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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.

Key words: 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).

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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 (Sreeramulu, 1982; Chweya and Mnzava, 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

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 in to 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 (Niemegeers *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 (Newbould, 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 thiobarbituric acid procedure of Ohkawa *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-nitro-anilide (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
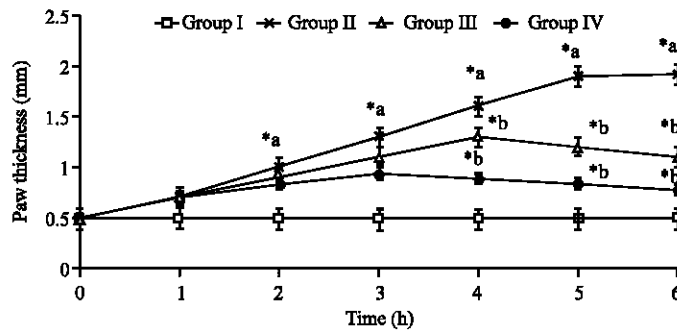

Fig. 1: Anti-inflammatory effect of *Cleome gynandra* and Indomethacin on carrageenan induced paw edema in experimental animals. Values are given as mean±SD for groups of six animals each. One way ANOVA followed by post hoc test LSD *p<0.001; #p<0.05. [a] Arthritic control (group II) was compared with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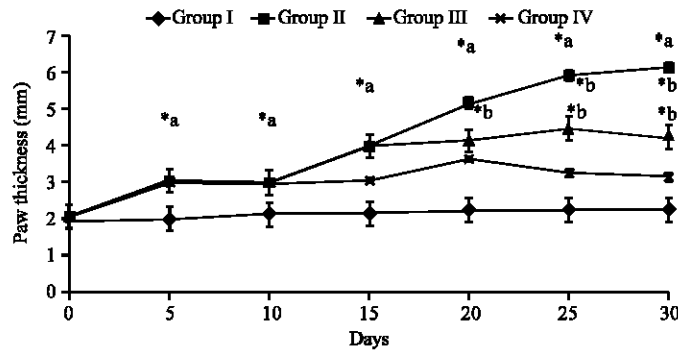

Fig. 2: Anti-arthritic effect of *Cleome gynandra* and indomethacin on the changes in adjuvant induced arthritis of control and experimental animals. Values are given as mean±SD for groups of six animals each. One way ANOVA followed by post hoc test LSD *p<0.001; #p<0.05. [a] Arthritic control (group II) was compared with normal rats (Group I). [b] Experimental groups (Group III and IV) were compared with control (Group II)

Table 1: Weight of cotton pellet in control and drugs treated rats

Cotton pellet (mg)	Control	Control±*C. gynandra*	Control±Indomethacin
Wet weight	260.8±8.1	153.4±7.4	129.5±6.3
Dry weight	72.7±3.7	45.9±1.8	57.2±2.1

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

	Serum lipid peroxide	Liver lipid peroxide	Exudate GGTP	Spleen lipid peroxide	Serum albumin	Serum acid phosphatase	Serum alkaline phosphatase
Normal	2.9±0.1	1.35±0.5	-	2.51±0.2	5.3±0.2	1.25±0.2	0.51±0.02
control	5.41±0.3[*a]	2.89±1.2[*a]	21.85±2.97	5.56±0.8[*a]	2.9±0.1[*a]	2.81±0.9[*a]	0.82±0.05[*a]
Control+Indomethacin	3.1±0.1[#b]	1.86±0.7[#b]	14.59±1.9[#b]	3.01±0.3[#b]	4.5±0.1[#b]	1.42±0.3[#b]	0.61±0.03[#b]
Control+*C. gynandra*	3.6±0.2[#b]	2.12±0.8[#b]	16.11±1.2[#b]	3.92±0.3[#b]	3.9±0.1[#b]	1.79±0.2[#b]	0.70±0.02[#b]

The level of lipid peroxides was expressed as milli moles of TBA reactants/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: [a] Arthritic rats compared with normal rats: [b] *C. gynandra* leaf treated arthritic rats were compared with arthritic rats
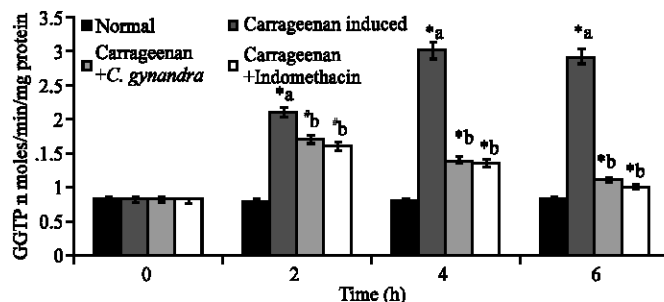

Fig. 3: Activity of γ-Glutamyl transpeptidase in carrageenan induced acute inflammation. Values are given as mean±SD for groups of six animals each. One way ANOVA followed by post hoc test LSD *$p<0.001$; #$p<0.05$. *[a] Arthritic control (group II) was compared with normal rats (Group I). [b] Experimental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

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. gynandra* 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. gynandra* 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. gynandra* leaf extract. The activity of lysosomal enzymes (acid phosphatase and alkaline phosphatase) in serum was markedly increased during inflammation and this increase was normalized by administration of *C. gynandra* (Table 2).
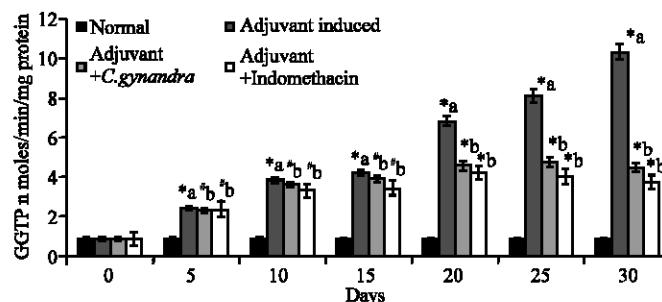
Fig. 4: Activity of γ-Glutamyl transpeptidase in adjuvant induced arthritis. Values are given as mean±SD for groups of six animals each. One way ANOVA followed by post hoc test LSD *p<0.001; #p<0.05. [a]Arthritic control (group II) was compared with normal rats (Group I). [b] Experimental groups (Group 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 progress, 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 Alcaraz, 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 Shiderman, 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 Shiderman, 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 (Hosseinzadeh *et al.*, 2000). Nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration/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 (Arrigoni-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 (Marhoffer *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 steroidal components in the extract of *C. gynandra* leaf. The activity of lysosomal enzymes (ACP and ALP) in tissues was elevated during inflammation (Nishikaze *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

Arrigoni-Martellie, E., 1977. Inflammation and Anti-inflammatory, New York. Spectrum Publishers Inc., pp: 119-120.

Becker, E.L. and P.M. Henson, 1973. *In vitro* studies of immunological-induced secretin of mediators from cells and related phenomena. Adv. Immunol., 17: 93.

Begum, V.H. and J. Sadique, 1988. Long term effect of herbal drug *Withania somnifera* on adjuvant induced arthritis in rats. Indian J. Exp. Biol., 26: 877-882.

Boelsterli, U. and G. Zbinden, 1980. Fine Needle Aspiration Biopsy of the Rat Liver: Cytological, Cytochemical and Biochemical Methods. Zbinden, G., G. Oxford (Eds.), Pergamon Press, pp: 59.

Chweya, J.A. and N.A. Mnzava, 1997. Cat's whiskers *Cleome gynandra* L. Promoting the conservation and use of underutilized and neglected crops 11. IPGRI: Italy, pp: 6-7.

Crofford, L.J. and R.L. Wilder, 1993. Arthritis and autoimmunity in animals. In: Arthritis and Allied Conditions. McCarty, D.J. and W.J. Koopman (Eds.). Lea and Febiger, London, pp: 525-539.

Dharmasiri, M.G., J.R.A.C. Jayakody, G. Galhena, S.S.P. Liyanage and W.D. Ratnasooriya, 2003. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. J. Ethnopharmacol., 87: 199-206.

Doumas, B.T., W.A. Watson and H.G. Biggs, 1972. Albumin standards and the measurement of serum albumin with bromocresol-green. Clin. Chim. Acta, 31: 87-96.

Dunne, M.W., 1990. Concepts of altered health states. Philadelphia: Lippincot, pp: 165-176.

Ferandiz, M.L., M.J. Alcaraz, 1991. Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. Agents Acta, 32: 283-288.

Ferreira, S.H., 1979. A new method for measuring variations of rats paw volumes. J. Pharm. Pharmacol., 31: 648.

Flower, R.J., S. Moncada and J.R. Vane, 1980. Analgesic, Antipyretics and Anti-inflammatory Agents: Drugs Employed in the Treatment of Gout. In: the Pharmacological Basis of Therapeutics. Goodman, L.S. and A. Gilaman (Eds.). New York, Macmillan, pp: 682.

Hosseinzadeh, H., M. Ramezani and G. Salmani, 2000. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* boiss extracts in mice and rats. J. Ethnopharmacol., 73: 379-385.

Katzung, B.G., 1988. Basic and clinical pharmacology, 7th Edn., Stanford, Connecticut, Appleton and Lange, pp: 578-579.

Kawamura, T., Y. Ohisa, Y. Abe, A. Ishimori, R. Shineha and K. Yokota, 1992. Plasma lipid peroxides in the operation of oesophageal cancer. Rinsho-Ayori, 40: 881-884. (In Japanese).

King, E.J., 1965. The Hydrolases-acid and Alkaline Phosphatases. In: Practical Clinical Enzymology. Van, D. (Eds.). London, Nostrand Company Ltd., pp: 191-208.

Lonac, M., M.J. Parnham, M. Plauchithiu and K. Brune, 1996. Oxaceprol, an atypical inhibitor of inflammation and joint damage. Pharmacol. Res., 33: 367-373.

Lowry, O.H., N.J. Rosenbrough, A.I. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Marhoffer, W., M. Stein and K. Federlin, 1993. Impaired function of polymorphonuclear neutrophilic granulocytes in rheumatoid arthritis. Immun. Infect., 21: 15-16.

Mizushima, Y., W. Tsukada and T. Akimoto, 1972. A modification of rat adjuvant arthritis for testing anti-rheumatic drugs. J. Pharm. Pharmacol., 24: 781-785.

Mukherjee, P.K., K. Saha, J. Das, M. Pal and B.P. Saha, 1977. Studies on the anti-inflammatory activity of rhizomes of *Nelumbo nucifera*. Planta Med., 63: 367-369.

Narendhirakannan, R.T., M. Kandaswamy and S. Subramanian, 2005a. Anti-inflammatory activity of *Cleome gynandra* L. on hematological and cellular constituents in adjuvant-induced arthritic rats. J. Med. Food., 8: 93-99.

Narendhirakannan, R.T., S. Subramanian and M. Kandaswamy, 2005b. Free radical scavenging activity of *Cleome gynandra* L. leaves on adjuvant induced arthritis in rats. Mol. Cell Biochem., 276: 71-80.

Nathan, C., 2002. Points of control in inflammation. Nature, 420: 846-852.

Newbould, B.B., 1963. Chemotherapy of arthritis induced by rats by injection of mycobacterial adjuvant. Br. J. Pharmacol., 21: 127-136.

Niemegeers, C.J.E., F. Awouters, F.M. Lenaerts and A.J. Janessey, 1975. The activity of suprofen on nystatin-induced paw edema in rats. Arzneimittel Forschung, 23: 1516-1519.

Nishikaze, O., H. Takita and T. Takase, 1980. Activity of newly discovered protease in carrageenan-induced inflammation in rats. IRCS Medical Science, Biochemistry; Connective Tissue; Skin and Bone; Pharmacology; Survey and Transplantation, 8: 725.

Ohkawa, H., N. Oshishi and K. Yag, 1979. Assay of lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Singh, J., J. Chander, S. Singh, G. Singh and C.K. Atal, 1986. γ-Glutamyl transpeptidase: A novel biochemical marker in inflammation. Biochem. Pharmacol., 35: 3753-3760.

Spector, W.G., 1964. The acute inflammatory response. New York, Acad. Sci., 116: 747-752.

Spector, W.G., 1969. The granulomatous inflammation exudates. Intl. Rev. Exp. Pathol., 8: 1-55.

Sreeramulu, N., 1982. Chemical composition of some green leafy vegetables grown in Tanzania. J. Plant Foods, 4: 139-141.

Suleyman, H., L.O. Demirezer, A. Kuruuzum, Z.N. Banoglu, F. Gocer and G. Ozbakir, 1999. Anti-inflammatory effect of the aqueous extract from *Rumex patientia*. J. Ethnopharmacol., 65: 141-148.

Swingle, K.F. and F.E. Shiderman, 1972. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. J. Pharmacol. Exp. Ther., 13: 226-234.

Tak, P.P., N.J. Zvaifler, D.R. Green and G.S. Firestein, 2000. Rheumatoid arthritis and p53: How oxidative stress might alter the course of inflammatory disease. Immunol. Today, 21: 78-82.

Vijayalakshmi, T., V. Muthulakshmi and P. Sachdanandam, 1997. Effect of milk extract of *Semicarpus anacardium* nuts on glycohydrolases and lysosomal stability in adjuvant arthritis in rats. J. Ethnopharmacol., 58: 1-8.

Vinegar, R., W. Schreiber and R. Hugo, 1969. Biphasic development of carrageenan edema in rats. J. Pharmacol. Exp. Ther., 166: 96-103.

Vinegar, R., J.F. Truax, J.H. Selph, P.R. Johnston, A.L. Vanable and K.K. McKenzie, 1987. Pathway to carrageenan induced inflammation of the hind-limb of the rat. Federation Proc., 6: 118-126.

Walsh, D.A. and C.I. Pearson, 2001. Angiogenesis in the pathogenesis of inflammatory joint and lung diseases. Arthritis Res., 3: 147-153.

Walz, D.T., M.J. Di Martimo and A. Misher, 1971. Adjuvant induced arthritis in rats, II: Drug effects of physiologic, biochemical and immunologic parameters. J. Pharmacol. Exp. Ther., 178: 223-231.

Weismann, G., 1967. The role of lysosomes in inflammation and disease. Ann. Rev. Med., 18, 97.

Winter, C.A., E.A. Risly and C.W. Nuss, 1962. Carrageenan-induced oedema in hind paw of the rats-an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 111: 544.