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EFFECT OF CINNAMALDEHYDE ON ADJUVANT INDUCED ARTHRITIS IN RATS

K. Padhi[1*], R. Somani[1] and D. Pradhan[2]

[1]Department of Pharmacology, All India Shri Shivaji Memorial Society's College of Pharmacy, Kennedy road, Near R.T.O., Pune 411 001 Maharashtra, India.

[2]University Department of pharmaceutical sciences, Utkal university, Bhubaneswar, India-751004.

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***Corresponding Author**
K. Padhi
Department of Pharmacology, All India Shri Shivaji Memorial Society's College of Pharmacy, Kennedy road, Near R.T.O., Pune 411 001 Maharashtra, India.

ABSTRACT

Cinnamaldehyde is a phytoconstituent that is abundantly found in various plant species like Cinnamomum osmophloeum Kaneh, Cinnamomum cassia Presl and various species of Cinnamomum. Cinnamaldehyde is a natural product from spices that has many pharmacological activities. It belongs to the terpene group of compounds. We investigated the effect of Cinnamaldehyde on adjuvant induced arthritis in rats. Female Wistar rats were divided into 6 groups having 6 animals in each group (n=6). Arthritis was induced by injecting 0.1 ml of CFA into the surface of the left hind paw. Drug or vehicle treatment was started on day 5 till day 28 in prophylactic protocol and on day 16 till day 25 for the therapeutic protocol. All the characteristic features of rheumatoid arthritis like weight loss, Rheumatoid factor and C-reactive protein, Radiological deformation and biochemical parameters are significantly attenuated by Cinnamaldehyde demonstrating its beneficial effect for rheumatoid arthritis.

KEYWORDS: Cinnamaldehyde, adjuvant induced arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, relapsing autoimmune disorder that is characterized by pain, synovial membrane inflammation and restricted joint movement due to tissue damages**.**

RA patients experience swelling in the joints, synovial tissue inflammation and subsequent damage to the cartilage. This result in significant disability and decrease in the quality of life. RA affects about 1% of the population around the world.[1] The efficacy of currently used anti-rheumatic therapy is often limited. Frequent problems are side effects, either cumulative or idiosyncratic, and high cost**.** In modern pharmacological research, C. cassia has exhibited diverse actions, including antioxidative stress, preventing mitochondrial dysfunction, antitumor properties, Cinnamaldehyde, one of the main constituents of C. cassia, is an aromatic aldehyde which has been reported to have multiple potential therapeutic activities**.**[2] It is found that cinnamaldehyde could decrease production of prostaglandin E2 stimulated by interleukin-1β and could down regulate the expression of transient receptor potential vanilloid subtype in the cerebral micro vascular endothelial cells of the mouse, which may contribute to its antipyretic effects. It has been reported that cinnamaldehyde has antioxidant and in vitro anti-inflammatory properties**.** Low concentrations of cinnamaldehyde can inhibit secretion of interleukin-1β, tumor necrosis factor α, and reduce reactive oxygen species. With regard to the circulation, several studies have shown that cinnamaldehyde has antiplatelet and antithrombotic activity**.** Cinnamaldehyde also showed a dose-dependent relaxation of the rat aorta contraction induced by nor adrenaline, potassium, and prostaglandin F2a.[3] Aim of the present investigation was to evaluate the potential activity of the Cinnamaldehyde in arthritis in laboratory animals by pharmacological screening methods.

2) MATERIALS AND METHODS

2.1 Chemicals

Cinnamaldehyde was purchased from Merck India. Freund's complete adjuvant was purchased from sigma. Diclofenac sodium Fine Chem. Industry. The other chemicals and solvents used were of analytical grade and purchased from commercial suppliers.

2.2 Animals

Female wistar rats weighing 225–250g and albino mice of Swiss stain weighing 25-35 g were used in the experiments. The animals were provided by the central animal house facility of National Institute of Biosciences, Pune, India. Animals were housed in groups of six in well-ventilated polypropylene cages with husk beds at an ambient temperature of 25 ± 2◦C and 45–55% relative humidity with 12 h light and dark cycle. They had free access to pellet chow and water ad libitum. The experimentations on animals were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No: CPCSEA/IAEC/PC-03/12-2K12) under the

regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.3 Preliminary Acute Oral Toxicity

Healthy adult male albino mice (25-30g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the Organization for Economic Co-operation and Development**. (OECD Guideline, 2000).** The mice were observed continuously for 2h for behavioural and autonomic profiles and for any sign of toxicity or mortality for seven days.

2.4 Anti-arthritic activity using Complete Freund's adjuvant induced arthritis in rats

The animals were divided into six groups of six animals each as follows:

(A) Non-arthritic animals:

- **Group I:** Normal animals: received 1% aqueous solution of Tween-80, p.o; (B) Arthritic animals.
- **Group II:** vehicle control animals: received 1% aqueous solution of Tween-80, p.o.
- **Group III:** Drug treated animals: received Cinnamaldehyde (50 mg/kg, p.o.).
- **Group IV:** Drug treated animals: received Cinnamaldehyde (100 mg/kg, p.o.).
- **Group V:** Drug treated animals: received Cinnamaldehyde (200 mg/kg, p.o.).
- **Group VI:** Drug treated animals: received diclofenac (4 mg/kg, p.o.).

Each rat was injected with 0.1 ml of Freund's complete adjuvant (FCA) in to subplantar region of left hind paw on day 0 under light ether anesthesia. The dosing of all the groups started from day 12 once daily orally. Anti-arthritic activity of Cinnamaldehyde was evaluated on joint weight change. On day 29th, blood was withdrawn by retro-orbital puncture for assessment of haematological, biochemical parameter and radiological analysis was done.[4,5,6,8]

3) RESULT

3.1 Acute oral toxicity test and dose fixation of cinnamaldehyde

In our study, mice treated with cinnamaldehyde were found to be free of any toxicity upto 2000 mg/kg dose and exhibited normal behaviour. Mice were alert with normal grooming, touch response, pain response. There was no abnormal change in motor activity, secretory signs as well as their body weight and water intake. Our results are in accordance with reported results; therefore from AOT we selected different concentrations of

cinnamaldehyde. We selected three doses; less than 1/10th of the above dose was selected as the highest dose (i.e. 150 mg/kg) and remaining 2 doses were selected as 50 and 100mg.

3.2 Effect of Cinnamaldehyde on Complete Freund's adjuvant Induced Arthritis

A) Protocol I- Prophylactic Effect on body Weight

Arthritic control group weighed substantially less on day 28 compared with normal control animals. The Cinnamaldehyde 150mg/kg and 100mg/kg and 50 mg/kg reduced the CFA induced body weight loss. The reference standard also reduced the loss of the body weight.

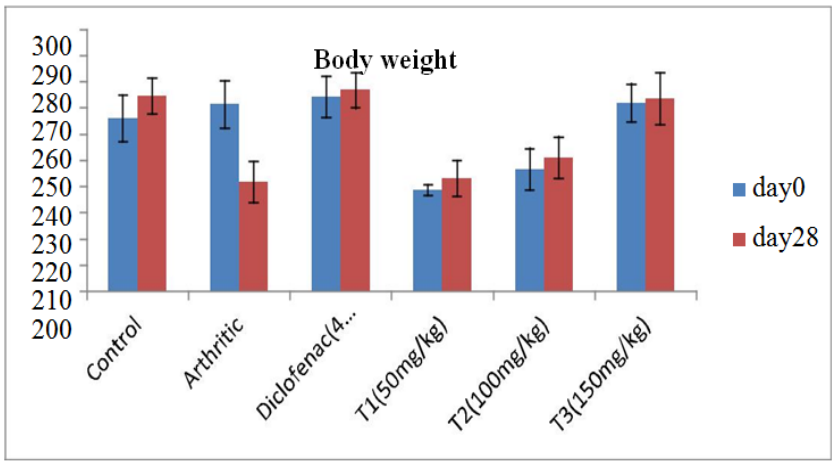


Figure.3.1: Effect of cinnamaldehyde, on mean change in body weight. n=6 in each group; *p<0.05 and **p<0.01 (compared to Arthritic control) #p<0.05 and ##p<0.01(compared to control) (ANOVA followed by Dunnett's test). All treatment groups compared to arthritic control.

Effect on Haematological Parameters

The haematological parameters were evaluated on day 29. The WBC count which was raised in arthritic group. Also, the increase in Erythrocyte sedimentation rate (ESR) of arthritic control group was remarkably counteracted by test drug. The RBC count which was decreased during arthritic condition was also effectively restored by Cinnamaldehyde 150 mg/kg. Haemoglobin (gm %) which was decreased in arthritic condition was also increased in the entire Cinnamaldehyde treated group and also in diclofenac group.

Table 3.1: Effect of Cinnamaldehyde on Haematological parameters in prophylactic protocol.

	Haematological parameters			
Treatments	**WBC Count**	**RBC Count**	**Hb (gm %)**	**ESR (mm/hr)**
(mg/kg)	**(1000/cu.mm)**		**(million/cu.mm)**	
Control	6.86±1.13	8.70±0.11	15.5±1.59	3.3±0.32
Induction control	16.21±0.42##	5.05±0.68##	8.5±0.77##	8±1.50##
Diclofenac sodium (4 mg/kg)	11.07±0.57**	6.52±0.85	12.76±0.71*	3.5±0.56**
T1(50 mg/kg)	13.05±0.27*	5.73±0.35	10.5±0.57	7±0.36
T2 (100 mg/kg)	12.46±0.52**	6.76±0.27	11.11±0.76	6.16±1.0
T3 (150 mg/kg)	10.93±0.43**	7.09±0.37*	12.53±0.44*	4.83±0.40*

Values expressed as Mean ± SEM, n=6 in each group; *p<0.05 and **p<0.01(compared to arthritic control). #p<0.05 and ##p<0.01(compared to control) (ANOVA followed by Dunnett's test). All treatment groups compared to arthritic control.

Effect on Rheumatoid factor and C-reactive protein

In arthritis there is an increase in the level of CRP and RF; but observation made on day 29th of treatment showed decreased level of both in all the treatment groups of Cinnamaldehyde.

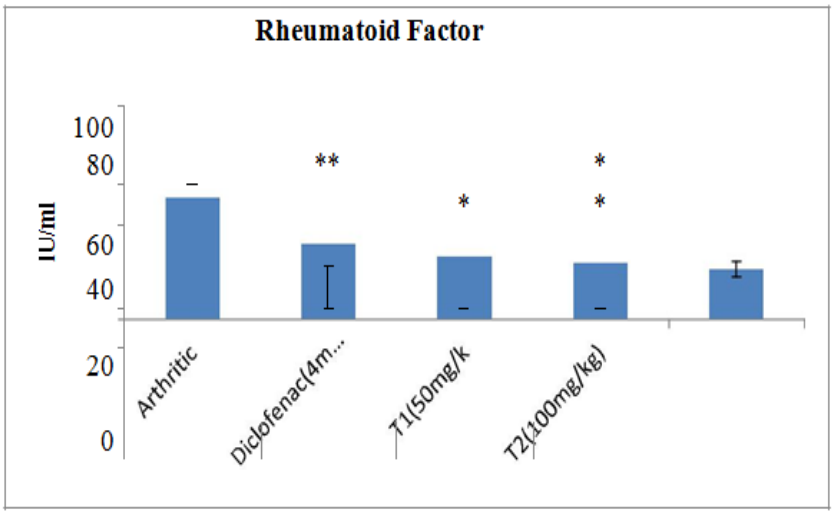
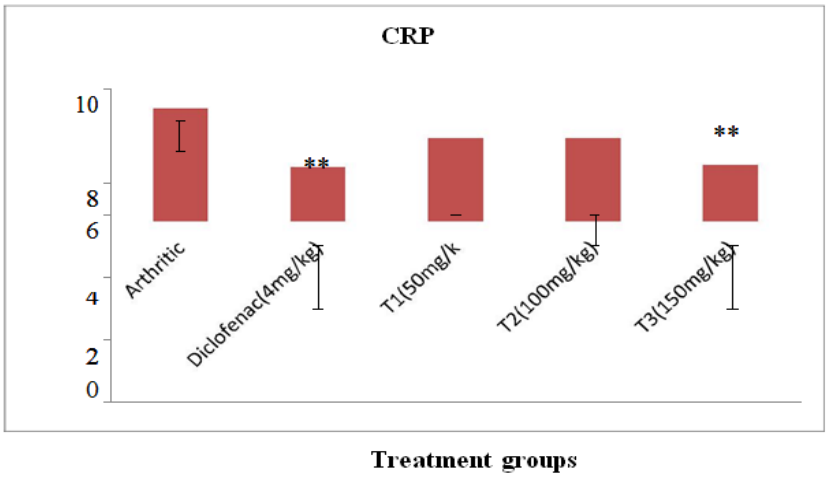


Figure 3.2: Effect of Cinnamaldehyde on C- reactive protein Values expressed as Mean ± SEM, n=6 in each group; *p<0.05 and **p<0.01(compared to arthritic control). #p<0.05 and ##p<0.01(compared to control) (ANOVA followed by Dunnett's test). All treatment groups compared to arthritic control.



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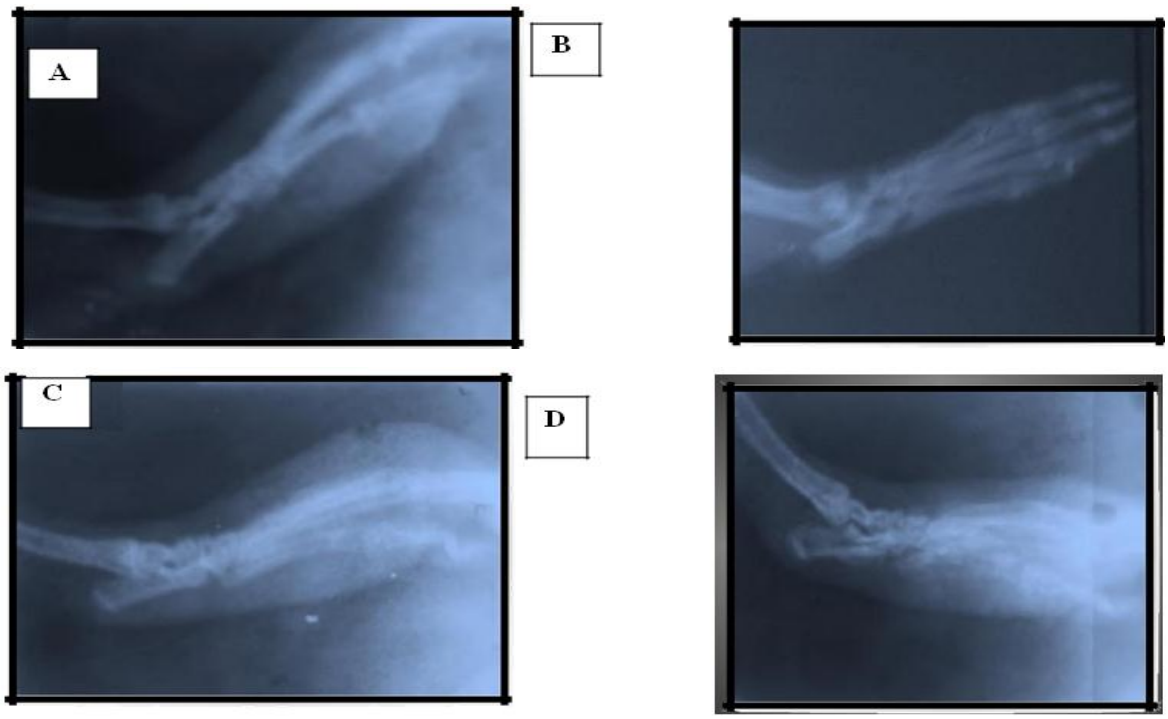
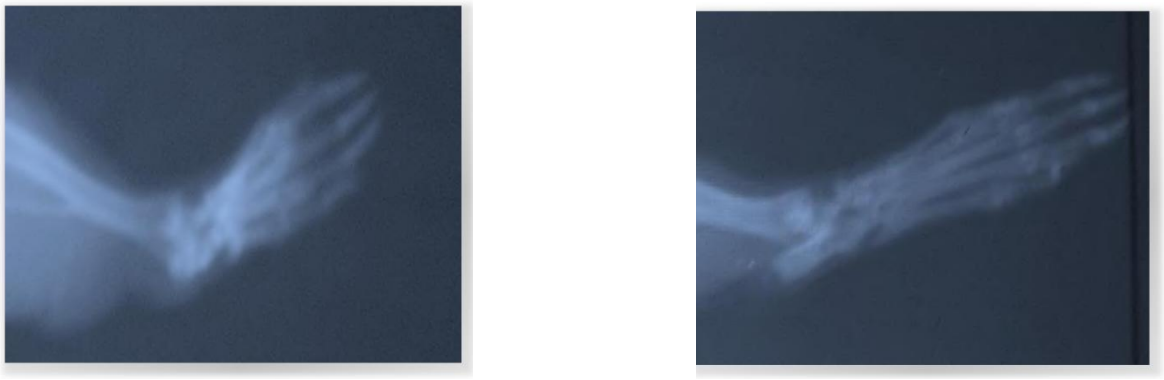
Radiological Assessment of Rat Paw in FCA Induced Arthritis

The radiographic features of the rat joints in FCA induced arthritic model are shown in fig. In disease control group soft tissue swelling is seen along with narrowing of the joint spaces, periosteal reaction is also seen. Bony erosion is seen. All this complications implies development of arthritisIn the present study X ray examination reveals more beneficial effects in group T2 (100mg/kg) as compared to other groups.





E **F**

Figure 3.4: Radiographical analysis of the joints of hind limb of various treatment groups. A – Normal control, B – Arthritic control, C – T1 (50mg/kg), D –T2 (100mg/kg) E – T3 (150mg/kg), F – Diclofenac (4mg/kg).

Table 3.2: Graded scores of the joints of hind limb of various treatment groups.

Group	Necrosis of bones erosions	Joint space narrowing	Periosteum inflammation
Control	00	00	00
Arthritic control	+++	+++	++++
T1(50mg/kg)	+++	+++	++++
T2(100mg/kg)	++	++	++
T3(150mg/kg)	++	++	+++
Diclofenac(4mg/kg)	++	+++	+

B) Protocol II – Therapeutic

protocol Effect on body weight

Decrease in body weight was observed during arthritic condition. All the doses of cinnamaldehyde inhibited the loss in body weight as compared to the arthritic control group.

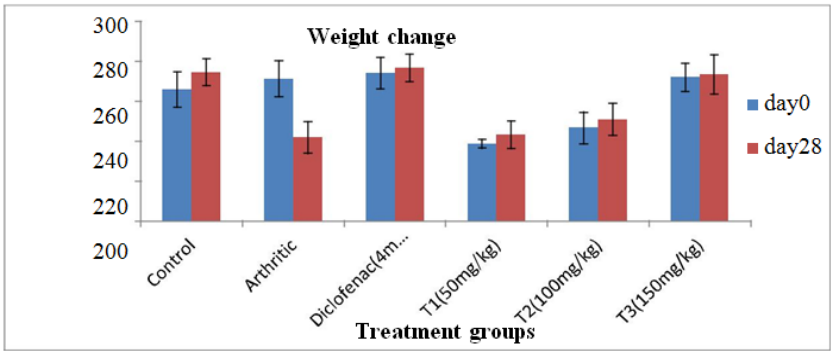


Figure.3.5: Effect of cinnamaldehyde, on mean change in body weight. n=6 in each group; values expressed as Mean ± SEM, n=6 in each group; *p<0.05 and **p<0.01(compared to arthritic control). #p<0.05 and ##p<0.01(compared to control) (ANOVA followed by Dunnett's test). All treatment groups compared to arthritic control.

Effect on Haematological Parameters

Table 3.3: Effect of Cinnamaldehyde on haematological parameters.

	Haematological parameters			
Treatments (mg/kg)	**WBC Count (1000/cu. mm)**	**RBC Count (million/cu.mm)**	**Hb (gm %)**	**ESR (mm/hr)**
Control	6.86±1.13	8.70±0.11	15.56±1.59	3.3±0.32
Induction control	16.21±0.42##	5.05±0.68##	8.58±0.77##	8.58±1.13##
Diclofenac sodium (4 mg/kg)	9.48±0.33**	6.5±0.18*	13.5±0.26**	3±0.36**
T1 (50 mg/kg)	12.36±0.46*	6.9±0.17*	12.03±0.49*	6.8±0.47
T2(100 mg/kg)	11.35±0.34*	7.4±0.15*	11.9±0.52*	5.6±0.42**
T3 (150 mg/kg)	9.22±0.68*	7.2±0.19**	13.15±0.12*	4.5±0.42**

Values expressed as Mean ± SEM, n=6 in each group; *p<0.05 and **p<0.01(compared to arthritic control). #p<0.05 and ##p<0.01(compared to control) (ANOVA followed by Dunnett's test). All treatment groups compared to arthritic control.

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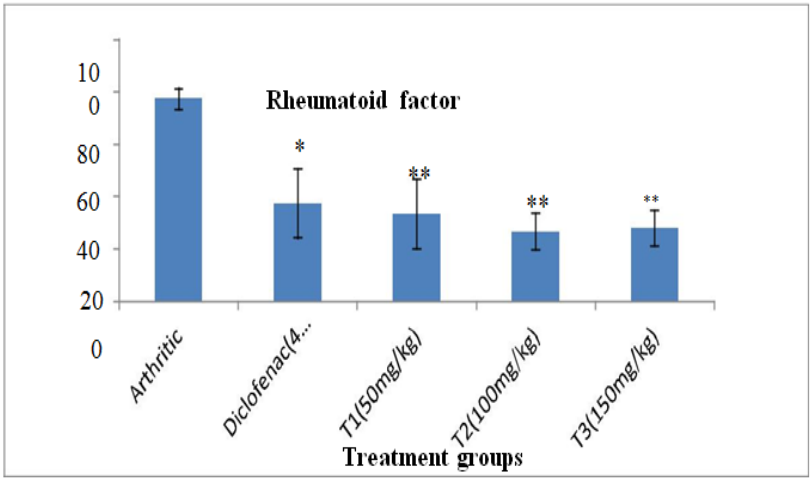


Figure 3.6: Values expressed as Mean ± SEM, n=6 in each group; *p<0.05 and **p<0.01(compared to arthritic control). #p<0.05 and ##p<0.01(compared to control) (ANOVA followed by Dunnett's test). All treatment groups compared to arthritic control.

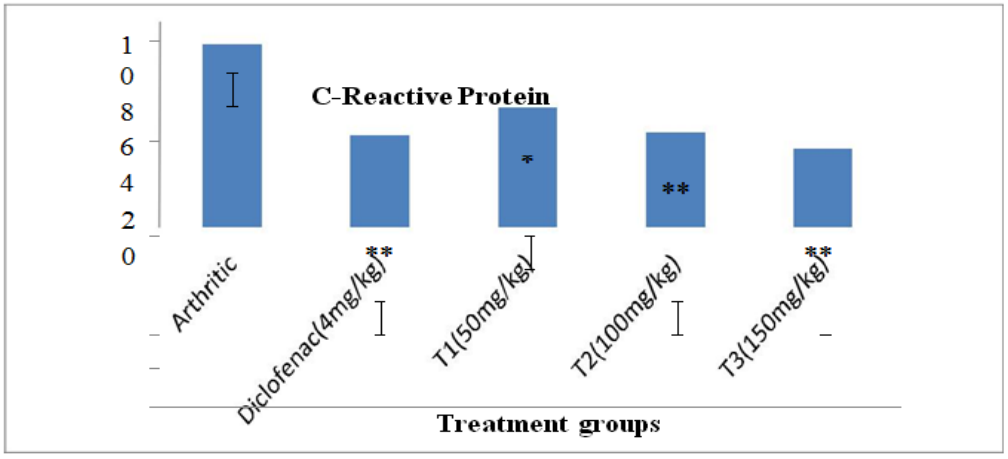
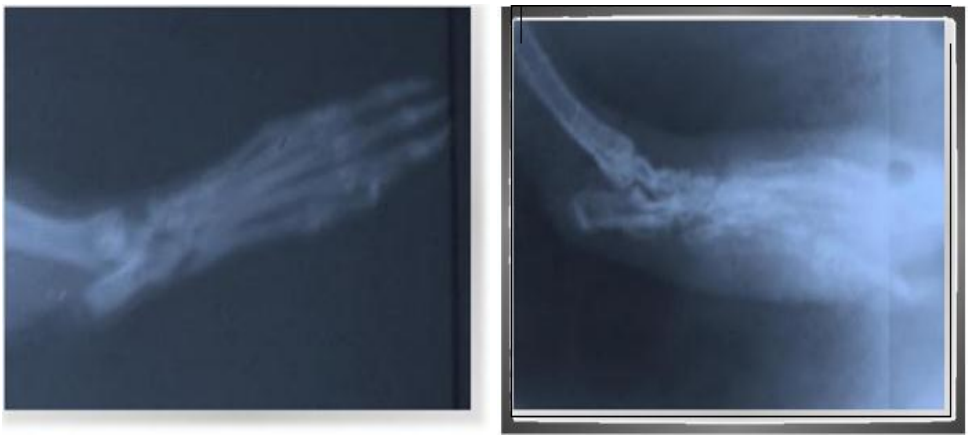
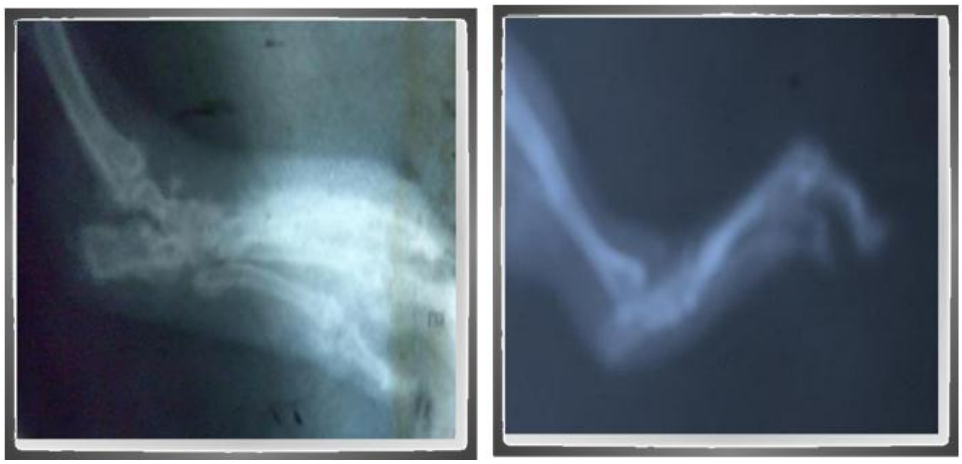


Figure 3.7 Values are expressed as Mean ± SEM, n=6 in each group; *p<0.05 and **p<0.01(compared to arthritic control). #p<0.05 and ##p<0.01(compared to control) (ANOVA followed by Dunnett's test). All treatment groups compared to arthritic control.

Radiological Assessment of Rat Paw in FCA Induced Arthritis



A ***B***



C ***D***

E ***F***

Figure 3.8 Radiographical analysis of the joints of hind limb of various treatment groups. A – Normal control, B – Arthritic control, C – T1 (50mg/kg), D –T2 (100mg/kg) E – T3 (150mg/kg), F – Diclofenac (4mg/kg).

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Group	Necrosis of bones – erosions	Joint narrowing	space	Periosteum inflammation
Control	00	00		00
Arthritic control	+++	+++		++++
T1(50mg/kg)	+++	+++		++++
T2(100mg/kg)	++	+		++
T3(150mg/kg)	++	++		++
Diclofenac(4mg/kg)	++	+++		+

0: no abnormality detected

+: damage changes up to more than 25 %

++: damage changes up to more than 50 %

+++: damage changes up to more than 75 %

++++: damage changes up to more than 75%

DISCUSSION

Rat adjuvant induced arthritis is an experimental model of numerous anti-arthritic agents. The hallmark of this model is reliable onset and progression of robust, easily measurable poly arthritic inflammation, mark bone desorption and periosteal bone proliferation. Complete Freunds reagent (CFA) is designed to provide continuous release of antigens necessary for stimulating a strong persistent immune response.[9] Interestingly heat shock proteins (HSP's) have been implicated in the pathogenesis of human RA as well.

The change in body weight was monitored as apparent indicator of arthritic symptoms and loss of body weight usually to appear at the onset stage of arthritis. A report suggests that the decrease in the body weight during inflammation is due to deficient absorption of nutrients through the intestine and that treatment with anti-inflammatory drugs normalizes the process of absorption. In the present study, decreased body weight of arthritic animals might be linked to systemic and local action of cytokines. The diminished weight in arthritic rats was reduced in Cinnamaldehyde treated groups. This effect of Cinnamaldehyde on weight gain, appears due to its anti-inflammatory activity, possibly involve an immunomodulatory role on the effect of cytokines in arthritic rats.[7]

In arthritis there is decrease in Hb and RBC, increase in WBC count and ESR levels. The decrease in Hb and RBC levels in arthritic rats reflect the presence of anaemia in these rats. The increase in total WBC count in AIA rats falls in line. The increase in WBC count is due to the stimulation of immune system against the invading pathogenic microorganism. This is evident by the infiltration of inflammatory mononuclear cells in the joints of AIA rats.[10]

ESR is an indirect measurement of acute phase response for determining the the disease activity in RA. ESR, the rate at which erythrocytes settle out of unclotted blood in a certain period of time, is influenced by increase in plasma concentration of acute phase reaction proteins (fibrinogen, alpha and beta globulin) and therefore, provides a measure of acute phase of inflammatory disease**.**[11]

We found that treatment that treatment of Cinnamaldehyde in CFA induced arthritis rats showed significant increase in level of Hb and RBC in a dose dependent manner in both the protocols, while significant decrease in the level of WBC, ESR was observed as compared to control group. The information of RF and CRP add further detail about chronicity of disease.

The changes in the level of CRP and RF were brought back to near normal levels upon treatment with Cinnamaldehyde, which emphasizes the beneficial effect of Cinnamaldehyde on AIA rats.

The radiological picture of AIA rats show the narrowing of joint space and soft tissue swelling. In Cinnamaldehyde treated animals partially cured in prophylactic protocol. The 100mg/kg animals showed reduction in the deformities. In the treatment protocol also similar results were observed. All the doses show beneficial effect.

The present study results indicate that Cinnamaldehyde possesses significant analgesic and anti-inflammatory activities. Cinnamaldehyde normalized the various biochemical and haematological abnormalities in both developing and developed phases of CFA induced arthritis in rats. Cinnamaldehyde also significantly attenuated CFA induced bone deformity and cartilage destruction. The current research work indicates that Cinnamaldehyde significantly combated CFA induced polyarthritis. This suggests that Cinnamaldehyde could be a valuable addition to the current anti-arthritic therapies.

CONCLUSION

All the characteristic features of rheumatoid arthritis like pain, joint swelling and bone destruction are significantly attenuated by Cinnamaldehyde demonstrating its beneficial effect for rheumatoid arthritis. From the present work, it can be concluded that Cinnamaldehyde acts as an anti-rheumatic agent with high analgesic, anti-inflammatory and anti-arthritic activity.

FUTURE SCOPE

Further research is required to know the exact mechanism of Cinnamaldehyde activity. Further studies using other models like genetically modified animals could be used to evaluate the activity of cinnamaldehyde. Also more data on safety and efficacy is required to be generated for its use in clinical trials and combined use with other anti-rheumatic agents.

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