Evaluation of Anti-inflammatory Activity of *Cleome gynandra* L. 
Leaf Extract on Acute and Chronic Inflammatory Arthritis Studied in Rats

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**Abstract:** The present study was aimed to evaluate the anti-inflammatory potential of *Cleome gynandra* leaf extract on both acute (carrageenan induced paw edema) and chronic (cotton pellet granuloma) experimental inflammatory models induced in rats. The effect of *C. gynandra* on adjuvant induced arthritis was also evaluated. The extract at a dose of 150 mg kg⁻¹ body weight was found to possess significant anti-inflammatory activity in all the experimental models and the results were comparable with indomethacin, a standard reference drug. The extract significantly decreased the carrageenan induced paw edema and cotton pellet granuloma. The increased activities of acid and alkaline phosphatase activity and decreased serum level albumin in cotton pellet granulomatous rats were reverted back to near normal levels after treatment with the extract. The extract significantly decreased the lipid peroxide (LPO) content of exudates, liver and spleen and the activity of γ-glutamyl transpeptidase in the exudates of cotton pellet granuloma. Moreover, *C. gynandra* also significantly suppressed the development of chronic arthritis induced by Freund’s complete adjuvant. These results demonstrated that the ethanolic extract of *Cleome gynandra* possess anti-inflammatory activity on both acute and chronic inflammation.

**Keywords:** Anti-inflammatory, *Cleome gynandra*, adjuvant arthritis, carrageenan, cotton pellet granuloma

**Introduction**

Inflammation is a coordinated response that protects and heals the host after infection or tissue damage and it involves several molecular cues generated from either host or disease agent (Nathan, 2002). Inflammation may be considered as homeostatic response designed to destroy or inactivate invading pathogens, removal of waste and debris and restoration of normal function. The initial inflammatory response is usually acute and may or may not evolve into chronic inflammation (Walsh and Pearson, 2001).

The modern drugs, both steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) available for the treatment of various inflammatory disorders, however, offer only temporary relief and often elicit undesirable side effects (Flower et al., 1980).

Therefore, increasing efforts are being directed towards the development of drugs with long acting anti-inflammatory effects with minimum side effects. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects (Dharmasiri, 2003). Recently we have reported hematological alterations and tissue defense activities of *Cleome gynandra* on adjuvant induced arthritic rats (Narendhirakannan et al., 2005a, b).
Cleome gynandra L. (Cat’s whiskers) is a common weed which grows in most tropical countries. It is comes under the family Capparidaceae. Cat’s whiskers, is an erect herbaceous annual herb, predominantly used as a leafy vegetable and finds use in the Indian system of medicine for the treatment of various ailments. The leaves and seeds of C. gynandra are used in many countries for earache, epileptic fits, stomachache, constipation, inflammation, fever, liver diseases, bronchitis, diarrhea and infantile convulsions (Sreeamulu, 1982; Chweya and Mzava, 1997).

However, no systemic studies have been carried out in the anti-inflammatory effect of this plant. Hence, in the present study, an attempt has been made to evaluate the anti-inflammatory activity of C. gynandra leaf extract in both acute and chronic inflammation induced in experimental rats.

Materials and Methods

Materials

Carrageenan and Freund’s complete adjuvant were obtained from Sigma Chemicals and Difco Laboratories, USA respectively. All other chemicals were of analytical grade.

Animals

Male albino rats of Wistar strain weighing around 160-180 g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai and housed in solid-bottomed polypropylene cages and acclimatized to animal house conditions. The rats were fed with commercial rat diet (Hindustan Lever Limited, Mumbai, India) and water ad libitum. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethical Committee approval (IAEC No. 01/032/04). Once arthritis developed, food was served on the bottom of the cages as arthritic rats have difficulty in feeding from the cage top.

Collection of Plant Materials

Fresh C. gynandra leaves were collected from a rural region of Dindigul district, Tamilnadu, India and the plant was identified by Prof. V. Kaviyarasan, Center for Advanced Studies in Botany, University of Madras, Chennai, India. The leaves were dried under shade. The voucher specimen of the plant is being retained in the department herbarium.

Preparation of Leaf Extract

The dry leaves were chopped and subjected to extraction with 95% methanol by Soxhlet apparatus for 15 h, nearly 85% of the solvent was recovered by distillation over a boiling water bath at atmospheric pressure and the remaining under reduced pressure (temperature at 40-50°C). The yield was 3.2 g/100 g. The extract was stored in a refrigerator until further use.

Anti-inflammatory Activity

Carrageenan Induced Paw Edema

Anti-inflammatory activity of C. gynandra on carrageenan induced paw edema was evaluated by the method of Winter et al. (1962). The rats were divided into four groups, each group consisting of six animals as follows:

Group I

Control rats

Group II

Carrageenan (0.1 mL of a 1% solution in saline) induced paw edema in rats

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Group III
Carrageenan induced rats treated with C. gynandra leaf extract (150 mg kg\(^{-1}\) body weight/rat)

Group IV
Carrageenan induced rats treated with indomethacin (10 mg kg\(^{-1}\) body weight).

Carrageenan was injected into the left rear plantar region of the right hind paw of the experimental rats using a glass syringe (2 mL) with the locking hubs and a 26 G needle. The measurement of foot volume was carried out following the plethysmography method described by Ferreira (1979) before carrageenan injection and at 1, 2, 3, 4 h after carrageenan injection. Drugs (C. gynandra and indomethacin) were administered orally 1 h prior to carrageenan injection to Group III and IV, respectively.

Cotton Pellet Granuloma Test
The effect of C. gynandra leaf extract on the proliferation phase of inflammation was investigated by the cotton pellet granuloma model (Niemegkens et al., 1975). The rats were divided into four groups, each group consisting of six animals as follows:

Group I
Normal rats,

Group II
Control rats implanted cotton pellets (measuring 5 mm of length and weighing 40 mg) into subcutaneous tissue on the back of rats,

Group III
Cotton pellets (measuring 5 mm of length and weighing 40 mg) implanted into subcutaneous tissue on the back of the rats treated with C. gynandra leaf extract (150 mg kg\(^{-1}\) body weight/rat),

Group IV
Cotton pellets (measuring 5 mm of length and weighing 40 mg) implanted into subcutaneous tissue on the back of the rats treated with indomethacin (10 mg kg\(^{-1}\) body weight).

The assay was performed under aseptic condition and anesthesia using diethyl ether. A ventral longitudinal scission was made in each animal and four rolls of hydrofoils white cotton (Johnson and Johnson), measuring 5 mm of length and weighing 40 mg each, were implanted through the diffusion of the subcutaneous tissue at four equidistant sites of the incision. Drugs (C. gynandra and indomethacin) were administered orally just before the induction of inflammation and then daily for 7 days to group III and IV, respectively. At the end of the experimental period the rats were fasted overnight and the anesthetized rats were sacrificed by cervical decapitation on the 8th eighth day and the pellets surrounded by granulomatous tissues were dissected out, weighed, dried for 24 h at 60°C temperature and again weighed. The increment between dry and wet pellet weights was taken as a measure of granuloma formation and compared with control.

Adjuvant Arthritis in Rats
Arthritis was induced by a single intra-dermal injection of 0.1 mL of Freund’s Complete Adjuvant (FCA) containing 10 mg mL\(^{-1}\) dry heat-killed Mycobacterium tuberculosis per milliliter sterile paraffin oil (Difco Laboratories, Detroit, MI, USA) into a foot pad of the left hind paw of male rats (Mizushima, 1972). A glass syringe (1 mL) with the locking hubs and a 26 G needle was used for
injection. The rats were anesthetized with ether inhalation prior to and during adjuvant injection, as the very viscous nature of the adjuvant exerts difficulty while injecting. The swelling in hind paws were periodically examined in each paw from the ankle using plethysmography (Newbold, 1963).
Animals were divided into four groups of six animals in each group as follows:

**Group I**
Control rats

**Group II**
Adjuvant induced arthritic rats

**Group III**
Adjuvant arthritic rats treated with extract of *C. gynandra* leaf. (150 mg kg^{-1} body weight/rat/day for 30 days)

**Group IV**
Adjuvant arthritic rats treated with indomethacin (10 mg kg^{-1} body weight).

**Collection of Serum and Preparation of Tissue Homogenate**
At the end of the experimental period the rats were fasted overnight and the anesthetized rats were sacrificed by cervical decapitation. Blood collected with EDTA was used for biochemical studies.

**Preparation of Tissue Homogenate**
Liver and spleen homogenates were centrifuged separately at 600 X g for 10 min. The sediment which containing nuclei, unbroken cells and plasma membranes (nuclear fraction) were separated and the supernatant was subjected to centrifugation at 16000 X g for 30 min. The sediment was suspended in 0.25 M sucrose buffer. Aliquots were withdrawn at 0 and 30 min. intervals, immediately cooled at 0°C and centrifuged at 16000 X g for 30 min. Enzyme activity in the supernatant was determined.

**Biochemical Parameters of Liver, Spleen Exudates and Serum**
Lipid peroxide content in the liver and spleen homogenate and in the exudates collected from the remaining pellets was assayed by thiorubarbituric acid procedure of Okawa et al. (1979). The level of lipid peroxides was expressed as milli moles of TBA reactants/100 g of wet tissue and nmol mL^{-1} in exudates and liver.

The activity of γ-glutamyl transpeptidase (EC 2.3.2.2) (GGTP) in the exudates of cotton pellet granuloma, carrageenan induced inflammation and adjuvant arthritis was estimated by colorimetric kinetic method described by Boelsterli and Zbinden (1980) using L-γ-glutamyl-3-carboxy-4-nitroanilide (Boehringer Mannheim) as substrate. The enzyme activity was expressed as nmoles/min/mg of protein available in the exudates.

For determining the activity of alkaline phosphatase (EC 3.1.3.1) in the serum, the procedure described by King (1965) was followed using p-nitrophenyl phosphate as substrate.

The activity of acid phosphatase (EC 3.1.3.2) in the serum and in the exudates was assayed colorimetrically as described by King (1965) using p-nitrophenyl phosphate as substrate. The enzyme activity was expressed both in serum and in the exudate as unit of enzyme/mg of protein available in the serum and in exudate.

The level of albumin in the serum of the experimental rats was determined using bromocresol-green method described by Doumas et al. (1972). The level of total protein in serum and in the exudate of the tested animals was estimated using folin phenol reagent by the method of Lowry et al. (1951).
Statistical Analysis

Results were presented as mean±SEM of six rats. The results were statistically evaluated using Student’s t-test and ANOVA. Statistical significance is expressed as p<0.001.

Results

The intraplantar injection in the hind paw with carrageenan induced progressive edema reaching a maximum thickness after 3 h (Fig. 1). This increase was observed at 1 h and was maximal at 4 h after administration. Animals treated orally with ethanolic extract of C. gynandra showed significant edema inhibition in all phases of the experiment as well as indomethacin.

The drug C. gynandra able to reduce the inflammatory process of granuloma formation in rats after the treatment period in comparison with control rats (Table 1); this was evident from the reduction of both wet and dry weights of the cotton pellets. Treatment with indomethacin also effectively reduced the granuloma. Pus was observed only in the control groups.

Figure 2 shows the time course of edema rate and inhibition rate after the administration of FCA and C. gynandra treated rats. The adjuvant injected paw became swollen gradually for more than

Fig. 1: Anti-inflammatory effect of Cleome gynandra and Indomethacin on carrageenan induced paw edema in experimental animals. Values are given as mean±SD for groups of six animals each. One way ANOVA followed by post hoc LSD test LSD *p<0.001, † p<0.05. Arthritic control (group II) was compared with normal rats (Group I). Experimental groups (Group III, IV, V and VI) were compared with control (Group II)

Fig. 2: Anti-arthritis effect of Cleome gynandra and indomethacin on the changes in adjuvant induced arthritis of control and experimental animals. Values are given as mean±SD for groups of six animals each. One way ANOVA followed by post hoc LSD test LSD *p<0.001, † p<0.05. Arthritic control (group II) was compared with normal rats (Group I). Experimental groups (Group III and IV) were compared with control (Group II)
Table 1: Weight of cotton pellet in control and drug treated rats

<table>
<thead>
<tr>
<th>Cotton pellet (mg)</th>
<th>Control</th>
<th>Control+C. gymnandra</th>
<th>Control+Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight</td>
<td>260.8±8.1</td>
<td>153.4±7.4</td>
<td>129.5±6.3</td>
</tr>
<tr>
<td>Dry weight</td>
<td>72.7±3.7</td>
<td>-45.9±1.8</td>
<td>57.2±2.1</td>
</tr>
</tbody>
</table>

Table 2: Effect of oral treatment of Cleome gymnandra leaf extract on biochemical parameters in exudates, spleen and liver of experimental animals

<table>
<thead>
<tr>
<th>Normal control</th>
<th>Serum lipid peroxide</th>
<th>Liver lipid peroxide</th>
<th>Exudate lipid peroxide</th>
<th>Spleen lipid peroxide</th>
<th>Serum albumin</th>
<th>Serum acid phosphatase</th>
<th>Serum alkaline phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9±0.1</td>
<td>1.35±0.5</td>
<td>-</td>
<td>2.51±0.2</td>
<td>5.3±0.2</td>
<td>1.25±0.2</td>
<td>0.51±0.02</td>
<td></td>
</tr>
<tr>
<td>Control+Indomethacin</td>
<td>3.1±0.4*</td>
<td>1.86±0.7*</td>
<td>14.59±1.0*</td>
<td>3.01±0.3*</td>
<td>4.54±0.1*</td>
<td>1.42±0.3*</td>
<td>0.61±0.03*</td>
</tr>
</tbody>
</table>

The level of lipid peroxides was expressed as milli moles of TBA reactive/100 g of wet tissue and nmol mL⁻¹ in tissues. Serum albumin levels was expressed as gram per dl. Acid phosphatase and alkaline phosphatase activities were expressed as micromoles of p-nitrophenol formed per hour per milligram of protein. Value represents mean±SD for six animals in each group. Values are statistically significant at *p<0.001; **p<0.05*. Statistical significance was compared within the groups as follows: *Arthritic rats compared with normal rats; **C. gymnandra leaf treated arthritic rats were compared with arthritic rats.

Fig. 3: Activity of γ-Glutamyl transpeptidase in carrageenan induced acute inflammation. Values are given as mean±SD for groups of six animals each. One way ANOVA followed by post hoc test LSD *p<0.001; **p<0.05. *Arthritic control (group II) was compared with normal rats (Group I). **Experimental groups (Group III and IV) were compared with control (Group II).

25 days. The curves of edema rate versus time could be divided into two phases. In the first phase, edema rate of the injected footpad increased and reached a peak during the first 3 days. Thereafter, swelling slowly subsided until 9th day when the paw began to swell again and peaked in the 3rd week (second phase). Administration of C. gymnandra leaf extract was started after the adjuvant injection, suppresses the secondary increase in swelling of the injected foot, which occurs at the appearance of polyarthritis and the arthritic score was also found to be significantly inhibited the development of joint swelling induced by FCA, except in the group treated with C. gymnandra.

Table 2 shows the levels of lipid peroxides in the plasma, liver and spleen of rats exposed to cotton pellet granuloma. Lipid peroxide level was found to be significantly increased (p<0.001) in serum, spleen and liver of cotton pellet granulomatous animals when compared to normal animals. These levels were restored to near normal in C. gymnandra administered rats. γ-glutamyl transpeptidase shows increased activity in carrageenan-induced edema, adjuvant arthritis and cotton pellet granuloma and that the level of this enzyme can be suppressed in a C. gymnandra treated rats. Table 2 exhibits the level of serum albumin in experimental animals. The decreased level of albumin was found in inflammatory rats and it was increased after the treatment with C. gymnandra leaf extract. The activity of lysozyme enzymes (acid phosphatase and alkaline phosphatase) in serum was markedly increased during inflammation and this increase was normalized by administration of C. gymnandra (Table 2).
Fig. 4: Activity of γ-Glutamyl transpeptidase in adjuvant induced arthritis. Values are given as mean±SD for groups of six animals each. One way ANOVA followed by post hoc test LSD

\*p<0.001; \*p<0.05. \* Arthritic control (group II) was compared with normal rats (Group I).
\* Experimental groups (Group III and IV) were compared with control (Group II).

Changes in the activity of γ-glutamyl transpeptidase during the progression of acute and chronic inflammation (carrageenan-induced edema and adjuvant arthritis) are shown in Fig. 3 and 4, respectively. γ-GT registered the remarkable increase in 2 h after carrageenan injection as well as adjuvant induction. This progressed to a maximum after 4 h of inflammation. Indomethacin and C. gynandra pretreated rats showing diminished rise of enzyme activity compared to the untreated animals.

Discussion

Inflammation comprises of three phases, namely acute inflammation, the immune response and chronic inflammation (Katzung, 1988). In acute inflammation, due to the changes in small blood vessels, fluid and granulocytic cells accumulate at the site of injury. This reaction often triggers a systemic response such as fever, leucocytosis, protein catabolism and altered hepatic synthesis of acute phase proteins such as C-reactive protein. Chronic inflammation is characterized by tissue infiltration by macrophages and lymphocytes (Walsh and Pearson, 2001).

The soft tissue formed around the ankle joints, during the acute phase of arthritis, was considered to be due to edema of periarticular tissues, such as ligaments and joint capsules. As the disease progresses, a more diffused demineralization developed in the extremities (Begaum and Sadique, 1988).

Carrageenan induced edema is often used as an experimental model for evaluating the anti-inflammatory potential of natural products (Mukherjee et al., 1977). The development of edema in the paw of the rat after injection of carrageenan was described by Vinegar et al. (1969).

According to Vinegar et al. (1987), the carrageenan-induced edema can be divided into two phases. The first phase occurs during 1 h after carrageenan injection. It derives from the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin in the inflammatory area. The second phase occurs 3-5 h after carrageenan injection. In this phase, the macrophages in carrageenan insulted dermal tissues release much interleukin-1 (IL-1) to induce accumulation of polymorphic nuclear cells (PMNs) into the inflammatory area. The activated PMNs then release the lysosomal enzymes and active oxygen, especially super oxide, to destroy connective tissue and induce paw swelling. The results of the present study indicate that C. gynandra extract and indomethacin play a crucial role as protective factors against carrageenan induced acute-inflammation. The significant suppressive activity of the extract in both phases shows its potent anti-inflammatory effect which can be attributed to the presence of flavonoids, terpenes (Ferandiz and Alcaruz, 1991) in the extract of C. gynandra.
Likewise, the granulomatous tissue formation is released to the chronic inflammatory process, which is characterized by several phases (Swingle and Shideman, 1972). The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation and is a typical feature of established chronic inflammatory reaction. The fluid absorbed by the pellet greatly influences the wet weight of the amount of granulomatous tissue formed (Spector, 1969; Swingle and Shideman, 1972).

*C. gynandra* leaf extract decreased both wet and dry weights of cotton pellets when compared to control group of rats. Monocyte infiltration and fibroblast proliferation rather than neutrophil infiltration and exudation take place in chronic inflammation (Dunne, 1990). This proliferation becomes widespread by proliferation of small vessels or granuloma (Hosseinizadeh et al., 2000). Nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration and inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides (Suleyman et al., 1999; Lonac et al., 1996). The extract exhibited significant anti-inflammatory activity in the cotton-pellet granuloma test. This reflected its efficacy to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Aragnoni-Martellie, 1977). These results indicate the potential of *C. gynandra* leaf extract which possesses anti-inflammatory activity.

Present results also showed that *C. gynandra* leaf extract significantly inhibited the development of chronic joint swelling induced by FCA. Adjuvant-induced arthritis is the most frequently used chronic inflammatory model. It seems that bacterial peptidoglycan and muramyl dipeptide are responsible for its induction (Crofford and Wilder, 1993). Since the composition of bacterial adjuvant is complex and the immune response is a multi-stage process of intercellular cooperation, the mechanism is unclear (Walz et al., 1971). It is previously known that as the arthritis proceeds, the redness and swelling of the joints and the body weight loss usually start to appear at the onset stage of arthritis (Tak et al., 2000).

Lipid peroxides are formed by auto oxidation of polyunsaturated fatty acids found primarily in cell membranes. An increase in the level of lipid peroxide in tissue therefore, reflects membrane damage (Kawamura et al., 1992). The lymphoid organs, spleen and liver are affected during arthritic conditions, which may be result of phagocytosis (Spector, 1964). A significant enhancement in the level of LPO was observed in arthritic animals. The increased lipid peroxides may be due to weakening of antioxidant defense system and failure of antioxidant defense system has been reported in RA (Vijayalakshmi et al., 1997). The increase may also be due to the release of free radicals from neutrophils and monocytes in inflammation. Granulocytes, which accumulate in the rheumatoid joints, are known to produce oxygen derived free radicals during phagocytosis of bacteria and immune complexes, which may lead to the increased lipid peroxide formation (Marhoff et al., 1993).

γ-Glutamyl transpeptidase plays an important role in the turnover of glutathione and protein biosynthesis. Because in inflammation both catabolic and anabolic steps are activated together with migration of cells, an alteration in γ-GT activity was postulated to occur at the site of inflammation with the development of inflammatory process (Singh et al., 1986). Alterations in γ-glutamyl transpeptidase activity and albumin levels may be attributed to the presence of specific steroid components in the extract of *C. gynandra* leaf. The activity of lysosomal enzymes (ACP and ALP) in tissues was elevated during inflammation (Nishikage et al., 1980). The role of lysosomal enzymes as mediators of inflammation is well documented (Weismann, 1967; Becker and Henson, 1973). The levels of ACP and ALP in the exudates have got much significance over its level in serum as far as cotton pellet granuloma is concerned (Becker and Henson, 1973).

In conclusion, the data obtained in this study demonstrated that methanolic extract of *Cleome gynandra* leaf might have anti-inflammatory activity against early phase (acute paw edema), late phase (cotton pellet granuloma) of inflammation and adjuvant arthritis without any deleterious side effects.
The anti-inflammatory activity could be ascribed to the presence of the previously reported flavonoids, terpenes and other related synergistic components. Further studies are necessary to elucidate the mechanism behind its traditional effects.

References


