**

Oral administration of CC significantly inhibited the carrageenan- induced paw oedema in rats at both doses (250 and 500 mg/kg) studied. At 250 mg/kg dose, 67% inhibition was observed, and at 500 mg/kg dose, 78% inhibition was obtained. The group treated with Indomethacin (5 mg/kg) showed maximum inhibition of oedema, 89% (Table 1).

**Analgesic Activity**

Intraperitoneal injection of acetic acid produced 64.0 ± 3.0 writhes in the control group, 20 min after injection. CC at both the doses used in the study significantly inhibited acetic acid induced writhing response in mice. Writhing response inhibition at the dose of 250 mg/kg was (73.44%) and the extent of writhing response inhibition at 500 mg/kg dose was 77%, whereas the % inhibition caused by the standard drug, acetyl salicylic acid (Aspirin) at 25 mg/kg dose was (92.18 %), when compared to the acetic acid control (Table 2).

**In vitro Anti-lipid Peroxidation Studies**

There was significant increase of malondialdehyde (MDA) in FeCl\(_2\)-AA treated rat liver homogenate, compared to normal control without FeCl\(_2\)-AA. CC showed very potent inhibition of FeCl\(_2\)-AA stimulated rat liver lipid peroxidation in vitro at 50 µg/ml dose. Lower doses studied did not produce significant anti-lipid peroxidation effects in vitro and doses higher than 50µg/ml did not significantly increase the anti-lipid peroxidation effect any further (Table 3).

**Behavioural and Toxic Studies**

In the toxicity study, no mortality occurred within 24 h with the 4 doses of CC tested. The LD\(_{50}\), of CC was therefore, greater than 1500 mg/kg p.o., in mice.

**Discussion**

Inflammation is a complex process and various mediators, e.g. prostaglandins, leucotrienes, kinins, platelet activating factor, etc. have been reported to be involved in the development of inflammatory diseases. Carrageenan assay is well suited for

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oral dose (mg/kg)</th>
<th>Difference in paw volume at 3 h (ml)</th>
<th>Percentage inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrageenan control</td>
<td>_</td>
<td>0.45±0.01</td>
<td>_</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>5</td>
<td>0.05 ± 0.02**</td>
<td>88.89</td>
</tr>
<tr>
<td>CC</td>
<td>250</td>
<td>0.15 ± 0.05**</td>
<td>66.67</td>
</tr>
<tr>
<td>CC</td>
<td>500</td>
<td>0.10 ± 0.01**</td>
<td>77.78</td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. \( n = 6 \). ANOVA **\( P \leq 0.01 \) vs Carrageenan control

**Table 2—Effect of Commiphora caudata ethanolic extract (CC) on acetic acid - induced writhing response in mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oral Dose (mg/kg)</th>
<th>Mean number of writhes in 30 min</th>
<th>Percent inhibition of writhes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid control</td>
<td>_</td>
<td>64.0 ± 3</td>
<td>_</td>
</tr>
<tr>
<td>Acetyl salicylic acid (aspirin)</td>
<td>25</td>
<td>5± 1**</td>
<td>92.18</td>
</tr>
<tr>
<td>CC</td>
<td>250</td>
<td>17.0 ± 1**</td>
<td>73.44</td>
</tr>
<tr>
<td>CC</td>
<td>500</td>
<td>15.0 ± 2**</td>
<td>77.00</td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. \( n = 6 \). ANOVA **\( P \leq 0.01 \) vs acetic acid control

**Table 3—Inhibitory effect of Commiphora caudata ethanolic extract (CC) on FeCl\(_2\)- ascorbic acid - induced lipid peroxidation in rat liver homogenate in vitro**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plant extract concentration (µg/ml)</th>
<th>MDA (n mol/mg protein)</th>
<th>MDA inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>_</td>
<td>1.44 ± 0.02</td>
<td>_</td>
</tr>
<tr>
<td>FeCl(_2)-AA control</td>
<td>_</td>
<td>3.75 ± 0.03</td>
<td>_</td>
</tr>
<tr>
<td>FeCl(_2)-AA + CC</td>
<td>25</td>
<td>2.45 ± 0.01</td>
<td>34.66</td>
</tr>
<tr>
<td>FeCl(_2)-AA + CC</td>
<td>50</td>
<td>1.74 ± 0.07**</td>
<td>53.60</td>
</tr>
<tr>
<td>FeCl(_2)-AA + CC</td>
<td>100</td>
<td>1.78 ± 0.01**</td>
<td>52.53</td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. \( n = 3 \). ANOVA **\( P \leq 0.01 \) vs FeCl\(_2\)-AA control

MDA= Malondialdehyde
comparative bioassay of anti-inflammatory agents, since the relative potency estimates obtained from most drugs tend to reflect clinical experience. The time course of oedema development in carrageenan induced paw oedema model in rats is generally represented by a biphasic curve. The first phase occurs within an hour of injection and is partly due to the trauma of injection and also due to the serotonin component. Prostaglandins play a major role in the development of the second phase of reaction which is measured around 3 h time. The presence of prostaglandin in the inflammatory exudates from the injected foot has been well demonstrated previously by other workers. The carrageenan induced paw oedema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit cyclooxygenase involved in prostaglandin synthesis. Based on these reports, it is inferred that the inhibitory effect of CC on carrageenan-induced inflammation in rats in the present study may be due to inhibition of the enzyme cyclooxygenase, leading to inhibition of prostaglandin synthesis.

Acetic acid-induced writhing response in mice is a simple and reliable model to evaluate peripheral type of analgesic action of herbal and other drugs rapidly. It was found that CC significantly inhibited the acetic acid induced writhing response at 250 and 500 mg/kg showing 69 and 73% inhibition of writhing, respectively. It has been reported that the abdominal constriction is related to the sensitization of nociceptive receptors by prostaglandins. Therefore in the present study it is possible that CC exerts the analgesic effect probably by inhibiting synthesis or action of prostaglandins.

CC significantly inhibited in vitro lipid peroxidation in rat liver homogenate in the present study. It has become evident that non-enzymatic or unspecified lipid peroxidation occurs during experimental inflammation in rats. Lipid peroxides may be pro-inflammatory and can damage tissues directly. Protection against free radical-induced lipid peroxidation by plant extracts is of great significance for their traditional use against inflammatory disorders, many of which are associated with membrane damage and tissue recovery. There is abundant evidence that peroxidative decomposition of structural lipids in cellular and subcellular membranes is catastrophic in a living system. Lipid peroxidation results in mitochondrial swelling and disintegration. Disintegration of lysosomes has been correlated with the peroxidative decomposition of lysosomal lipids. It can therefore be concluded from the present study that the beneficial effects of CC may be from their role in the stabilization of lysosomes. The combination of anti-inflammatory and analgesic effects of CC indicates the likelihood of intervention of prostaglandin synthesis as prostaglandins have been established as a common mediator in all these responses. However, this possibility remains to be investigated in detail.

The acute toxicity study indicated that CC is fairly non-toxic. This is not surprising, as *C. caudata* is being used in traditional medicine of Kerala as an anti-inflammatory and analgesic agent.

It has been reported that the leaves and bark of *C. caudata* contain oleoresins. Many oleoresins are used in anti-inflammatory and analgesic agents in modern medicine. Preliminary phytochemical studies at our laboratory indicated the presence of gum resins in CC. It is reported that destructive distillation of the gum resin of *C. caudata* gives dark brown oil with odour of birch tar. Volatile oils, resins, flavonoids and terpenoids isolated from plant extracts are known to produce anti-inflammatory and analgesic effects. It is likely that *C. caudata* may contain some or most of the above mentioned phytocompounds which are responsible for its anti-inflammatory and analgesic effects. Detailed studies are warranted in this direction to decipher the exact nature of the phytochemical compounds responsible for the anti-inflammatory and analgesic effects of CC.

**Conclusion**

The findings of the present study have demonstrated that *C. caudata* has potent anti-inflammatory and analgesic activity and justify its use in traditional medicine to treat inflammatory and painful conditions. The results also furnish evidence that the beneficial effects of this plant may be due to its free radical scavenging activity.

**Acknowledgements**

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References