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Research Article

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Effect of *Flueggea Leucopyrus* Leaf on Inflammatory Markers in Freund's Complete Adjuvant Induced Arthritic Rats

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ABSTRACT

Many plant-derived compounds have been used as drugs, either in their original or semi-synthetic form. Plant secondary metabolites can also serve as drug precursors, drug prototypes and pharmacological probes. Due to these side-effects, there is need for the search of newer drugs with less or no side-effects. In the present study to investigate the anti-arthritic activity of Flueggea leucopyrus leaf on Freund's Complete Adjuvant (FCA) induced arthritic rats. The arthritic markers as tumour necrosis factor (TNF-α), Interleukin (IL-6), Homocystine, Serum Cortisol, N-acetyl-β-glucosaminidase, β-glucuronidase, nitric oxide and Cathepsin – D were investigated. The current evidence from experimental studies demonstrate that supplementation of Flueggea leucopyrus leaf extract (FLLE) has potential anti- arthritic activity in Freund's Complete Adjuvant (FCA) induced rats. Our study confirms that FLLE plays duel role by blocking inflammatory reaction. Over all, the experimental studies suggest that Flueggea robustra leaf extract (FLLE) possess anti-arthritic activity.

Keywords: *Flueggea leucopyrus* leaf; Arthritic markers Inflammation; Freund's Complete Adjuvant

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INTRODUCTION

The inflammatory process is a combination of many pathways like a synthesis of prostaglandin, interleukin or other chemo toxin, adhesive protein receptor action, platelet-activating factors. All can act as chemotactic agonists. Inflammation initiates with any stress on the membrane or by other trigger or stimuli, these activate hydrolysis of membrane phospholipid by phospholipase A into arachidonic acid, which further substrate for cyclooxygenase and lipooxygenase enzyme and byproduct of these are prostaglandins PGE2, PGH2 and leukotrines like LTC4, LTB4 etc. Several cytokines also play essential roles in orchestrating the inflammatory process, especially interleukin-1 (IL-1) and tumor necrosis factor-a (TNF-a). IL-1 and TNF are considered principal mediators of the biological responses to bacterial lipopolysaccharide (LPS, also called endotoxin). They are secreted by monocytes and macrophages, adipocytes, and other cells [1]. The majority of naturally occurring phenolics retain antioxidative and anti- inflammatory properties which appear to contribute to their chemopreventive or chemoprotective activity. Since inflammation is closely linked to tumor promotion, substances with potent anti-inflammatory activities are anticipated to exert chemopreventive effects on carcinogenesis, particularly in the promotion stage. Examples are curcumin, a yellow pigment of turmeric (*Curcuma longa L*), the green tea polyphenol epigallocatechin gallate (EGCG) and resveratrol from grapes (*Vitis vinifera*) that strongly suppress tumor promotion [2]. Thus, searching for inflammatory inhibitors with chemotherapeutic potential from natural sources is an alternative approach in the development of anti-arthritic.

Because of the adverse side effects of NSAIDs, traditional medicines and natural products used in folklore medicine have been studied as potential alternatives to these drugs; these having minimal toxicity related to the gastrointestinal tract [3]. Many plant-derived compounds have been used as drugs, either in their original or semi-synthetic form. Plant secondary metabolites can also serve as drug precursors, drug prototypes and pharmacological probes [4]. Due to these side-effects, there is need for the search of newer drugs with less or no side-effects. In the present study to investigate the anti-arthritic activity of *Flueggea leucopyrus* leaf on FCA induced arthritic rats.

MATERIALS AND METHODS

Plant Material

The leaves of the *Flueggea leucopyrus* were collected from the Maraimalai Nagar, Tamil Nadu, during the month of July 2014. The collected plants were identified and authenticated by a Dr. S.Jhon Brito, Director. The Rapinat Herbarium and Centre for Molecular Systematics, St.Joseph's College (Campus) Tiruchirappalli-620 002, Tamil Nadu, India. The plants were cut into small pieces and shade dried at room temperature for 15 days. The powdered plants were used for the preparation of extract.

Preparation of Plant Extract

The collected plant materials were washed, sliced and completely dried in a hot-air oven at 37°C. The dried materials was ground into make a fine powder and used for extraction. Three hundred grams (300 g) of the powered plants were extracted with ethanol (70%) using "Soxhlet Apparatus" for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The extract contains both polar and non-polar phytocomponents. For experiments 500 mg/kg body weight of *Flueggea leucopyrus* leaf extract (FLLE) was used. This effective dose was selected based on dose dependent studies of FLLE carried out in our laboratory.

Freund's Complete Adjuvant (FCA) Induced Arthritic Model

Adult Wistar male rat with an initial body weight of 180 to 220 g 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 0.1 ml of Freund's complete adjuvant. This consists of *Mycobacterium butyricum* suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 6 mg/ml [5]. Dosing with the *Flueggea leucopyrus* extract (500 mg/kg) and standard compound indomethacin (3 mg/kg body weight) 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 *Flueggea leucopyrus* (500mg/kg body weight/rat/day for 21 days p.o.).
Group IV: Arthritis induced rats administered with indomethacin (3 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.

$$\frac{Vc - Vt}{Vc} \times 100$$

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

Collection of Samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50 mg/kg). The blood was collected with or without EDTA as anticoagulant. Blood, plasma and serum were separated for the estimation of various biochemical parameters.

Biochemical Estimation

Plasma TNF-α levels were determined using an enzyme linked immunosorbent assay (ELISA) kit from R&D Systems Inc., Minneapolis, USA. Antibodies specific for rat TNF-α and IL 6 were coated onto the wells of the microtiter strips and the samples including standards of known rat TNF-α was pipetted into the wells, incubated and washed. Intensity of the colour was determined at 450 nm with a correction wavelength of 540 nm. Homocysteine (Hcy) was quantitatively estimated in plasma by Enzymatic Immunosorbant Assay (ELISA). Homocysteine Microplate Enzyme immunoassay provided by BIO-RAD (BIO-RAD, USA). Protein bound Hcy was reduced by dithiotheritol to free Hcy and enzymatically converted to S-Adenosyl-L-Homocysteine (SAH) in a separate procedure prior to the immunoassay. The total corticoterone from the homogenate of brain was estimated by following the method of Silber et al. [6] as modified by Katyare and Pandya [7]. The N-acetyl-β-glucosaminidase activity was determined by the method of Walker and Pugh [8]. The β –glucuronidas activity was determined earlier by the method of Fishman et al. [9]. NO concentration in the serum was measured by the method of Sastry et al. [10].

RESULTS AND DISCUSSION

In the present study observed that FLLE treated rats restored the altered level of Tumour necrosis factor (TNF-α), Interleukin (IL-6), Homocystine, Serum Cortisol, N-acetyl-β-glucosaminidase, β-glucuronidase, nitric oxide and Cathepsin - D. From these observations it can be concluded that FLLE reduced the inflammatory markers confirmed through the antioxidant activity and reduce MDA production may prove the anti-inflammatory action of FLLE. Thus, FLLE may be beneficial anti-inflammatory agent (Figures 1-3).

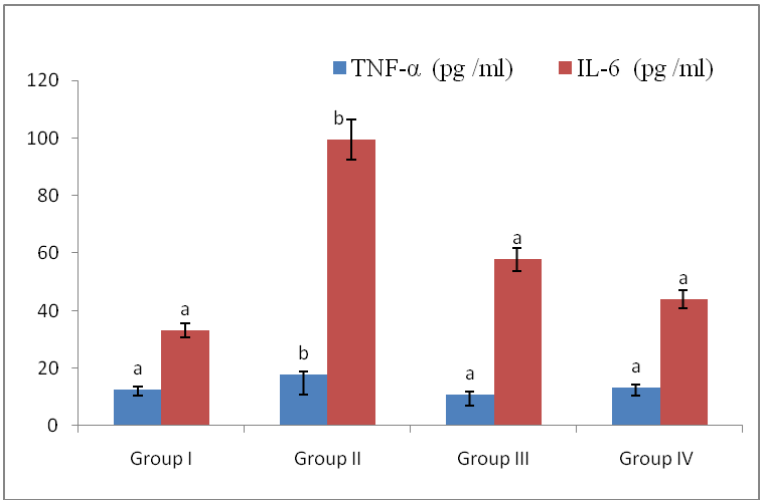

Figure 1: Effect of *Flueggea leucopyrus* leaf extract on TNF-α and IL-6 in control and experimental rats

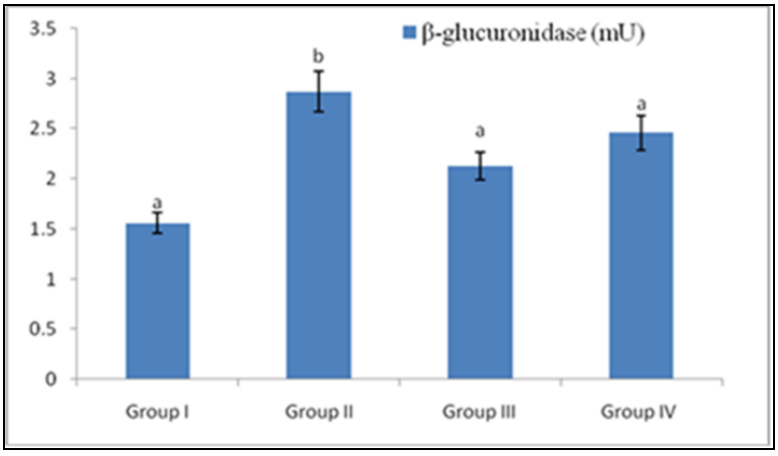

Figure 2: Effect of *Flueggea leucopyrus* leaf extract on β- glucuronidase in control and experimental rats

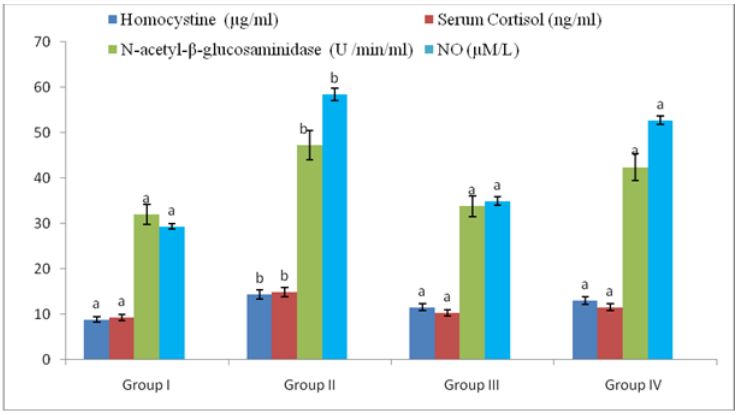

Figure 3: Effect of *Flueggea leucopyrus* leaf extract on N acetyl β- glucosaminidase, homocytine and cortisol in control and experimental rats

a: p<0.05 significantly different compared with Group II animals
b: p<0.001 significantly different compared with Group I animals
a: p<0.05 significantly different compared with Group II animals
b: p<0.001 significantly different compared with Group I animals
a: p<0.05 significantly different compared with Group II animals
b: p<0.001 significantly different compared with Group I animals

Analysis of inflammatory markers can be used as an indicator of inflammatory response to therapy. Analysis of these markers reflects mechanisms of inflammation and subsequent release of proteins and extracellular turnover. Thus, the purpose of present study is to evaluate the effect of FLLE on the level of inflammatory markers during inflammatory process. Several biochemical markers have been suggested for biomonitoring the actions of anti-inflammatory agents. Numerous molecules are involved in the induction and maintenance of the inflammatory response. In addition to pivotal cytokines such as interleukin-1, -6 and tumor necrosis factor-α (TNF-α), thromboxanes, prostaglandins, leukotrienes and nitric oxide (NO) are important chemical mediators of inflammation [11]. PGs are synthesized by cyclooxygenases (COX), which exist as at least two isoforms, COX-1 and the inducible COX-2. NO is synthesized from arginine by nitric oxide synthases, among which the inducible isoform of nitric oxide synthase (iNOS) is primarily responsible for producing large amounts of NO in inflammatory lesions. Therefore, inhibition and/or down-regulation of these proinflammatory molecules may exert anti-inflammatory effects. Adjuvant-induced arthritis involves a single injection of CFA into an area of potent lymphatic drainage in susceptible rats that results in arthritis-like symptoms for many weeks in distal joints. The infiltration of leukocytes into the synovial fluid and tissues is a hallmark of chronic joint inflammation. Initiation of a "flare" or reactivation of the inflammatory reaction in arthritic disease has been attributed to activation responses of polymorph nuclear neutrophils (PMNs) recruited into the joint space by local production of inflammatory cytokines such as TNF- α and IL-1 in response to an antigen driven immune complex deposition [12]. Neutrophils can then contribute to joint damage by the production of reactive oxygen metabolites and the production of cytokines that further amplify the inflammatory response by their effects on lymphocytes and macrophages [13]. Results of the current study of increased TNF- α and IL-6 in the serum of arthritic rats is found to be in accordance with the previous studies of Cai et al. [14] and Bush et al. [15], respectively. The significant reduction in the paw edema and lysosomal enzymes activity after *Flueggea leucopyrus* leaf extract treatment should have eventuated by its membrane stabilizing action by fusing with the plasma membrane and inhibiting the release of lysosomal enzymes. Present finding is in agreement with Pragasam et al. [16] and Manal et al. [17] studies.

Inflammation is known to result in increased in production of nitric oxide (NO) and prostaglandins. NO is an important mediator of diverse physiologic and pathologic processes, including arthritis. Joint inflammation in autoimmune adjuvant induced in arthritis is dependent on the enhanced production of NO. NO is ideally suited as a potent Inflammation mediator because of its strong⇓in reactivity with oxygen, superoxide, and iron containing compounds [18]. Growing evidence implicates NO in immune regulation, inflammation, autoimmunity, and arthritis [19]. Raised levels of NO in serum and synovial fluid have been reported in patients with rheumatoid arthritis (RA) and animals with experimentally induced arthritis and in autoimmune arthritis [20]. Several cell types present within

the joint, including synovial fibroblasts, endothelial cells and chondrocytes, can be induced by proinflammatory cytokines to produce NO *in vitro* [21]. In experimental arthritis, administration of NOS inhibitors profoundly reduces disease activity. In humans, the beneficial effects of NOS inhibition are inferred from indirect evidence: glucocorticoids, auranofin, salicylates, indometacin, and methotrexate inhibit induction of the inducible NOS and/or reduce enhanced NO synthesis and disease activity in different ways. Thus selective inhibition of the pathologically enhanced NO synthesis is a new experimental therapeutic approach in the treatment of inflammatory joint diseases [22]. In the present study decline the nitric oxide upon treatment with *Flueggea leucopyrus* leaf extract. Present finding is similar to the Jeong et al. [23] study.

CONCLUSION

The current evidence from experimental studies demonstrate that supplementation of *Flueggea leucopyrus* leaf extract (FLLE) has potential anti-inflammatory activity in inflammatory rats. Significantly decreased the paw volume in carageenan, FCA and cotton pellet method. Our study confirms that FLLE plays duel role by blocking inflammatory reaction. Over all, the experimental studies suggest that Flueggea robustra leaf extract (FLLE) possess anti-inflammatory activity.

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