Research Article

In Vivo Anti-arthritic activity of Ethanolic Extracts of *Elaeocarpus serratus L.*

DH. Geetha*, Indhiramuthu Jayashree, M. Rajeswari
PG and Research Department of Botany, Vellalar College for Women, (Autonomous), Erode- 638012, Tamil Nadu, India.
***Corresponding author's E-mail:** geethadhandapani2010@gmail.com

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ABSTRACT

The anti-arthritic effect of oral administration of ethanolic extracts of leaf and seed of *Elaeocarpus serratus* was evaluated using Freund's adjuvant arthritis model in Wistar rats. The acute toxicity studies were carried out according to the OECD, 423 guidelines. Arthritis was induced by injecting 0.1ml of 1% Freund's complete adjuvant into the subplantar region in the right hind paw. The oral administrated with indomethacin (10mg/ kg/day p.o.) daily for 15days which served as the standard reference group. The ethanolic extracts of leaf and seed of *E.serratus* at the doses of 200 and 400 mg/kg/day p.o., respectively daily for 15days. The increase in joint diameter was measured daily starting from day 1, by using vernier calipers. Fresh blood samples were treated with various biochemical parameters like SOD, CAT, GPx and GST was significantly reduced in the animals treated with the ethanolic plant extracts. The lipid peroxidase was also significantly decreased in the experimental rats. The results of the present study support the traditional use of this plant and it can be used as anti-arthritic drug.

Keywords: In vivo study, anti-arthritic, *Elaeocarpus serratus*, Freund's complete adjuvant.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, relapsing inflammatory and autoimmune multisystem illness that affects the joints, characterized by inflammation of the synovial membrane, pain and restricted joint movement[1]. RA is systemic inflammatory disease in which the destruction of articular cartilage leads to bone deformity and loss of joint function and ultimately severe pain. It is the most common inflammatory arthritis affecting approximately 1-2% of the general population worldwide i.e. 20 million people worldwide. Incidence increases with age, with women being affected three times more than men[2]. RA is a frequent inflammatory condition being classically treated with anti-inflammatory and immune suppressive drugs, whose side effects are well known[3]. It would therefore be highly desirable to find less toxic alternatives. Some medicinal botanicals might be candidates for such an alternative.

Plants are one of the most important sources of medicines. India is known as the "Emporium of Medicinal plants" due to availability of several thousands of medicinal plants in the different bioclimatic zones. The use of natural remedies for the treatment of inflammatory and painful conditions has a long history, starting with ayurveda treatment, and extending to the European and other systems of traditional medicines. Natural products serve as a 'gold mine' in the management of inflammatory diseases as they are effective, nontoxic and are considered being excellent candidates for arthritis therapy [4].

Elaeocarpus serratus L. commonly known as Ceylon-olive (Tamil: Karamaramm) belongs to family Elaeocarpaceae grows up to 18m tall in evergreen to semi-evergreen forests. The leaves are used in the treatment of rheumatism, diuretic and as antidote to poison, while the fruits are locally prescribed for the treatment of diarrhea and dysentery. The fruit juice is given for stimulating secretions from taste buds thus increasing appetite in patients[5]. The therapeutic basis of herbal medication are by the presence of diverse bioactive compounds in plants and also for the treatment of diseases which are still incurable, medicinal plants can serve as a source of novel therapeutic agent. Hence, the present study was envisaged to evaluating the leaf and seed of *E. serratus* for its anti-arthritic activity.

MATERIALS AND METHODS

Plant material

The leaf and seed of *Elaeocarpus serratus* L. were collected from Upper Palani Hills of Western Ghats (Kodaikanal Forest Division), India and were authenticated at Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and the herbarium of Voucher specimen number BSI/SRC/5/23/2011-12/Tech.239 has been deposited at the PG and Research Department of Botany, Vellalar College for Women, Erode (T.N), India.

Sample Preparation

Collected leaf and seed of *Elaeocarpus serratus* were converted into moderately coarse powder and extracted with solvent ethanol (78.5°C) for 27 hours by soxhlet apparatus. The powder materials were dried in a hot air oven at 40°C. The extracts were dried over anhydrous

sodium sulfate, stored in sealed vials in refrigerator (5-8°C) until analysis [6].

Drugs and chemicals

The chemicals used in the present work were acquired from E. Merck (INDIA) Limited, Hi-media, Pvt. Ltd, India and Sigma-Aldrich, India.

Animal Model

Experimental study was carried out using adult male wistar rats weighing between 150-200g were used for toxicity studies and anti-arthritic activity. The rats were procured from the Small Animals Breeding Station, Mannuthy, Kerala, India. Animal experiments were done in compliance with the Institutional Ethical Committee-CPCSEA (Reg.No.722/02/a/CPCSEA). The animals were housed in polypropylene cages (38x 23 x 10cm) with not more than six animals per cage and maintained under standard environmental conditions (14hr. dark/ 10hr. light cycles; temp 25+ 2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s.Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The rats were acclimatized to the environment for two weeks prior to experimental use. Animals were fasted overnight before the experimental schedule, but had free access for water *ad libitum.*

Acute toxicity studies

The Acute Toxicity studies were performed in order to establish the therapeutic index of a test drug. The experiment was conducted according to the OECD, 423 guidelines. It was administered as 1000, 2000, 3000, 4000 and 5000 mg/kg b.w. orally.

Freund's complete adjuvant (FCA)-induced Paw Edema Assay

The anti-arthritic activity of ethanol extracts of leaf and seed of *E. serratus* was investigated Freund's complete adjuvant model [7]. Wistar male rats (150g) were divided into seven groups of six animals each (n=6). Group I served as control. Arthritis was induced in rats by injecting 0.1ml of 0.1% Freund's complete adjuvant (FCA) into the subplantar region in the right hind paw of group II - group V rats on the first day of the experiment. Group III was administrated with indomethacin (10mg/ kg/day p.o.) daily for 15days which served as the standard reference group. Groups IV, V, VI and VII were administrated with ethanolic extracts of leaf and seed of *E. serratus* at the doses of 200 and 400mg/kg/day p.o., respectively daily for 15 days. The increase in joint diameter was measured daily starting from day 1, by using vernier calipers. The mean changes in injected paw edema with respect to initial and final paw volume, were calculated on respective days.

Antioxidant Enzymes

The rats were anaesthetized under light chloroform anesthesia and blood was collected by cardiac puncture and allowed to clot for 20-30minutes and centrifuged in a refrigerated centrifuge (4°C) at 3000 rpm for 10minutes. Fresh serum samples were stored at-20°C and used to estimate various biochemical parameters viz., Total protein [8], Superoxide Dismutase[9], Catalase[10], Glutathione Peroxidase[11], Glutathione-S-Transferase[12] and Lipid Peroxidation levels[13].

Statistical Analysis

For anti-arthritic assays the values are expressed as means of triplicate analysis of the samples (n=3) ± Standard Deviation (SD). For *in vivo* anti-arthritic study the values are expressed as mean (n=6) ± Standard Deviation (SD). Statistical significance of difference between groups was determined by one way analysis of variance (ANOVA). p values of <0.05 are considered significantly different.

RESULT

Toxicological study

A single dose (1000, 2000, 3000, 4000, and 5000 mg/kg b.w. p.o./day) of ethanolic extracts of leaf and seed of *E. serratus* administered to Wistar male rats showed no death up to 72 hours. At the dose, there were no abnormal clinical signs which include changes in skin colour and behavioral changes like alertness, grooming, restlessness, tremors, convulsions and writhing at any time during the observation period. The effect of plant extract on touch response, torch response, pain response, and righting reflex, gripping strength, pinna reflex, corneal reflex, pupils, urination, salivation and lacrimation were also found to be normal. These results indicated that the plant extract were quite safe even at a high dose of 5000 mg/kg b.w.p.o. and had no acute toxicity.

Freund's complete adjuvant (FCA)-induced Paw Edema Assay

Paw swelling is one of the arthritic symptoms. The determination of paw swelling is apparently simple sensitive and quick procedure in evaluating the degree of the inflammation and assessing the therapeutic effect of drugs. Measurement of paw only gives induction of edematous changes in this region. In this present study, Freund's Complete Adjuvant (FCA) model was used. Standard drug is indomethacin and the anti-arthritic potential of ethanolic plant extracts of leaf and seed (200, 400 mg/kg b.w.p.o.) were assessed Table 1. Following FCA-induction, the animals showed arthritis development as seen by the increase of paw volume from the 1st day onwards. Observations of paw volume were recorded in the regular interval from the day of adjuvant injection. The paw diameter reached maximum up to 11th day of adjuvant injection and after that it was slightly decreased. The chronic inflammation developed on the 10th day in group II (11.84±0.07mm). The ethanolic plant extracts inhibited chronic inflammation response. The leaf extract at 400 mg/kg b.w. reduced the paw volume on 7th day (8.08±0.05mm) group V but the seed extract at 400 mg/kg b.w. showed reduction in paw volume after the 7th

day (8.31±0.07mm) group VII. On comparison with the indomethacin standard which showed an inhibition of 5.12±0.06mm at 10 mg/kg p.o., the leaf and seed extracts caused a significant decrease in the paw volume (5.55±0.05 and 5.86±0.04mm, respectively) at 400 mg/kg b.w. on the 15th day.

Table 1: Effect of ethanolic extract of leaf of *Elaeocarpus serratus* on FCA-induced paw edema in rats

Days	Paw edema (mm)						
	Control Group - I	Induced Group - II	Standard Group- III	ESL LD Group- IV	ESL HD Group- V	ESS LD Group- VI	ESS HD Group- VII
0	3.28 ± 0.16	3.16 ± 0.12^{ns}	3.40 ± 0.08^{ns}	3.44 ± 0.15^{ns}	3.15 ± 0.15^{ns}	3.54 ± 0.17*	3.33 ± 0.09^{ns}
1	3.28 ± 0.16	6.96 ± 0.24*	6.60 ± 0.21^{ns}	6.43 ± 0.11*	5.54 ± 0.18*	7.05 ± 0.08*	6.53 ± 0.12*
2	3.28 ± 0.16	7.54 ± 0.23*	7.27 ± 0.14^{ns}	7.48 ± 0.19^{ns}	6.36 ± 0.13*	7.32 ± 0.16^{ns}	6.95 ± 0.04*
3	3.28 ± 0.16	7.95 ± 0.02*	7.82 ± 0.06^{ns}	7.89 ± 0.04^{ns}	6.95 ± 0.12*	7.69 ± 0.18^{ns}	7.19 ± 0.11*
4	3.28 ± 0.16	8.60 ± 0.09*	8.40 ± 0.09^{ns}	8.35 ± 0.07^{ns}	7.56 ± 0.08*	7.98 ± 0.10*	7.41 ± 0.13*
5	3.28 ± 0.16	9.33 ± 0.05*	8.16 ± 0.03*	8.62 ± 0.09*	7.95 ± 0.12*	8.26 ± 0.12*	7.77 ± 0.17*
6	3.28 ± 0.16	9.91 ± 0.05*	7.89 ± 0.05*	8.95 ± 0.07*	8.26 ± 0.06*	8.59 ± 0.11*	8.18 ± 0.09*
7	3.28 ± 0.16	10.25 ± 0.10*	7.73 ± 0.11*	9.16 ± 0.09*	8.08 ± 0.05*	8.96 ± 0.09*	8.59 ± 0.09*
8	3.28 ± 0.16	10.76 ± 0.05*	7.24 ± 0.07*	9.02 ± 0.07*	7.93 ± 0.08*	8.72 ± 0.07*	8.31 ± 0.07*
9	3.28 ± 0.16	11.44 ± 0.08*	6.88 ± 0.10*	8.83 ± 0.09*	7.55 ± 0.07*	8.43 ± 0.07*	7.97 ± 0.05*
10	3.28 ± 0.16	11.84 ± 0.07*	6.52 ± 0.13*	8.44 ± 0.08*	7.20 ± 0.10*	8.20 ± 0.12*	7.65 ± 0.07*
11	3.28 ± 0.16	11.23 ± 0.08*	6.15 ± 0.05*	8.01 ± 0.06*	6.94 ± 0.10*	7.99 ± 0.08*	7.27 ± 0.08*
12	3.28 ± 0.16	10.73 ± 0.07*	5.72 ± 0.06*	7.63 ± 0.12*	6.65 ± 0.08*	7.75 ± 0.07*	6.82 ± 0.09*
13	3.28 ± 0.16	10.12 ± 0.06*	5.44 ± 0.06*	7.10 ± 0.07*	6.39 ± 0.08*	7.48 ± 0.07*	6.45 ± 0.06*
14	3.28 ± 0.16	9.85 ± 0.08*	5.24 ± 0.09*	6.75 ± 0.05*	6.11 ± 0.17*	7.11 ± 0.07*	6.25 ± 0.10*
15	3.28 ± 0.16	9.31 ± 0.08*	5.12 ± 0.06*	6.46 ± 0.07*	5.55 ± 0.05*	6.80 ± 0.08*	5.86 ± 0.04*

Values are expressed as mean ± SD for six animals

Groups compared: GII vs. GI; GIII, GIV, GV, GVI, GVII vs. GII

Statistical significance: * - Significant at 5% level ($p<0.05$) ns - non significant

Induced (G-II) - (0.1ml 1 % Freund's complete adjuvant)

Standard (G-III) - Indomethacin (10 mg/kg /b.w. p.o.)

ESL LD (G-IV) - Low dose (200 mg/kg b.w. p.o.) of *E.serratus* leaf

ESL HD (G-V) - High dose (400 mg/kg b.w. p.o.) of E. *serratus* leaf

ESS LD (G-VI) - Low dose (200 mg/kg b.w. p.o.) of E. *serratus seed*

ESS HD (G-VII) - High dose (400 mg/kg b.w. p.o.) of E. *serratus seed*

Percentage Protection of Paw Volume

The investigation is based on the need for newer anti-arthritic agents from natural source with potent activity and lesser side effect. The percentage protection of paw volume by the ethanolic extracts of leaf was 50.86 and 60.84%, respectively at 200 and 400 mg/kg p.o. In the leaf extract the maximum protection percentage of paw edema due to FCA administration, was more pronounced at 400 mg/kg b.w. which was comparable with the standard drug indomethacin as depicted in Figure. 1.

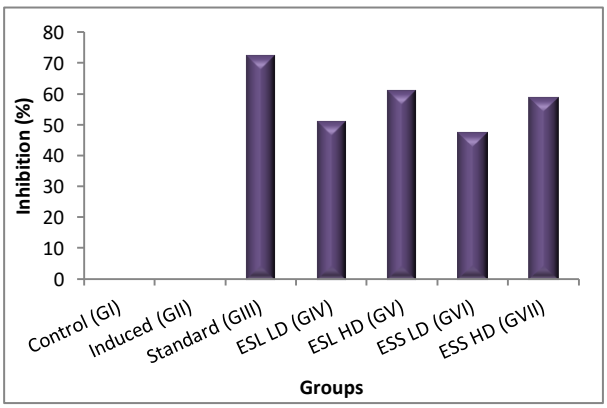

Figure 1: Effect of ethanolic extract of *E. serratus* on inhibition of FCA-induced paw edema in rats

Induced (G-II) - Administration of 0.1ml 1% Freund's complete adjuvant

Standard (G-III) - Administration of Indomethacin (10 mg/kg /b.w. p.o.)

ESL LD (G-IV) - Administration of Low dose (200 mg/kg b.w. p.o.) of *E. serratus* leaf

ESL HD (G-V) - Administration of High dose (400 mg/kg b.w. p.o..) of *E. serratus* leaf

ESS LD (G-VI) - Administration of Low dose (200 mg/kg b.w. p.o.) of *E. serratus* seed

ESS HD (G-VII) - Administration of High dose (400 mg/kg b.w. p.o.) of *E. serratus* seed

Evaluation of Biochemical Parameters in Blood Serum

In order to explore the effect of antioxidant defenses in the body of experimental animals during inflammation process, the antioxidant levels (SOD, CAT, GPx, GST and LPO) in the blood serum of normal group (group I) and experimental groups (group II to group VII) of rats were evaluated and depicted in Table 2. This table shows a highly significant reduction ($p< 0.05$) in the antioxidant status (SOD, CAT, GPx, and GST) in the FCA-induced arthritic rats (group II) as compared with the control animals (group I). However, the oral administration of the both the doses (200 and 400 mg/kg p.o.) of ethanolic extract of leaf and seed (group IV to group VII) significantly increased ($p<0.05$) the levels of SOD, CAT, GPx, and GST enzymes to near normalcy as in group III treated with the reference drug indomethacin (10 mg/kg b.w. p.o.). High dose (400 mg/kg p.o.) of leaf extracts exhibited remarkable antioxidant enzyme augmenting ability which was comparable to the standard drug.

Table 2: Antioxidant enzymes and LPO level in the blood serum of rats administrated with FCA, indomethacin and ethanolic extracts of leaf and seed of *E. serratus*

Groups	SOD µmoles/min/mg protein	CAT µmoles/min/mg protein	GPx µmoles/min/mg protein	GST µmoles/min/mg protein	LPO µmoles/mg protein
Control Group - I	0.62 ± 0.01	18.29 ± 0.16	88.23 ± 0.43	30.12 ± 0.10	10.40 ± 0.10
Induced Group - II	0.38 ± 0.02*	6.74 ± 0.39*	59.48 ± 0.58*	15.53 ± 0.44*	23.59 ± 0.28*
Standard Group- III	0.58 ± 0.01*	16.71 ± 0.36*	85.53 ± 0.51*	28.75 ± 0.38*	12.09 ± 0.11*
ESL LD Group- IV	0.45 ± 0.01*	10.02 ± 0.25*	70.35 ± 0.49*	20.53 ± 0.21*	19.30 ± 0.10*
ESL HD Group- V	0.57 ± 0.01*	15.49 ± 0.18*	83.20 ± 0.41*	25.48 ± 0.32*	12.24 ± 0.09*
ESS LD Group- VI	0.44 ± 0.01*	9.53 ± 0.30*	65.32 ± 0.36*	18.39 ± 0.18*	20.52 ± 0.34*
ESS HD Group- VII	0.47 ± 0.01*	11.64 ± 0.12*	76.68 ± 0.20*	22.51 ± 0.19*	15.47 ± 0.32*

Values are expressed as mean ± SD for six animals.

Groups compared: GII vs. GI; GIII, GIV, GV, GVI, GVII vs. GII

Statistical significance: * - Significant at 5% level ($p<0.05$) ns - non significant

Induced (G-II) - (0.1ml 1 % Freund's complete adjuvant)

Standard (G-III) - Indomethacin (10 mg/kg /b.w. p.o.)

ESL LD (G-IV) - Low dose (200 mg/kg b.w. p.o.) of *E. serratus* leaf

ESL HD (G-V) - High dose (400 mg/kg b.w. p.o.) of *E. serratus* leaf

ESS LD (G-VI) - Low dose (200 mg/kg b.w. p.o.) of *E. serratus* seed

ESS HD (G-VII) - High dose (400 mg/kg b.w. p.o.) of *E. serratus* seed

On the contrary, the level of LPO was found to be significantly increased ($p<0.05$) in the induced group (group II) as compared to the control rats (group I). The administration of high and low doses (200 and 400 mg/kg p.o.) of the ethanolic extract of leaf and seed (group IV to group VII) was significantly reduced ($p<0.05$) the levels of lipid peroxidation comparable to the standard. High dose of the leaf extract reduced the LPO level to 12.24 ±

0.09µmoles/mg protein which was closer to that of the standard drug treated group (12.09 ± 0.11µmoles/mg protein).

DISCUSSION

The Freund's complete adjuvant (FCA)-induced arthritis model in rat is the common mode[14]. Freund's Complete adjuvant (FCA) contains heat killed mycobacteria is a water-in-oil emulsion. After subcutaneous injection, FCA induces arthritis that can serve as a model to test the anti-arthritic and anti-inflammatory effects of investigational substances. The effects observed in this model seem to be parallel to that observed human diseases[15,16]. Due to inoculation of FCA, there was an increase in the ankle diameter where sings as an inflammation of ankle joint. The determination of swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation[17]. The increase in edema of hind paw after adjuvant infection in rat is paralleled by increased extra cellular activities of lysosomal enzymes. These enzymes are involved in the degradation of structural macromolecules in connective tissue and cartilage proteoglycans. They are also capable of destroying extra cellular activities by increased extra cellular activities of lysosomal enzymes. They are also capable of destroying extra cellular structures and may participate in mediating tissue injury in rheumatic diseases[18].

In the present findings, the anti-arthritic activity of ethanolic extracts of leaf and seed of *Elaeocarpus serratus* was estimated using the FCA-induced rat paw edema model. Both the plant extracts gave significant reduction ($p < 0.05$) of rat paw edema at all valuation times. High doses (400mg/kg p.o.) of plant extracts displayed profound anti-arthritic effect as compared to the control group. The significant ameliorative activity of the plant extracts and standard drug indomethacin detected in the present study may be due to inhibition of the mediators of inflammation. In a corresponding study, the anti-arthritic effects of ethanolic extract of seed of *Elaeocarpus sphaericus* at a dose of 250mg/kg p.o. on FAC-induced rat [19]. The ethanolic extract showed significant reduction in rat paw edema volume when compared to the standard drug prednisolone. A similar trend of observation was made in plants like *Merremia emarginata* [20], *Asystasia dalzelliana* [21], *Moringa oleifera*[22], *Piptadeniastrum africanum* [23] and *Schleichera oleosa*[24]. From the results it could be confirmed that the extract of *Elaeocarpus serratus* at the dose, 400 mg/Kg b.wt. Possessed a significant anti-arthritic activity.

CONCLUSION

This study confirmed the efficacy of *E. serratus* extracts as an anti- arthritic agent and also scientifically justified the use of this plant as an anti-edematous agent in traditional medicine. This finding justifies the preclinical efficacy and safety data, the *E. serratus* could be considered as safe and effective intervention for arthritis. Etiopathogensis of rheumatoid arthritis still remains obscure despite extensive research. Although the pathophysiology basis of rheumatoid arthritis is not yet fully understood, reactive oxygen species have been implicated in its pathogensis. A further research is on underway with its active principles to identify the extract mechanism of action.

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