Available online at www.ijpsdr.com

International Journal of Pharmaceutical Sciences and Drug Research 2015; 7(2): 172-175

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Anti Arthritic Activity of *Caesalpinia pulcherrima* against Type II Collagen Induced Arthritis in Experimental Rats

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ABSTRACT

Caesalpinia pulcherrima is a medicinal plant belonging to the family *Caesalpiniaceae*. The main aim of the present study was to investigate the Anti Arthritic activity of the ethanolic extract of *Caesalpinia pulcherrima* (ECP) at the dose of 200 mg/kg and 400 mg/kg on Type II Collagen induced Arthritis in experimental rats. In collagen induced arthritic rats, there was significant enhance in rat paw volume and reduce in body weight, whereas ECP and Aspirin treated groups showed significant decrease in paw volume and normal gain in body weight. The altered hematological parameters (Hb, RBC, WBC, ESR and CRP) in the arthritic rats were significantly recover to normal by administration of ECP. Further the radiological studies revealed the anti arthritic activity of ECP by indicating less abnormality in extract treated groups when compared to the arthritic control group. Findings from the present investigations suggest that the ethonolic extract of *Caesalpinia pulcherrima* exhibits significant anti arthritic activity.

Keywords: Anti Arthritic, ECP, Collagen, CRP.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder that can cause chronic inflammation of the joints and other tissues of the body. It can affecting 0.8-1% of the human population and characterized by a symmetric polyarthritis affecting multiple joints, synovial hyperplasia, permanent joint destruction and deformity. [1] Treatment of RA is spotlight on controlling symptoms and preventing joint damage.

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Received: 31 January, 2015; **Accepted:** 12 March, 2015

Non-steroidal anti-inflammatory drugs (NSAIDs), Disease-Modifying Anti-Rheumatic Drugs (DMARDS) and steroidal agents are generally used in treatment of RA. However, their side effects and toxicity identify for alternative, safer and more effective herbal products. [2] As a result, there is radically growing attractions in exploration of herbal medicines within the RA research area. Whole plant of *Caesalpinia pulcherrima* (family: Caesalpiniaceae) has been extensively used for various disease in indigenous system of medicine. Phytochemical examinations reveal the presence of pulcherrimin, flavonoids, terpenoids, carotenoids, glycosides, steroids, and phenols. [3] However there was no scientific report existing for its Anti-Arthritic activity. Type II collagen experimentally induces autoimmune disease within the suitable rodent strains.

[4] When compared by means of other animal models, the distinctiveness of the collagen induced arthritis (CIA) model look a lot like human RA more closely in its pathological, immunological, clinical and histological aspects. [5] In adding together, CIA facilitates the thoughtful of RA pathogenesis in human and help to make new therapeutic agents for RA. [6] Hence, this research was designed to study the anti arthritic effect of *C.pulcherrima* in experimental rats.

MATERIALS AND METHODS

Plant material

Whole plant of *Caesalpinia pulcherrima* was collected from local area of Kadapa District, Andhra Pradesh, India and taxonomically recognized and authenticated by Dr. K. Madava Chetty, botanist, Department of Botany, SV University, Tirupathi, India. A voucher specimen (no.SV-30472) was deposited in the Department of Pharmacology, PRRM College of Pharmacy, Kadapa, India. The whole plant was shade dried and coarsely powdered and stored in air tight vessels for further use.

Preparation of the extract

Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of the whole plant (1 kg) was soxhlet extracted with 90% ethanol. The extract obtained was concentrated under reduced pressure to yield ethanolic (18.70%) extract.

Experimental Animals

Healthy adult male albino rats of Wistar strain (150-200 g) were procured from Raghavendra enterprises, Bangalore, and they were housed under standard husbandry conditions, 25 ± 5°C temperature, and 12 h light/dark cycle with standard rat feed (Pranav Agro Ltd. India) with water *ad libitum*. The study was accepted by Institutional Animal Ethical Committee of PRRM College of Pharmacy (Ref no: 1423/PO/a/11/CPCSEA/02/2013).

Induction of arthritis

Rats were injected with 0.1 ml of type II collagen emulsified with Incomplete Freund's adjuvant (IFA) into the left hind foot on the initial day. Paw swelling in each animal was recorded with plethysmograph (PLM 01 Plus). Administration of test and standard drugs were ongoing on the next day and continued for 28 days. [7]

Experimental design

Total animals were randomly divided into five groups, each group comprising 6 animals and treated for 28 days. The standard drug aspirin and ECP were suspended in 1% carboxymethyl cellulose (CMC), made as a suspension and administered immediately. Group I served as normal (without treatment); Group II served as control (Arthritic control); Group III is treated with Aspirin (Standard); Group IV treated with ECP (200 mg/kg, *p.o*, Low dose); Group V administered with ECP (400 mg/kg, *p.o*, High dose). Paw was marked with ink at the level of the malleoluslaterials and paw volumes were recorded by the plethysmometer immediately after injection and on 7th, 14th, 21stand 28th day. [8] The body weight changes were observed on every week.

Hematological Parameters

White blood cell (WBC) and Red blood cell (RBC) counts were estimated as stated in the method of Chesbrough and McArthur. [9] Drabkin and Austin method was used to confirming the Hemoglobin (Hb) content. [10] Estimation of erythrocyte sedimentation rate (ESR) was carried out by the method of Westergren. [11] C-reactive protein (CRP) levels estimated by using ELISA kit obtained from Erba diagnostics, Delhi.

Radiographic analysis

The rats were anaesthetized using Ketamine (50 mg/kg, *i.p*.) and radiograph was recorded on a digital system and seimen's X-ray machine.

Statistical analysis

Results were analyzed using one way analysis of variance (ANOVA) followed by tukey test using statistical software package, Graph Pad Prism; version 5.03. Values were expressed as mean ± SEM and the $p<0.05$ were considered as statistically significant.

RESULTS

Paw volume

There was a significant increase in rat paw volume in collagen injected control rats when compared to normal groups. Groups treated with ECP at the dose of 200 mg/kg and 400 mg/kg showed significant reduction in paw volume when compared with the control group (Table 1) & (Figure 1).

Body weight changes

The challenge with collagen injected control rats showed remarkable decrease in the body weight as compared to the normal group. Under similar conditions, aspirin and all the ECP treated groups showed significant gain in body weight (Figure 2).

Hematological Parameters

In collagen induced untreated group, there was an incredible decrease in RBC count and hemoglobin and also a marked raise in WBC count and ESR. However treatment with ECP reversed these altered hematological parameters and the effects for ECP were found to be dose dependant. In control group rats showed significantly increasing the levels of CRP. However, a significant protective effect against this increase was observed in extract treated groups and standard treatment group (Table 2).

Radiographic analysis

The results were observed from X- ray was the normal group animals showed absence of soft tissue swelling and bony destruction. The arthritis control group animals was found with soft tissue swelling along with narrowing of joint spaces and sign of bony destruction. The treatment groups have shown prevention against bony destruction and narrowing of joint spaces by showing less soft tissue, swelling (Figure 3).

Table 1: Effect of ECP on Collagen Induced Paw volume

Groups	Paw volume (ml) (mean ± SEM) on				
	0 d	7th d	14th d	21st d	28th d
Group I	0.20±0.04	0.20±0.04	0.20±0.04	0.20±0.04	0.20±0.04
Group II	0.22±0.02	1.95±0.09###	2.27±0.08###	2.35±0.10###	2.32±0.06###
Group III	0.27±0.02	2.12±0.07	1.82±0.06**	1.22±0.06***	0.52±0.04***
Group IV	0.25±0.02	2.22±0.06	2.15±0.06	1.87±0.04**	1.85±0.11**
Group V	0.27±0.02	1.97±0.14	1.97±0.08*	1.32±0.04***	0.6±0.07***

All values are shown as mean ± SEM and n=6. ### indicate $p<0.001$ when compared to normal group. * indicate $p<0.05$, ** indicate $p<0.01$, *** indicate $p<0.001$ when compared to control group.

Table 2: Effect of ECP on hematological parameters

Groups	RBC (millions/mm³)	WBC (thousands/mm³)	Hb (g/dl)	ESR (mm/h)	CRP(µg/ml)
Group I	5.91±0.36	5.35±0.07	14.38±0.175	2.95±0.17	4.90±0.21
Group II	3.31±0.08###	13.38±1.3###	10.73±0.33###	10.67±0.45###	10.10±0.42###
Group III	5.50±0.12***	7.01±0.09***	13.94±0.17***	3.5±0.21***	5.74±0.40***
Group IV	4.25±0.09**	10.4±0.52*	11.78±0.143*	8.9±0.23**	6.90±0.71**
Group V	5.16±0.09***	7.33±0.14***	12.13±0.209**	4.47±0.12***	5.86±0.33***

All values are shown as mean ± SEM and n=6. ### indicate $p<0.001$ when compared to normal group. ** indicate $p<0.01$, *** indicate $p<0.001$ when compared to control group.

DISCUSSION

Collagen induced Arthritis in rats is a well reputable animal model for study the pathophysiology of RA. [8] The initial reaction of edema and soft-tissue thickening at the depot site in this model is caused by the irritant effect of the adjuvant, whereas the late-phase arthritis and flare in the injected foot are presumed to be immunologic events of cell-mediated immunity. It results in noticeable cartilage destruction connected with immune complex deposition on articular surfaces, bone resorption as well as inflammation. Considering these advantages the study was designed to assess the anti arthritic potential of *Caesalpinia pulcherrima* against experimental induced arthritis in rats.

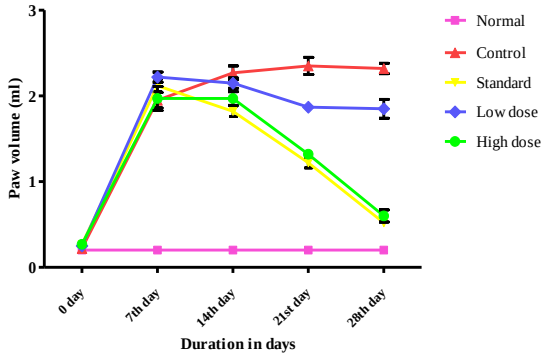

Fig. 1: Effect of ECP on Paw Volume

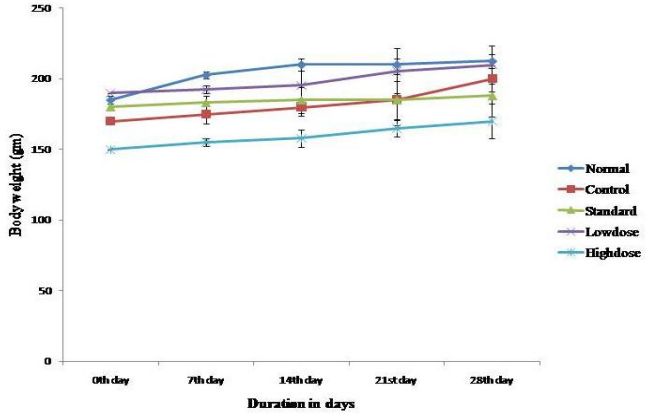

Fig. 2: Effect of ECP on Body Weight

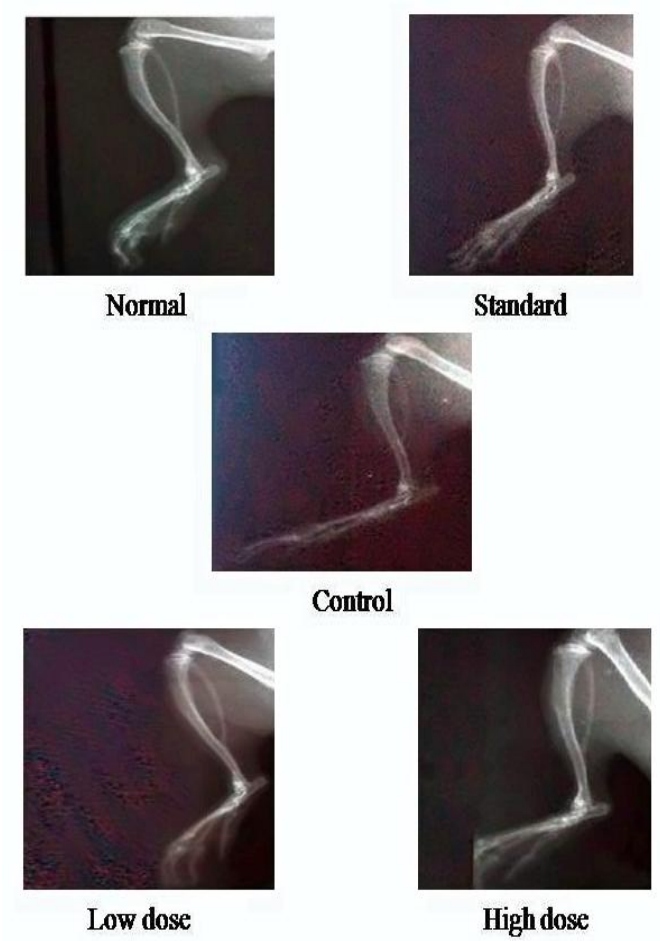

Fig. 3: Radiology of hind legs in collagen induced arthritic rats

Paw swelling is one of the indexes for the measurement of anti arthritic activity of various drugs and here it is employed to determine the anti arthritic activity of ECP at the dose of 200 mg/kg & 400 mg/kg. Extract administered groups showed marked decrease in paw volume when compared with the arthritic control group. Body weight changes have been used to evaluate the course of disease and the response to therapy of drugs. Treatment with ECP has been shown significant increase in body weight gain when compared to control.

This study demonstrated that control rats showed a drastic reduced RBC count, reduced Hb levels, and an increased erythrocyte sedimentation rate (ESR). All these symptoms indicate an anemic condition. [12] The *C. pulcherrima* extract administered groups showed a significant improvement from the induced anemia.The significant increase in leukocyte count in collagen induced arthritic rats may be due to the stimulation of immune system against the invading antigens and significant reduce in *C. pulcherrima* treated groups showed its immunomodulation effect. This clearly indicates the anti-arthritic potential of *C. pulcherrima*.

C-reactive protein is a constituent of the class of acute phase proteins. Its levels increase dramatically during inflammatory processes. [13] In the present study the concentration of C-reactive protein was found to be significantly decreased in the extract treated as well as the aspirin treated groups.

Radiographic changes in RA conditions are useful diagnostic measures which indicate the severity of the disease. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages of arthritis. [14] In adjuvant induced arthritic rat, soft tissue swelling along with narrowing of the joint spaces were observed which implies the bony destruction in arthritic condition. The group receiving ECP at dose 400 mg/kg has shown significant prevention against bony destruction by showing less soft tissue swelling and narrowing of joint spaces when compared to arthritic control group. The radiological studies strongly support that the plant having anti arthritic activity.

Phytochemical investigations on *C. pulcherrima* have shown the presence of pulcherrimin, flavonoids, terpenoids, carotenoids, glycosides, steroids, and phenols. [3] These components may exert its anti-inflammatory activity by inhibiting the 5-lipoxygenase pathway, which together with the COX-2 pathway, is very important in producing and maintaining inflammation. This previously has been shown to possess anti-inflammatory property of *C. pulcherrima.* [3] The presence of these compounds in the *C. pulcherrima* extract may explain the anti-arthritic properties of this plant. Taken together, our results strongly support the anti-arthritic potential of the plant *C. pulcherrima* and its use in traditional medicine.

Both doses of ECP showed significant reduction in rat paw volume and it could normalize the hematological abnormalities in collagen induced arthritic rats. Further the radiological studies confirmed the anti arthritic activity of ECP in collagen induced arthritis. From the observed findings, it may be concluded that *C. pulcherrima has* anti arthritic activity.

ACKNOWLEDGMENT

The author thanks to the management of P. Ramireddy Memorial College of Pharmacy for providing facilities and grants necessary to carry out the work.

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Source of Support: Nil, Conflict of Interest: None declared.