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RESEARCH ARTICLE

Anti-inflammatory and Anti-ulcerogenic Effect of *Crotalaria juncea* Linn. in Albino Rats

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ABSTRACT

The aim of this study was to evaluate the antiarthritic activity of ethanolic extract of the leaves of *Crotalaria juncea* (CJE) in Complete Freund's Adjuvant (CFA) induced arthritis model in rats, and also to evaluate anti-ulcerogenic activity of CJE.Arthritis was induced in male albino Wistar rats by injection of CFA (0.1 mL) into the left foot pad of the animals. Treatment with CJE at 200 and 400 mg/kg and standard indomethacin (0.3 mg/kg) was started on the same day and continued up to day 12. The paw volume was measured on day 1, 5, 12 and 21 for both the paws and antiarthritic activity was evaluated. Anti-ulcerogenic potential of CJE was also evaluated. For possible mechanism of anti-ulcerogenic potential, appetite suppressant activity was recorded. The drug CJE produced reduction in the inflammation of the paw produced due to CFA. The antiarthritic action started on the day 5 and continued till day 12 and the activity was comparable to that of the standard on both days. In indomethacin treated animals ulcer was observed, where as CJE was found to protect the animals from ulcer formation which may be due to appetite suppressant activity.CJE significantly inhibited adjuvant induced arthritis and has significant anti-inflammatory effect (p<0.001). It has anti-ulcerogenic property compared to indomethacin, which may be due to appetite suppressant activity.

Keywords: *Crotalaria juncea, Antiarthritic, CFA*

Crotalaria juncea is commonly known as Sun hemp or Indian hemp growing wild in South India that belongs to the family Leguminoceae. It is a short, erect, shrubby annual herb, generally 1 to 4 m in height. Leaves are simple, spirally arranged along the stem and available throughout the year [1]. *Crotalaria juncea* seed is reported to have several pyrrolizidine alkaloids [2]. It is also reported to possess antispermatogenic activity [3] in men and contraceptive activity in female [4]. Since this plant is used for antiarthritic effect in the folklore medicine, the present study is focused a3t its antiarthritic and anti-ulcerogenic property, hence suitability of the extract for chronic therapy in animal model.

Rheumatoid arthritis (RA) is a chronic autoimmune disease in which there is inflammation of joints, synovial proliferation and destruction of articular cartilage [5]. Although a number of drugs (non-steroidal or steroidal anti-inflammatory agents and immunosuppressants) being used in the treatment of RA have been developed over the past few decades, there is still an urgent need for more effective drugs with lower side effects [6].

One of the widely used models for studying the anti-inflammatory/anti-rheumatic properties of compounds is Complete Freund's Adjuvant (CFA) induced arthritis in rats [7]. It is an experimental immunopathy that is thought to share many features with human rheumatoid arthritis [8].

Most of the anti-inflammatory drugs are ulcerogenic. From this viewpoint, in the present study, antiulcerogenic property of CJE is also recorded.

MATERIAL AND METHODS

Animals

Male albino Wistar rats weighing between 150–250 g were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*. The experiments were performed followed by approval from Animal Ethical Committee of the Establishment.

Table 1. Table showing the data of antiarthritic activity of *Crotalaria juncea* extract

Treatment	% Edema Rate (ER)					
	Left (Injected) Paw			Right (Non-Injected) Paw		
	Day 5	Day 12	Day 21	Day 5	Day 12	Day 21
Normal control	0±0	0±0	0±0	0±0	0±0	0±0
Arthritic Control	100±0	400±68.32	500±68.30	0±0	150±42.88	183.34±16.66
Standard (Indomethacin 10 mg/kg)	116.66±40.82	100±22.36***	83.32±27.88**	25±17.08	16.66±16.66**	16.66±16.66*
CJE (200 mg/kg)	183.32±98.32	283.32±98.32*	450±34.16	0±0	66.66±21.08*	116.66±16.66
CJE (400 mg/kg)	183.32±147.20	100±25.82***	433.33±102.20	0±0	16.66±16.66**	166.66 ± 33.34
One-way ANOVA F	0.8626	2.917	15.904	2.143	6.618	18.36
df	(4, 25)	(4, 25)	(4, 25)	(4, 25)	(4, 25)	(4, 25)
p	0.4998	0.4998	0.0001	0.1052	0.0009	0.0001

Values are mean ± SEM of 6 animals.
One-way ANOVA followed by Newman-Keuls' Multiple Comparison test.
* $p<0.05$, ** $p<0.01$, ***$p<0.001$ as compared to arthritic control.

Preparation of the Plant Extract

Leaves of *Crotalaria juncea* Linn were collected during July 2004 from Madurai, Tamil Nadu and identified by Society for Health, Environment and Research on Biodiversity, Pondicherry, India, where a voucher specimen was deposited. The leaves were carefully dried under shade and were extracted with alcohol three times. The extracts were combined and distilled to remove alcohol. The filtrate was concentrated, dried under vacuum and final extract was dissolved in distilled water for use.

Acute Toxicity Studies

Acute toxicity studies were carried out following OECD guidelines [9] and was found to be safe up to 2000 mg/kg body weight in albino Wistar rats.

Antiarthritic Activity

Antiarthritic activity of CJE was studied using Complete Freund's adjuvant induced arthritis model [10].

Thirty rats were randomly divided into five groups of six animals each and treated for 12 days. Group I served as normal control, Group II as arthritic control, Group III as standard which received 10 mg/kg indomethacin (p.o.), Group IV and V received 200 mg/kg and 400 mg/kg of CJE respectively orally and served as test groups.

All the animals except normal control group were injected with 0.1 mL of CFA in the subplantar region of the left hind paw.

On day 1, body weight and paw volumes of all the animals were measured using plethysmograph. The treatment with standard drug and CJE started on the same day and continued till day 12. The body weight and paw volumes were measured on day 5, 12 and 21. The edema rate (ER) and inhibition rate (IR) of each group were calculated as follows [11]:

$$ER\% = \frac{V_t - V_0}{V_0} \times 100$$

where V_o is the volume before CFA injection (mL); V_t the volume at day t after CFA injection (mL).

$$IR\% = \frac{E_c - E_t}{E_c} \times 100$$

where E_c is the edema rate of control group and E_t is the edema rate of treated group.

Anti-ulcerogenic Activity

Twenty four albino Wistar rats were divided into 4 groups of six animals each and treated for 12 days with standard and CJE. Group I served as normal control, Group II was treated with indomethacin 10 mg/kg orally, Group III and IV were treated orally with 200 mg/kg and 400 mg/kg of CJE respectively.

At the end of 12 days treatment, the animals were fasted for 24 hours and sacrificed. The stomach was cut open along the lower curvature and ulcer index was calculated. Scoring of the ulcers was done as follows [12]:

0 – Normal colored stomach
0.5 – Red colouration
1 – Spot ulcers
1.5 – Hemorrhagic streaks

Table 2. Appetite suppressant activity of *Crotalaria juncea* Linn

Treatment	Difference in Body Weight (g)		
	Day 5	Day 12	Day 21
Normal control	3.75 ± 1.25	2.50 ± 1.44	0 ± 0
Arthritic Control	10.0 ± 3.54	5.0 ± 2.04	6.24 ± 3.15
Standard (Indomethacin 10 mg/kg)	0 ± 0	5.84 ± 1.52	4.16 ± 5.38
CJE (200 mg/kg)	-26.66 ± 3.08**	-21.66 ± 7.82**	20.0 ± 2.88**
CJE (400 mg/kg)	-9 ± 2.92*	-6 ± 5.1	21 ± 1.86**
One-way ANOVA F	31.528	6.916	9.108
df	(4, 25)	(4, 25)	(4, 25)
p	0.0001	0.0007	0.0001

'–' indicates decrease in body weight
Values are mean ± SEM of 6 animals.
One-way ANOVA followed by Newman-Keuls' Multiple Comparison test.
* $p<0.05$, ** $p<0.001$ as compared to arthritic control.

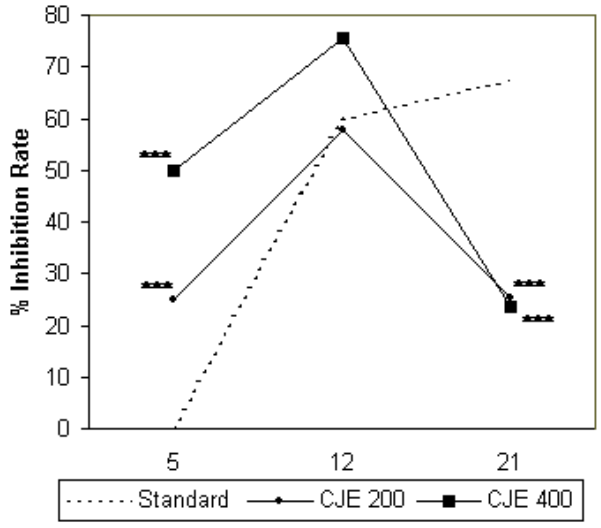

Fig 1. Percentage inhibition rate of *Crotalaria juncea* Linn. on CFA induced ANOVA followed by Student Newman-Keul's multiple comparison test. n = 6. *** $p < 0.001$ compared to standard.

2 – Ulcers > 3 but < 5
3 – Ulcers > 5

Ulcer index was calculated [10].

Statistical Analysis

The results are expressed as mean ± SEM. The data were analysed by One-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test.

RESULTS

On day 5, there was no significant decrease in percentage edema rate compared to arthritic control in all the treatment groups. On day 12, there was a significant ($p<0.05$ and $p<0.01$) decrease in the percentage edema rate of both the groups of 200 and 400 mg/kg CJE respectively compared to the arthritic control (Table 1). None of the animals showed signs of development of secondary lesions.

The anti-inflammatory activity of CJE at both the doses were comparable to that of the standard on day 12. Percentage edema rate in the non-injected paw was also reduced in CJE 200 mg/kg and 400 mg/kg treated animals significantly ($p<0.05$ and $p<0.001$ respectively) compared to the arthritic control on day 12.

There was no significant antiarthritic activity on day 21, in paws of the CJE treated groups compared to arthritic control. Indomethacin treated animals showed decrease in edema rate ($p<0.01$) even on day 21 which was not observed with CJE treated animals.

IR at both the doses was found to be more than the standard on day 5, whereas on day 12, IR at both doses was comparable to that of standard and on day 21, after the treatment was withdrawn, IR of the treatment groups was less than the standard as shown in (Fig 1).

Oral administration of indomethacin (10 mg/kg body weight) caused ulcer formation and ulcer index was found to be 17 compared to 0 of both 200 mg/kg and 400 mg/kg of CJE and 1 of normal control as shown in Fig 2A, 2B, 2C and 2D.

CJE treated animals (200 and 400 mg/kg) significantly ($p<0.001$ and $p<0.05$) decreased the body weight, which was observed on day 5 and day 12. However the decrease in body weight was observed only with CJE treated groups and not with arthritic control or standard.

On withdrawal of CJE after 12 days treatment, there was a significant ($p<0.01$) increase in the body weight of the animals when measured on day 21 as shown in (Table 2).

DISCUSSION

Rat adjuvant-induced arthritis is a model of chronic polyarthritis with features that resemble RA [13]. Therefore, therapeutic efficacy was mainly investigated in the rat adjuvant arthritis model in present study. All animals tolerated the experimental procedures well and no death up to the study termination by day 21 was observed. Dosage selection for CJE (200 and 400 mg/kg) was based on acute toxicity studies.

CJE significantly inhibited the development of chronic joint swelling induced by CFA in rats for 12 days whereas the standard indomethacin produced anti-arthritic effect till day 21. The effect of CJE was dose-dependent and linear, but for a short period compared to the standard.

During antiarthritic activity, the body weight of all groups of animals were recorded. Treatment with CJE for 12 days significantly reduced the body weight of the animals and the body weight returned to normal after withdrawal of CJE. This decrease in body weight was not observed in any other animals in normal and standard groups. This may be due to CJE alone and not due to any biological factor or disease progression. This indicates the role of CJE in appetite suppressant activity.

Since indomethacin is a well-known ulcerogenic drug, the possibility of CJE producing ulcer was evaluated. CJE was found to be anti-ulcerogenic compared to the normal control and indomethacin group of animals, a definite advantage in chronic therapy in RA.

The current study relates the anti-ulcerogenic effect of extract under study to appetite suppressant effect, thus inhibiting gastric acid secretion, an important factor in ulcer formation, as observed in Garcinia cambogia treated rats [14].

A significant reduction in the number of ulcer lesions in the pretreated Garcinia cambogia animals may be due to the appetite suppressant effect of the drug, thus inhibiting gastric acid secretion, an important factor in ulcer formation [14] have been reported.

In conclusion, this report clearly showed CJE significantly inhibited adjuvant induced arthritis in rats. It also possesses anti-ulcerogenic property which may be due to its appetite suppressant effect. This indicates that the significant decrease in the paw volume on day 12 was due to the CJE alone and not due to any other biological reasons. However, the present investigation requires the clinical trails to substantiate the report.

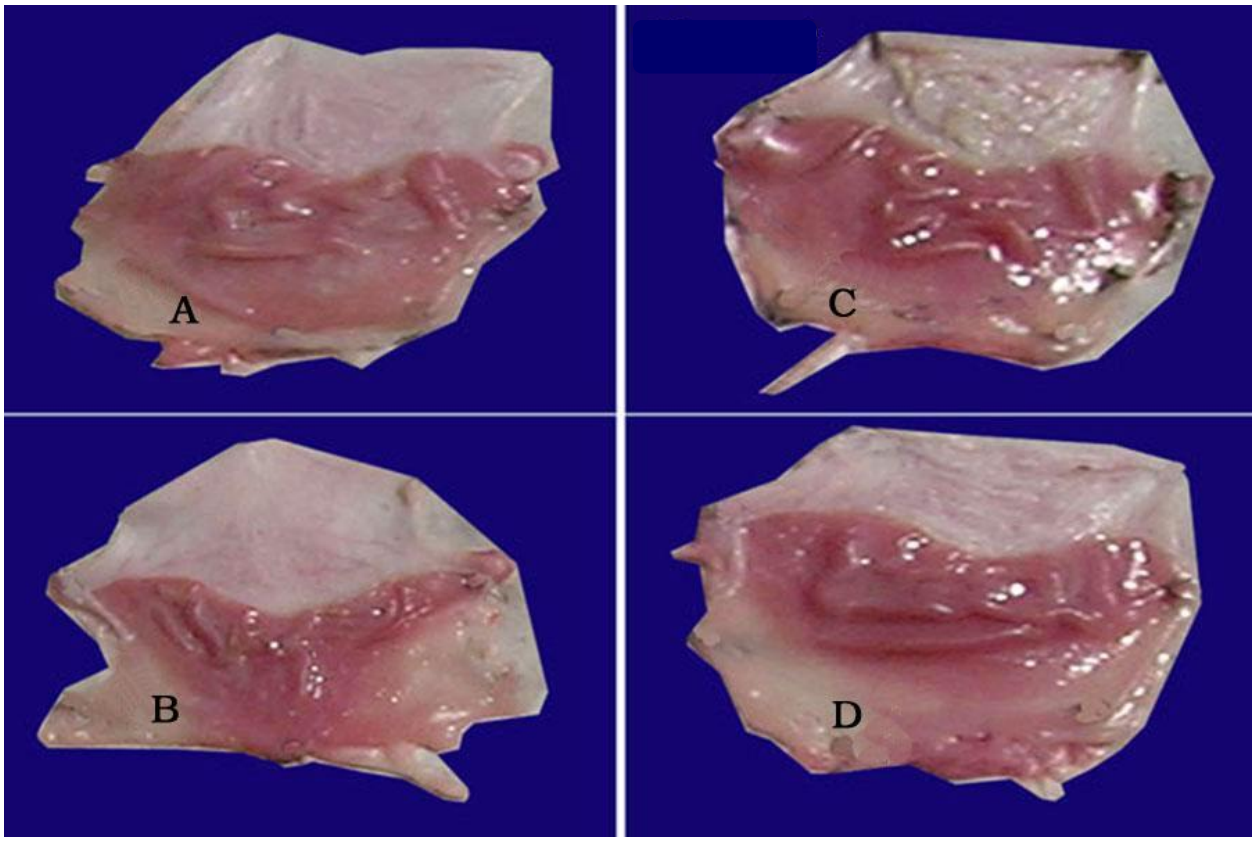

Fig 2. Photograph showing anti-ulcer activity of *Crotalaria juncea*. (A) *Crotalaria juncea* extract (200 mg/kg) treated. (B) *Crotalaria juncea* extract (400 mg/kg) treated. (C) Indomethacin (10 mg/kg) treated. (D) Normal control.

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