Anti-arthritic activity of *Cayratia pedata* leaf extract in Freund’s adjuvant induced arthritic rats

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Abstract

In the present study, ethanolic extract of *Cayratia pedata* was assessed for anti-arthritic activity in rat. The anti-arthritic activity of *Cayratia pedata* (500 mg/kg p.o) was evaluated using Frund’s complete adjuvant (FCA) induced arthritis in rats. The herbal extracts at dose 500 mg/kg p.o was administered for 21 days after the injection of FCA in the rats paws. A significant (*P* ≤ 0.05) inhibition of paw edema volume was observed from day 4th to 21st in the treated groups. The biochemical parameters like arthritis index, erythrocyte sedimentation rate (ESR), alkaline phosphatase (ALP), acid phosphatase (ACP), malondialdehyde (MDA), reduced glutathione (GSH), rheumatoid factor (RF), C-reactive protein (CRP) and total WBC count was observed which are the major markers of arthritis. A significant increase in the level of all the markers were found in the arthritic rats whereas in case of prednisolone and *Cayratia pedata* treated groups a marked decrease in the level was observed. These results indicate that the ethanolic extract of *Cayratia pedata* has anti-arthritic activity.

INTRODUCTION

Typically arthritis is a common inflammatory joint disease characterized by inflammation of the synovial membrane, pain and restricted joint movement (Pearson, 1956). Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial socio-economic impact (Buuch and Emery, 2002) The prevalence of arthritis is approximately in the West (Lipsky, 2005) The prevalence of RA in India subcontinent is 1.5-2 percent of population. The epidemiological ratio of arthritis in female and male is 3:1 and the prevalence is 1% of the world population. Adjuvant induced arthritis in rats is a chronic inflammatory disease characterized by infiltration of synovial membrane in association with destruction of joint resembles RA in humans (Katz and Piliero, 1969). Presently many non steroidal, steroidal and immunosuppressive drugs are used to control inflammatory symptoms and pain; they are associated with certain undesirable side effects. With these difficulties, the field of arthritis research has progressed exponentially towards herbal therapies that have been considered safe and effective in all elevating chronic pain associated with arthritis (Rao et al., 1999).

Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate that about 80 % of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. The use of herbal medicine becoming popular due Medicinal plants play an important role in the development of potential therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications (Verma and singh, 2008). Agents derived from plants (table 2 & 3) that can modulate the expression of pro-inflammatory signals clearly have potential against arthritis. These include flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins and anthoxanthins, all of which are known to have anti-inflammatory effects. Some of these polyphenols which have been tested for the treatment of arthritis (Khanna et al., 2007). The medicinal value of chosen plant *Cayratia pedata* belonging to the family of Vitaceae. The present study has been carried out to evaluate the antiarthritic
activity of *Cayratia pedata* on the Freund’s adjuvant induced arthritis in rats.

**MATERIALS AND METHODS**

**Chemicals**

Dexamethasone as standard (Dexona, Cadila Healthcare Ltd., India), Complete Freund’s adjuvant was obtained from Sigma Aldrich (Saint Louis, Missouri, USA).and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

**Plant materials:**

The fully mature *Cayratia pedata* leaves were collected in April 2013 from Vandayar Iruppu, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Botanist, Dr. S John Britto, Department of Botany, St. Josephs College, Tiruchirappalli, Tamil Nadu, India. A Voucher specimen (SR 001) has been deposited at the Rapinat Herbarium, St. Joseph’s College, Tiruchirappalli, Tamil nadu, India.

**Preparation of plant extract**

The collected leaves of *Cayratia pedata* were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. The powder material of *Cissus vititiginea* leaves were macerated with 70% ethanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45°C. A semi solid extract was obtained after complete elimination of alcohol. The *Cayratia pedata* leaves extract (CPLE) was stored in refrigerator until used.

**Freund’s Complete Adjuvant Induced Arthritic Model**

Adult Wistar male rat with an initial body weight of 180 to 220g were taken, and divided into four groups each containing six animals. On day zero, all rats were injected into the sub plantar region of the left hind paw with 0.1ml of Freund’s complete adjuvant. This consist of *Mycobacterium butyricum* suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 6mg/ml (Shivanand Pandey et al., 2010).

Dosing with the *Cayratia pedata* extract and standard compound dexamethasone was started on the first day and continued for 21 days according to the following schedule:

| Group I | Normal rats. |
| Group II | Adjuvant induced arthritic rat |
| Group III | Arthritis induced rats administered with extract of *Cayratia pedata* (500mg/kg body weight/rat/day for 21 days p.o.). |
| Group IV | Arthritis induced rats administered with dexamethasone (5 mg/kg body weight/ rat/day for 21 days p.o.). |

The degree of inflammation was measured by a mercury displacement method. The edema formation and the percentage of inhibition were calculated as follows.

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

Where *Vc* is the edema volume of the control group and *Vt* is the edema volume of the treated group.

**Primary and Secondary Lesions**

Primary lesion refers to the edema formation in the injected hind paw that peaks 3-5 days after injection of the phlogistic agent and is measured on days 3, 5, 9, 13 and 21 by calculating percent inhibition of the edema volume of the injected paw using the formula described above. Secondary lesions are immunologically mediated changes characterized by inflammation of the non injected sites (hind leg, forepaws, ears, nose and tail). Accordingly secondary lesions were evaluated by calculating the percent inhibition of paw volume of the non-injected right paw over control on day 21 as follows.

**Arthritic Index**

An arthritic index is calculated as the sum of the scores as indicated in table 1 for each animal.

| Table 1 shows the scores of arthritic index |
| Lesion site | Nature of lesion | Score |
| Ears | Absence of nodules and redness | 0 |
| | Presence of nodules and redness | 1 |
| Nose | No swelling of connective tissue | 0 |
| | Intensive swelling of connective tissue | 1 |
| Tail | Absence of nodules | 0 |
| | Presence of nodules | 1 |
| Forepaws | Absence of inflammation | 0 |
| | Inflammation of at least 1 joint | 1 |
| Hind paws | Absence of inflammation | 0 |
| | Slight inflammation | 1 |
| | Moderate inflammation | 2 |
| | Marked inflammation | 3 |

An arthritic index is calculated as the sum of the scores as indicated above for each animal. The average of treated animals is compared with the control group (Gerhard, 2002)

**Biochemical estimation**

MDA released from endogenous lipoperoxides, reflecting the lipid peroxidation process, were assayed by Beuge and Aust, (1978). The levels of non-enzymatic antioxidant GSH was estimated by the method of Moron et al. (1979). Acid phosphatase (ACP) and Alkaline phosphatase (ALP) activities were measured according to the method described by Annon (1963) and Kind and King’s (1954). ESR sedimentation rate and WBC counted by the method of Ochei and Kolhatkar, (2000).

**Rheumatoid factor**

The latex turbidimetry method was used in the present study using RF turbilatex kit of SPINREACT Company. Calibration was carried out for linear range up to 100 IU/ml the reading of RF factor of all the groups obtained was compared with the control animals. Values were expressed as IU/ml

**C - Reactive protein**

The biochemical analysis of CRP was done using high sensitive CRP turbilatex agglutination kit manufactured by Agappe (Switzerland). Latex principles coated with specific human anti-CRP in agglutination
The elevated plasma lipid peroxidation observed in the present study in Freund’s Adjuvant (FA) induced arthritis can be related to excessive lipid peroxidation observed in erythrocytes and erythrocyte membranes, with consequent leakage into plasma or as a result of excessive generation and diffusion of lipid peroxides from the inflamed or injured joints of rheumatoid arthritis. In the present study, we have observed a multidirectional change in enzymatic antioxidants has been well documented (Hassa et al, 2001). Hence, the decrease in plasma non enzymatic antioxidants can be correlated to impairment in the antioxidant defence mechanism, due to excess utilization of lipoproteins and other biomembranes against peroxidative damage by intercepting oxidants before they can attack the tissues (Wagner et al., 1998). Lower concentration of vitamin E has been reported in the joint fluid of Freund’s Adjuvant (CFA) induced arthritis. An inverse relationship between lipid peroxidation and non enzymatic antioxidants has been well documented (Hassa et al 2001). Hence, the decrease in plasma non enzymatic antioxidants can be correlated to impairment in the antioxidant defence mechanism, due to excess utilization by the inflamed tissues to scavenge the excessive lipid peroxides that are generated at inflammatory sites, or to scavenge accumulated lipid peroxides in plasma. Administration of Cayratia pedata decreased lipid peroxidation and increased the glutathione content in Freund’s Adjuvant (FA) induced arthritis.

To characterize the pharmacological profile of the Cayratia pedata this experiment provides to screen for anti-inflammatory activity which is characterized by primary lesion on day 5 and anti-arthritic activity which is characterized by secondary lesion on day 21 (Murray, 2001).
Cayratia pedata having just anti-inflammatory activity don not inhibit secondary lesion, which are prevented or diminished by immunosuppressive agents. The current experimental data reveals that there is a significant decrease in the primary lesion as evident on day 5 was observed in both Cayratia pedata at 500 mg/kg b.w., and the dexamethasone treated group as compared to control. Thus the above data explains the anti-inflammatory action of Cayratia pedata. With regards to the secondary lesion, it could only be partially evaluated as no edema formation was significant in the contra lateral hind paw of control animals.

The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. The freund’s adjuvant model is chosen as it develop chronic swelling in multiple joints with influence of inflammatory cells with erosion of joint cartilage and bone destruction. Chronic inflammation involves the release of number of mediators like cytokines (IL-1B and TNF-alpha), GM-CSF, interferon’s and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability (Lam et al., 2004). On the 21st day, a significant decrease in edema volume was observed in Cayratia pedata and the dexamethasone treated group as compared to the FA injected control rats (Table 1).

Increased white blood cell counts are a common feature of inflammatory reactions, especially those induced by microbial infection. So in arthritic group an increase in total leukocyte number was found. A significant reduction in total leukocyte number was found in case of treated groups (Table 2). In our study, it was found that the administration of Cayratia pedata leads to inhibition of leukocyte migration which may have beneficial effect for joint preservation. The activity may be due to presence of steroidal glycoside.

Erythrocyte sedimentation rate (ESR) in the FCA treated group several fold high compared to drug treated groups (Table 2). This may be due to the flavonoid content of the Cayratia pedata. These flavonoids are having the surface charge neutralizing effects. ESR is strongly related with the ability of red cells to aggregate into orderly stacks or rouleaux. Proteins are thought to affect the repellant surface charges on red cells and cause them to aggregate into rouleaux and hence the sedimentation rate increases (Grant et al., 1970). The rate of sedimentation was increased in arthritis control where as in case of treated groups, the ESR level was significantly decreased.

With regard to mean arthritic index, the average arthritic score of control is 2.30 which indicate arthritic (Table 2). In case Cayratia pedata and dexamethasone treated group it showed a profound anti-arthritic activity with the average arthritic score of 0.22 and 0.20 respectively, thereby these two groups decreased the arthritic index almost 10 fold when compared to that of control score being 2.30.

Serum rheumatoid factor (RF) is the immunological expression of an individual's immune system reaction to the presence of an immunoglobulin molecule that is recognized as "non-self." This response to the “non-self” immunoglobulin results in the presence of immune complexes. These, in turn, bind complement and may eventually lead to synovium, cartilage, and bone destruction. Higher the levels of serum rheumatoid factor, higher are the development of inflammation (Shivnanand Pandey et al., 2010). Cayratia pedata treated animal showed significantly lesser serum RF when compared to disease control animals (Table 2).

C-reactive protein (CRP), a hepatically derived marker of systemic inflammation, is the prototypic inflammatory biomarker. It is a member of the class of acute phase reactants, (precisely positive acute phase protein) as its level rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages(Pepys and Hirchfield, 2003) as well as adipocytes (Lau et al., 2005). Freund’s adjuvant (FA) induced arthritic rats increased the CRP level as evidenced in the inflammatory process. A significant (P < 0.01) reduction of CRP level was observed (Table 2) after administration of Cayratia pedata (500 mg/kg body weight), suggested that decreased the inflammatory reactions.

Lysosomes are membrane enclosed cytoplasmic organelles, which possess an acidic interior that contain many hydrolytic enzymes. Lysosomal enzymes are widely distributed in tissue and circulating blood cells and are responsible for intracellular breakdown of complex macromolecules. They also degrade endothelial membrane glycol-conjugates. The altered enzyme activities in arthritis can be regarded as an index of lysosomal enzyme activation occurring in response to metabolic need of degrading various constituents of cells such as mucopolysaccharides and glycoproteins accumulated in tissue due to arthritis associated with vasculopathies (Naparstek et al., 1984).

Acid phosphatase (ACP) seem to be an important index for the examination of the integrity of the lysosomal membrane and are responsible for the tissue damage and necrosis of hepatic tissue. Cytoplasmic cellular enzymes, such as alkaline phosphatase (ALP) membrane bound indicator of type II cell secretary activity or the lysosomal enzyme β-glucuronidase, an indicator of phagocytic activity, can also be used as sensitive markers of cellular integrity and cellular toxicity induced by pathological conditions. A significant (P < 0.01) reduction of ALP level was observed after administration of Cayratia pedata (500 mg/kg body weight) (Table 2).

Increased activities of plasma ACP were observed in arthritic rats. This may be attributed towards persistent inflammation. These changes are in agreement with the decreased lysosomal stability in adjuvant induced arthritis (Olsen et al., 1990) In the present study, the activity of lysosomal enzymes in plasma was markedly increased in the adjuvant induced arthritic rats and significantly (P < 0.05) reduced after treatment with Cayratia pedata (Table 2). An important mechanism of antiarthritic activity is the membrane stability modulating effect (Subrata et al., 1994). The administration of Cayratia pedata may exert its effects
by modifying the lysosomal membrane in such a way that it is capable of fusing with the plasma membrane and thereby preventing the discharge of acid hydrolase or by inhibiting the release of lysosomal enzymes (Carevic and Djokic, 1988). The activity is probable due to presence of flavonoids.

The results of the present experiment indicate that *Cayratia pedata* possesses significant antiarthritic activity. The possible mode of anti-arthritis activity of ethanolic extract of *Cayratia pedata* appears to be, possessing anti-inflammatory activity showed in arthritic parameters like Paw edema, Arthritic index, Rheumatoid factor and By normalization of pro-oxidant and improving anti-oxidant parameters indicating its anti-oxidant potency. The potential activity of various ingredients in *Cayratia pedata* acting synergistically and working in concert for overall antiarthritic activity.

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**References**


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