

Anti-arthritic property of the ethanolic leaf extract of *Cardiospermum halicacabum* Linn

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ABSTRACT

In this study, the anti-arthritic effect of oral administration of ethanolic extract of *Cardiospermum halicacabum* leaves (CEE) at the dose of 125 mg/kg and 250 mg/kg on Freund's complete adjuvant (FCA) induced arthritis has been studied in rats. The treatment is assessed by measuring the paw volume and by using various hematological parameters like hemoglobin (Hb) content, total red blood cell (RBC) count, white blood cell (WBC) count and erythrocyte sedimentation rate (ESR). The investigated result showed that the extract inhibited the FCA induced arthritis in a dose dependent manner and this effect was more significant ($p < 0.001$) with 250 mg/kg dose. Administration of extract improved the body weight significantly when compared to FCA induced arthritis rats. The results were compared to that of Indomethacin (10 mg/kg). The results suggest that the ethanolic extract of *Cardiospermum halicacabum* leaves exhibits significant anti-arthritic effect.

Key words: *Cardiospermum halicacabum*, Freund's complete adjuvant, Arthritis, Erythrocyte sedimentation rate

INTRODUCTION

Inflammatory diseases including different types of rheumatic diseases are a major cause of morbidity of the working force throughout world. This has been called the 'King of Human Miseries'¹. The immune system is a well-organized and well-regulated system. The deregulation of the immune system may lead to the development of autoimmune diseases. Rheumatoid arthritis is a prototype of such groups of illness with chronic systemic disorders to be considered an autoimmune disease with destructive inflammatory polyarticular joint potentially resulting in progressive destruction of articular and periarticular structure. Persistent inflammation produces swollen joints with severe synovitis, decreased nociceptive threshold and massive sub-synovial infiltration of mononuclear cells, which along with angiogenesis leads to pannus

formation. Expansion of the pannus induces bone erosion and cartilage thinning, leading to the loss of joint function^{2,3}.

In India, many Ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic conditions⁴. Although the applications of these medicaments have sound tradition and a rational background according to the Indian system of medicine, perhaps it is essential to investigate the rationality of their use in modern scientific terms.

The plant *Cardiospermum halicacabum* Linn. (Family: Sapindaceae) is a climbing plant widespread distributed in tropical and subtropical Africa and Asia, often found as a weed along roads and rivers. Indian system of medicine recommends *Cardiospermum halicacabum* leaves for

rheumatism, chronic bronchitis, stiffness of limbs and snakebite⁵. It is known to contain saponin, quebrachitol, apigenin, proanthocyanidin, stigmasterol^{6,7}, trace of alkaloids, flavanoids, proanthocyanidin and phytosterols. Plant leaf possesses saponins, alkaloids, several flavanoids such as apigenin, pinitol, luteolin and chrysoeriol⁸. In the indigenous system of medicine, the leaf extract of *Cardiospermum halicacabum* reported to be useful in the treatment of rheumatoid arthritis. In recent years, the extract of leaves of *C. halicacabum* has been extensively studied for anti-inflammatory activity^{9,10}.

In view of the importance of this herbal plant the present study aims to evaluate the comparative therapeutic effects of *Cardiospermum halicacabum* against Freund's complete adjuvant induced arthritis in rat model which is the best and most widely used experimental model for arthritis with clinical and laboratory features which closely mimic the clinical features of human rheumatoid disease^{11,12}.

MATERIAL AND METHODS

Plant material

Leaves of *Cardiospermum halicacabum* were collected from the local areas of Berhampur, Orissa, India. The plant was botanically identified and authenticated by Prof. K. Srinivasa Rao, Roland Institute of Pharmaceutical Sciences and voucher specimen was deposited in the department herbarium.

Preparation of plant extract

The plant leaves were shade dried at room temperature ($32\pm 2^\circ\text{C}$) and the dried leaves were ground in to fine powder using pulverizer. The powdered part was sieved and kept in deep freezer until use. 100 g of dry fine powder was suspended in 300 ml of ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at $40\pm 5^\circ\text{C}$.

Animals

Albino rats (175-200 g) procured from Mahaveer Enterprises, Hyderabad, India were used in the study. They were maintained under standard laboratory conditions at ambient temperature of

$25\pm 2^\circ\text{C}$ and $50\pm 15\%$ relative humidity with a 12-h light/12-h dark cycle. Rats were fed with a commercial pellet diet (Rayans Biotechnologies Pvt Ltd., Hyderabad) and water *ad libitum*. The experiments were performed after prior approval of the study protocol by the institutional animal ethics committee of Roland Institute of Pharmaceutical Sciences, Berhampur, India. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs/Chemicals

Indomethacin was supplied by Recon, Bangalore, India. All other chemicals used for this study were of analytical grade.

Pharmacological experiment

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines of CPCSEA.^[13] One tenth of the medium lethal dose (LD_{50}) was taken as an effective dose¹⁴.

Freund's complete adjuvant (FCA)

5 mg of heat killed mycobacterium tuberculosis cell (being killed at 60°C in 15-20 min in the autoclave) was finely ground using a mortar and pestle. Sufficient liquid paraffin was added and thoroughly triturated to make a 5 mg/ml suspension.

Incomplete Freund's adjuvant

The liquid paraffin is referred in the study as Incomplete Freund's adjuvant.

Induction of arthritis

Albino rats of either sex were divided in to five groups of six animals each.

Group-1	:	Vehicle control (0.5 ml normal saline)
Group-2	:	Arthritis control (0.5 ml normal saline)
Group-3	:	CEE-125 mg/kg/day, p.o
Group-4	:	CEE-250 mg/kg/day, p.o
Group-5	:	Indomethacin-10 mg/kg/day, p.o

The method described by Newbould¹⁵ was employed with some modifications. Adjuvant arthritis was induced by subcutaneous injection of FCA (0.1

ml) in to sub plantar tissue of the right hind paw of each rat. The test groups consisted of FCA-injected rats challenged with the respective doses of the test drugs administered orally 24 h before FCA injection while, the vehicle control rats were injected with 0.1 ml of liquid paraffin (incomplete Freund's adjuvant) only. The drug treatments were continued daily on the same time after the challenge for 20 more days.

The swelling in the injected and contralateral hind paws of the rats were monitored daily using liquid displacement plethysmometer (Ugo Basile, Italy). Increase in the extent of erythema and edema of the tissues shows the severity of the inflammation. The difference in severity of arthritis between the experimental groups and arthritis control group were statistically analyzed. The changes in body weight were recorded daily.

Biochemical parameters

At 20th day blood was withdrawn through retro orbital vein puncture of all groups and the biochemical parameters were analyzed. Hemoglobin (Hb) content was estimated by the method of Drabkin and Austin¹⁶. Red blood cell (RBC) and White blood cell (WBC) counts were

estimated according to the method of Chesbrough and Mc Arther¹⁷ in an improved Neubauer chamber. Estimation of erythrocyte sedimentation rate (ESR) was followed by the method of Westergren¹⁸.

Statistical analysis

The experimental results were expressed as mean \pm SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnet's 't' test.

RESEULTS

Acute toxicity studies

From the acute toxicity study, the LD₅₀ cut-off dose for ether extract was found to be 2500 mg/kg body weight. Hence one tenth of LD₅₀ dose (250 mg/kg) of extract was selected as maximum therapeutic dose and 125 mg/kg was selected as lower dose for the study.

Effect of cee on primary response of FCA induced arthritis rat

The paw volume of the right paw was measured and taken into consideration for evaluating the possible anti-inflammatory effect of CEE on rheumatoid arthritis. After the onset of inflammation the peak incidence in swelling reached during 4th-6th day with the increase in paw volume

Table 1: Effect of cee against freund's complete adjuvant induced paw edema on injected paw (primary response)

Post insult time in days	Swelling volume (ml) on injected paw			
	Arthritis Control	CEE-125 (mg/kg)	CEE-250 (mg/kg)	Indomethacin-10 (mg/kg)
01	0.69 \pm 0.03	0.63 \pm 0.06	0.57 \pm 0.04*	0.54 \pm 0.03*
02	0.83 \pm 0.04	0.72 \pm 0.03*	0.69 \pm 0.04**	0.65 \pm 0.05**
04	1.01 \pm 0.06	0.81 \pm 0.03**	0.77 \pm 0.05**	0.71 \pm 0.03**
06	1.14 \pm 0.08	0.89 \pm 0.03**	0.73 \pm 0.04**	0.69 \pm 0.04**
08	0.88 \pm 0.02	0.76 \pm 0.04*	0.70 \pm 0.03**	0.64 \pm 0.03**
12	0.80 \pm 0.02	0.65 \pm 0.04*	0.61 \pm 0.04**	0.54 \pm 0.04**
16	0.81 \pm 0.02	0.62 \pm 0.06**	0.56 \pm 0.04**	0.58 \pm 0.04**
18	0.83 \pm 0.04	0.66 \pm 0.05**	0.53 \pm 0.04**	0.49 \pm 0.05**
20	0.87 \pm 0.03	0.67 \pm 0.04**	0.50 \pm 0.03**	0.45 \pm 0.06**

CEE: Ethanolic extract of *Cardiospermum halicacabum* leaves

All values as mean \pm SEM

* Significance at p<0.05

** Significance at p<0.001

at the maximum of 1.14 ml for the arthritis control. The edema on the animals treated with CEE (250 mg/kg) and Indomethacin (10 mg/kg) group began to subside gradually ($p < 0.001$) when compared with arthritic control. The effect of CEE-250 mg/kg on this primary reaction was found to be high at the earlier of 2nd day after FCA injection and was maintained until the termination of the experiment. Therefore CEE-250 mg/kg observed for its significant effect in preventing the primary systemic response and it is capable of inhibiting the development phase of arthritis and this effect was

compared with the standard indomethacin-10 mg/kg (Refer Table 1).

Effect of cee on secondary response of FCA induced arthritis rat

The latent secondary response that occurs after few days and characterized by joint swelling and nodule formation in the contralateral paw was first evident on the 7th day. The administration of CEE (250 mg/kg) significantly ($p < 0.05$) protected against joint swelling in arthritis-induced paw when compared with arthritis control group. But the

Table 2: Effect of cee against freund's complete adjuvant induced paw edema on contralateral paw (secondary response)

Post insult time in days	Swelling volume (ml) on injected paw			
	Arthritis Control	CEE-125 (mg/kg)	CEE-250 (mg/kg)	Indomethacin-10 (mg/kg)
07	0.29 ± 0.03	0.24 ± 0.05	0.17 ± 0.05*	0.18 ± 0.03
09	0.38 ± 0.03	0.31 ± 0.04	0.21 ± 0.05*	0.19 ± 0.03*
11	0.46 ± 0.04	0.42 ± 0.05	0.30 ± 0.03*	0.25 ± 0.03*
13	0.70 ± 0.04	0.56 ± 0.06*	0.49 ± 0.05*	0.37 ± 0.04**
15	0.87 ± 0.06	0.68 ± 0.05*	0.53 ± 0.06**	0.42 ± 0.04**
17	0.92 ± 0.05	0.75 ± 0.05*	0.57 ± 0.05**	0.51 ± 0.05**
20	1.2 ± 0.07	0.79 ± 0.05*	0.64 ± 0.04**	0.59 ± 0.05**

CEE: Ethanolic extract of *Cardiospermum halicacabum* leaves

All values as mean ± SEM

* Significance at $p < 0.05$

** Significance at $p < 0.001$

Table 3: Effect of cee on hematological parameters against freund's complete adjuvant induced arthritis in rats

Group	Changes in hematological parameters			
	Total WBC count (Cells/cu.mm)	RBC count (Million/cu.mm)	Hb(gm%)	ESR(mm/hr)
Arthritis Control	7.86 ± 0.09	5.21 ± 0.11	13.74 ± 0.26	4.06 ± 0.18
CEE-125 mg/kg	7.26 ± 0.12**	5.38 ± 0.10	14.11 ± 0.13	3.51 ± 0.18*
CEE-250 mg/kg	7.04 ± 0.09**	5.59 ± 0.15	14.51 ± 0.14**	3.23 ± 0.16**
Indomethacin-10 mg/kg	7.16 ± 0.12**	5.23 ± 0.13	14.09 ± 0.13	3.26 ± 0.13**

CEE: Ethanolic extract of *Cardiospermum halicacabum* leaves

All values as mean ± SEM

* Significance at $p < 0.05$

** Significance at $p < 0.01$

significant reduction first found only from day 11 to 13. However the effect of CEE-250 mg/kg treatment was found to be significant ($p < 0.001$) from the initial stage of secondary response and maintained through out the experiment and shows $p < 0.001$ level significance during 15 to 19 days after FCA injection as that of the group treated with the reference standard Indomethacin-10 mg/kg (Refer Table 2).

Biochemical parameters

As shown in Table 3 standard drug (Indomethacin) and ethanolic extract have shown the increase in hemoglobin content and RBC count compared to arthritic control. The total WBC counts were remarkably increased in arthritic control group.

However, indomethacin and CEE treated group significantly decreased ($p < 0.01$) the total WBC count. The drastic increase in ESR count in arthritic control group has been remarkably counteracted by the standard and ethanol extract, restoring it back to normal thus justifying its significant roles in arthritic conditions.

Effect on body weight:

The arthritic control animals exhibited a significant decrease in body weight when compared with vehicle control group. The result showed the indomethacin-10 mg/kg and CEE-250 mg/kg could ameliorate the weight loss occurred during arthritis (Refer Table 4).

Table 4: Effect of cee on body weight against freund's complete adjuvant induced arthritis in rats

Group	Body weight (g)		% increase in body weight
	Initial	Final	
Vehicle Control	191.50 ± 7.12	246.83 ± 9.27*	29.04
Arthritis Control	188.83 ± 7.46	194.00 ± 10.33	2.75
CEE-125 mg/kg	195.17 ± 8.08	211.50 ± 10.69	11.5
CEE-250 mg/kg	190.33 ± 9.26	235.26 ± 11.41*	23.73
Indomethacin-10 mg/kg	193.00 ± 7.75	231.30 ± 12.47*	20.15

CEE: Ethanolic extract of *Cardiospermum halicacabum* leaves

All values as mean ± SEM

* Significance at $p < 0.05$

DISCUSSION

Freund's complete adjuvant (FCA) induced arthritis models are extensively used to study the pathogenesis of rheumatoid arthritis for testing therapeutics [19]. One of the reasons for the wide utilization of this model is due to the strong correlation between the efficacy of therapeutic agents in this model and in rheumatoid arthritis in humans and it is characterized by very rapid erosive disease²⁰. Paw swelling is one of the major factors in assessing the degree of inflammation and therapeutic efficacy of the drugs²¹.

After FCA injection on the rat hind paw, a pronounced swelling and hyperalgesia appeared with no involvement of contralateral paw. This response is usually considered as a primary

reaction. There is also a delayed hypersensitive response which is considered as latent secondary systemic response known to induce arthritis occurs after few days on the contra lateral paw and characterized by tibiotarsal joint swelling and nodule formation in the tail. According to our result and investigation more pronounced and reliable anti-inflammatory activity was observed in CEE-250 mg/kg, which significantly ($p < 0.001$) inhibited the development phase of chronic joint swelling induced by FCA on both the paws. The activity exhibited by extract was in dose-dependent manner. Adjuvant arthritis is characterized by reduced weight loss [22] and the body weight loss is associated with increased production of pro-inflammatory cytokines such as TNF- α and interleukin-1²³. Treatment with CEE extract showed significant ($p < 0.05$) increase in body weight as that of vehicle control group.

In the present study, the arthritic rats exhibited a reduced RBC count, reduced Hb level and an increased ESR. All these indicate the anemic condition which is a common diagnostic feature in patients with chronic arthritis^{24,25}. The treatment with the *Cardiospermum halicacabum* extract improved the RBC count, Hb level and the ESR to a near normal level indicating the significant recovery from the anemic condition thus justifying its significant role in arthritic conditions²⁶.

White blood cells (WBC) are a major component of the body's immune system. Indications for a WBC count include infectious and inflammatory diseases²⁷. WBC count was increased in arthritic group. The migration of leukocytes is significantly

suppressed in extract treated groups as seen from the significant decrease in the WBC count.

From the results observed in the current investigation, it may be concluded that the ethanolic extract of *Cardiospermum halicacabum* leaves possesses potentially useful anti-arthritic activity. This study warrants the investigation to isolate and identify the active principles and to elucidate the exact mechanism of action.

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