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COMBINATION OF TRANS-CINNAMALDEHYDE AND PIPERINE AGAINST COMPLETE FREUND'S ADJUVANT INDUCED ARTHRITIS ANIMAL MODEL ILLUSTRATING ANTI-ARTHRITIC ACTIVITY.

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ABSTRACT
An autoimmune disease, rheumatoid arthritis (RA) causes chronic inflammation of the joints characterized by hyperplasia of synovial cells and angiogenesis in the affected joints, which eventually leads to the erosion of cartilage and bone. The purpose of the present study was to examine anti-arthritic activity of trans-cinnamaldehyde (3mg/kg) and piperine (8mg/kg) combination using *in vivo* Complete Freund's Adjuvant (CFA) induced arthritis animal model in male Wistar rats. Paw volume and percentage inhibition of paw edema in CFA-injected and non-injected paws, arthritic index, and body weight measurement were estimated and conclusions from histopathological analysis were drawn. The study involves induction of arthritis to rats of all groups using CFA, followed by subsequent treatment with piperine (15mg/kg), trans-cinnamaldehyde (5mg/kg) and {piperine (8mg/kg) + trans-cinnamaldehyde (3mg/kg)} combination respectively. Indomethacin is used as a reference standard. The (piperine + trans-cinnamaldehyde) combination significantly reduce the swelling in the CFA-injected paws and inhibited the inflammation in non-injected sites and also, inhibited the activation and proliferation of T-cells thereby inhibiting the secondary lesions. Additionally, the histopathological studies support and justify the anti-arthritic effect of (piperine + trans-cinnamladehyde) combination. Thus, the (piperine + trans-cinnamaldehyde) combination showed significant anti-arthritic activity and the results were comparable with the standard drug indomethacin.

KEYWORDS: Rheumatoid arthritis, Complete Fruend's Adjuvant, Trans-cinnamaldehyde, Piperine, Indomethacin.

INTRODUCTION
Rheumatoid arthritis is mainly inflammation caused from an overactive immune system.[1] It is a chronic inflammatory disease predominantly affecting the joints and peri-articular tissue. RA remains a formidable disease, being capable of producing severe crippling deformities, functional disabilities and cartilage destruction commonly leading to significant disability, caused by number of pro-inflammatory molecules released by macrophages including eicosanoids (prostaglandins, leukotrienes, cytokines) and reactive oxygen species. Rhematoid Arthritis is a complex process which involves synovial cell proliferation and fibrosis, pannus formation and cartilage and bone erosion. In 2005, approximately 1.293 million adults aged 18 and older had RA globally. Rheumatoid arthritis occurs in all races and ethnic groups. Although the disease begins in middle age and occurs with increased frequency in older people, children and young adults also develop it. Rheumatoid arthritis occurs more frequently in women than men. About two or three times as many women as men have the disease.[2]

Available medicines for the treatment are focused towards decreasing the disease activity or decreasing the inflamed condition along with minimization of joint destruction and finally improving the physical condition and quality of life. A tactical treatment plan is in use for the treatment of the disease which includes four different classes of drugs: Non-steroidal anti-inflammatory agents (indomethacin), Corticosteroids (methyl prednisolone, prednisone), Disease modifying anti-rheumatic drugs (methotrexate, gold sodium thiomalate), and Biological agents/biologic response modifiers (etanercept, infliximab). All the above mentioned therapies, which are employed in the treatment of arthritis such as the use of NSAIDs, corticosteroids, DMARDs and biological agents are helpful in decreasing the joint stiffness and pain. But, one of the main drawbacks of using these drugs is that while they reduce the symptoms of the disease but the progression of the disease continues. All

these drugs come with a line of side effects which includes gastrointestinal ulcers, osteoporosis, serious infections like sepsis, development of various lymphomas etc. Till date none of the drug available in the market is able to cure rheumatoid arthritis completely. Thus, there is need for potential therapeutic agents, which need to be carefully scrutinized for efficacy and safety that would promote more potential anti-rheumatic activity. This in part has stimulated the search for natural plant-based "phytopharmaceuticals" which can be anti-inflammatory and anti-rheumatic in therapeutic action. As consumers perceive that such products will be of lower cost and safe, those in the industry will also see opportunity in that such products are easier to introduce into the marketplace than the traditional pharmaceutical market. When reviewing adverse reports on herbs, particularly common culinary herbs and spices, few such adverse reports have been recorded. Given the increasing interest in natural products to improve health, nutrition and the availability, the search for natural anti-inflammatory agents that could have application for RA treatment is relevant.

Cinnamomum zeylanicum Nees, commonly known as cinnamon, the evergreen tree of tropical area, a member of family Lauraceae, has been used as a spice and condiment in India. Cinnamon chiefly contains essential oils cinnamic acid, cinnamaldehyde and cinnamate. Trans-cinnamaldehyde has been reported to reduce myeloperoxidase (MPO) and malondialdehyde (MDA) level activity in the paw[3,4], avert protein expression of {inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), nuclear transcription factor kappaB (NF-κB), and IκBα}[5], escalate the activities of {catalase (CAT), glutathionse peroxidase (GPx) and superoxide dismutase (SOD)} in the edema paw after Carr injection[6] and inhibit {tumor necrosis factor (TNF-α), nitric oxide (NO) and prostaglandin E2 (PGE2)}.[6,7]

Piperine is an alkaloid found naturally in plants belonging to the family Piperaceae, such as *Piper longum L*, commonly known as long pepper and *Piper nigrum L*, commonly known as black pepper.[8] Piperine has been reported to inhibit prostaglandin(PGs) release[9,10] and reduce the expression of {(interleukin) IL6 and (matrix metalloproteinase) MMP13} in synoviocytes.[11] Based on these findings, present work is an attempt to study the anti-rheumatic activity of two phytoconstituents - trans-cinnamaldehyde and piperine, suggesting that both the phytoconstituents have potential for treatment of rheumatoid arthritis and its combination would be unique and beneficial to treat chronic inflammatory disorder like rheumatoid arthritis.[12]

METHODOLOGY

ANIMALS

Healthy male albino Wistar rats (*Rattus norvegicus*) of weight 150-200 grams and of 3-4 months old were used for research purpose. Animals were obtained from the Breeding Facility of Bombay Veterinary College, Parel, India and were accustomed to the laboratory environment for a period of 6 days prior to the commencement of dosing. The animals were housed in polypropylene cages with stainless steel grid lid at 20-25^{0}C with relative humidity of 65-70% under 12-h light: 12-h dark cycles. They were fed with Nutrilab Rodent pellet diet feed with free access to filtered aqua-guard water throughout the study. The study was carried out in the Department of Pharmacology after approval of the protocol by the (IAEC) Institutional Animal Ethics Committee.

DRUGS, CHEMICALS and PREPARATION OF STOCK SOLUTION

Indomethacin, trans-cinnamaldehyde, piperine and complete Freund's adjuvant were obtained from Sigma Aldrich Chemicals Pvt. Ltd., USA. Carboxy methyl cellulose and tween 20 were obtained from S D Fine Chem Ltd., India. Three main drug solutions were prepared for administration during *invivo* studies. Standard drug indomethacin and treatment drug piperine was prepared as suspension using 0.5%w/v carboxy methyl cellulose (CMC) as vehicle. Trans-cinnamaldehyde drug was administered as an emulsion and was prepared with tween 20 and distilled water in a ratio of 3:1 {Tween 20:distilled water (3:1)}, which was used as vehicle. Every time the drug solutions were freshly prepared, in required concentration of active ingredient, prior to dosing.

EXPERIMENTAL DESIGN

COMPLETE FREUND'S ADJUVANT - INDUCED ARTHRITIS MODEL

Adjuvant arthritis exhibits many similarities to human rheumatoid arthritis. Injection of CFA into the rat paw induces inflammation as primary lesion with a maximum after 3 to 5 days. After a delay of approximately 11 to 12 days, secondary lesions occur which are characterized by inflammation of non-injected sites (hind legs, forepaws, ears, nose and tail), a decrease of weight and immune responses. Adjuvant arthritis has gained wide usage for assessing a drug's potential anti-arthritic activity.[13]

Procedure

Male rats with initial bodyweight of 150 to 200 grams were used. CFA was injected 0.1 ml per animal subplantarly in left hind paw on day 0 and 0.05 ml per animal subplantarly in same hind paw on day 9 to all groups. Dosing with the test compounds and the standard drug was started on the same day and continued for 12 days. Paw volume of injected paws was measured on day 1, 3, 7, 11, 14 and 21, and that of non-injected paws was measured on day 11, 14, and 21, with the help of plethysmometer. On day 3, the volume of the injected paws indicated the primary lesion and the influence of therapeutic agents on this phase. Secondary lesions (paw volume of non-injected paws) pointed the severity of the induced adjuvant on day 11. From day 13 to 21, the animals were not dosed with the test compound or the standard drug. Body weight of the rats was also

measured with interval of 7 days starting from day 0 to day 21 (i.e. day 0, day 7, day 14 and day 21) and the observed body weight of all the animals under treatment including induction control group was compared with the normal rats which were provided with only standard rodent feed and water *ad libitum*. The severity of the secondary lesions was assessed visually on day 21 and graded according to the following scheme as mentioned.

Table 1: Arthritic assessment scoring for CFA - induced arthritis model.

BODY PART	PARAMETER	SCORE
EARS	Absence of nodules and redness	0
	Presence of nodules and redness	1
NOSE	No swelling of connective tissue	0
	Intensive swelling of connective tissue	1
TAIL	Absence of nodules	0
	Presence of nodules	1
FORE PAWS	Absence of inflammation	0
	Inflammation of at least one joint	1
HIND PAWS	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked inflammation	3

Evaluation

a) For primary lesions: Percent inhibition of paw volume of the injected left paw over vehicle control was measured on day 3.

b) For secondary lesions: The percentage inhibition of paw volume of the non-injected right paw over control was measured on day 11.

c) An arthritic index was calculated as the sum of the scores as indicated above for each animal. The average of the treatment group was compared with the control group.

d) The total percentage change was calculated by addition as follows: the percent inhibition of the injected paw on day 3 + the percent inhibition of the non-injected paw on day 11 + the percent change of the arthritic index.[14,15]

HISTOPATHOLOGICAL EXAMINATION

At the end of the *invivo* study, the rats were sacrificed by cervical dislocation. The hind paws of rats were amputated, secured with neutral-buffered 10% formalin, and then decalcified for 10 days with EDTA and embedded in paraffin for histopathological analysis. The paraffin sections were then stained with haematoxylin and eosin.[16]

STATISTICAL ANALYSIS

The raw data was processed to get the group means and standard error means with significance between the control and treatment groups. The data was processed using Graph Pad Prism 6.05 Trial statistical software. Appropriate statistical methods (One way ANOVA followed by Dunnett's multiple comparisons test or Tukey's multiple comparisons test) were employed to measure the significance among different groups with significance level of $P<0.05$.

RESULTS

CFA-Injected Paws

Table 2: Effect of trans-cinnamaldehyde and piperine combination on paw volume (ml) - in CFA -injected paw of rats.

GROUPS	Mean paw volume(ml) ± SEM					
	Day 1	**Day 3**	**Day 7**	**Day 11**	**Day 14**	**Day 21**
Group I (Control- 2ml distilled water)	0.5816 ± 0.008724	0.7716 ± 0.006009	1.005 ± 0.01945	1.155 ± 0.01057	1.2916 ± 0.007032	1.155 ± 0.01408
Group II (Standard- 3mg/kg indomethacin p.o.)	0.4316 ± 0.004773 ++++	0.5083 ± 0.006540 ++++	0.6583 ± 0.009458 ++++	0.7383 ± 0.007032 ++++	0.5983 ± 0.007032 ++++	0.4683 ± 0.008724 ++++
Group III (15mg/kg piperine p.o.)	0.55 ± 0.006831 ****	0.695 ± 0.006708 ****, ++++	0.8816 ± 0.007032 ****, ++++	0.9383 ± 0.009458 ****, ++++	0.7866 ± 0.008433 ****, ++++	0.6216 ± 0.009661 ****, ++++
Group IV (5mg/kg trans-cinnamaldehyde i.p.)	0.5083 ± 0.00654 ****, ++++	0.66 ± 0.01125 ****, ++++	0.7583 ± 0.009458 ****, ++++	0.8233 ± 0.008028 ****, ++++	0.6933 ± 0.009888 ****, ++++	0.5716 ± 0.007032 ****, ++++
Group V (8mg/kg	0.4633 ±	0.5483 ±	0.6983 ±	0.7733 ±	0.6216 ±	0.5166 ±

piperine p.o. + 3mg/kg trans-cinnamaldehyde i.p.)	0.01022 ++++	0.006009 **, ++++	0.006009 ++++	0.006667 ++++	0.006009 ++++	0.007601 *, ++++

Values are expressed as "mean ± SEM", N=6, One way ANOVA followed by Tukey's multiple comparison test. ++++P<0.0001 Vs Control group I, *P<0.05, **P<0.01, ****P<0.0001 Vs Standard Group II.

Table 3: Percentage inhibition of paw volume in the CFA - injected paws of treatment groups.

TREATMENT GROUPS	Percent Inhibition (%) of paw volume					
	Day 1	Day 3	Day 7	Day 11	Day 14	Day 21
Group II (Standard- 3mg/kg indomethacin p.o.)	25.79	34.12	34.49	36.07	53.67	59.45
Group III (15mg/kg piperine p.o.)	5.43	9.92	12.27	18.76	39.09	46.18
Group IV (5mg/kg trans-cinnamaldehyde i.p.)	12.60	14.46	24.54	28.71	46.32	50.51
Group V (8mg/kg piperine p.o. + 3mg/kg trans-cinnamaldehyde i.p.)	20.34	28.93	30.51	33.04	51.87	55.27

Statistically significant increase was observed in injected paw volume of rats of all groups from day 1 to 11, where as in induction control group, the increase was observed till third week of the study. There was significant decrease in paw volume of rats of treatment groups from day 13 to 21 when compared with the induction control group. Also, the rats of induction control group showed slight decrease in paw volume on day 21.

Non-Injected Paws

Table 4: Effect of trans-cinnamaldehyde and piperine combination on paw volume (ml) - in non-injected paw of rats.

GROUPS	Mean paw volume(ml) ± SEM		
	Day 11	Day 14	Day 21
Group I (Control- 2ml distilled water)	0.475 ± 0.005627	0.7883 ± 0.006009	0.5566 ± 0.006667
Group II (Standard- 3mg/kg indomethacin p.o.)	0.25 ± 0.01155 ++++	0.4566 ± 0.009189 ++++	0.33 ± 0.007303 ++++
Group III (15mg/kg piperine p.o.)	0.4416 ± 0.008724 ****	0.7266 ± 0.006667 ****, ++++	0.47 ± 0.008165 ****, ++++
Group IV (5mg/kg trans-cinnamaldehyde i.p.)	0.3733 ± 0.009189 ****, ++++	0.6066 ± 0.005578 ****, ++++	0.4283 ± 0.006540 ****, ++++
Group V (8mg/kg piperine p.o. + 3mg/kg trans-cinnamaldehyde i.p.)	0.3133 ± 0.004216 ***, ++++	0.5266 ± 0.009888 ****, ++++	0.3816 ± 0.006009 ***, ++++

Values are expressed as "mean ± SEM", N=6, One way ANOVA followed by Tukey's multiple comparison test. ++++P<0.0001 Vs Control group I, ***P<0.001, ****P<0.0001 Vs Standard Group II.

Table 5: Percentage inhibition of paw volume in the non - injected paws of treatment groups.

TREATMENT GROUPS	Percent Inhibition (%) of paw volume		
	Day 11	Day 14	Day 21
Group II (Standard- 3mg/kg indomethacin p.o.)	47.36	42.07	40.71
Group III (15mg/kg piperine p.o.)	7.03	7.82	15.55
Group IV (5mg/kg trans-cinnamaldehyde i.p.)	21.41	23.04	23.05
Group V (8mg/kg piperine p.o. + 3mg/kg trans-cinnamaldehyde i.p.)	34.04	33.19	31.44

Significant statistical decrease of paw volume was observed in rats of all treatment groups after day 14 of the study, when compared with the induction control group, which also showed decrease in paw volume after day 14.

Table 6: Arthritic Index scoring of rats.

TREATMENT GROUPS	Mean Arthritic Index					Total Mean Arthritic Index	Percent Change of Arthritic Index Compared to Control Group I
	Ears	Nose	Tail	Fore paws	Hind paws		
Group I (Control- 2ml distilled water)	0.833	0.5	1	1	2.833	6.166	---
Group II (Standard- 3mg/kg indomethacin p.o.)	0	0	0.66	0.66	1.33	2.65	57.02
Group III (15mg/kg piperine p.o.)	0.66	0.33	1	0.833	2.33	5.153	16.42
Group IV (5mg/kg trans-cinnamaldehyde i.p.)	0.166	0	0.66	0.66	1.833	3.319	46.17
Group V (8mg/kg piperine p.o. + 3mg/kg trans-cinnamaldehyde i.p.)	0	0	0.833	0.833	1.166	2.832	54.07

A substantial decrease in edema volume was observed on the 21st day in the injected paw and non-injected paw of all the animals in the treatment groups. With respect to mean arthritic index, the average arthritic score of piperine treated Group III was 5.153 whereas that of control Group I was 6.166 which indicate piperine having insignificant anti-arthritic activity. In case of Group V (piperine + trans-cinnamaldehyde) and Group II (indomethacin) treated group it showed a profound anti-arthritic activity with the average arthritic score of 2.832 and 2.65 respectively, thereby explaining that these two groups decreased the arthritic index almost 3 fold when compared to that of arthritic control, score being 6.166. Thus, the standard drug indomethacin and the combination (piperine + trans-cinnamaldehyde) treatment had significantly reduced the CFA-induced primary and secondary lesions in the respective treatment groups as compared with the induction control group. It can also be noted that the reduction of the secondary lesions was comparable in the indomethacin-treated group and the combination treated group.

Table 7: Total percentage change in CFA - induced arthritis model by the treatment groups.

TREATMENT GROUPS	Primary lesion: Percent inhibition of the injected paw on day 3	Secondary lesion: Percent inhibition of the non-injected paw on day 11	Percent change of the arthritic index	Total percentage change
Group II (Standard - 3mg/kg indomethacin p.o.)	34.12	47.36	57.02	138.5
Group III (15mg/kg piperine p.o.)	9.92	7.03	16.42	33.37
Group IV (5mg/kg trans-cinnamaldehyde i.p.)	14.46	21.41	46.17	82.04
Group V (8mg/kg piperine p.o. + 3mg/kg trans-cinnamaldehyde i.p.)	28.93	34.04	54.07	117.04

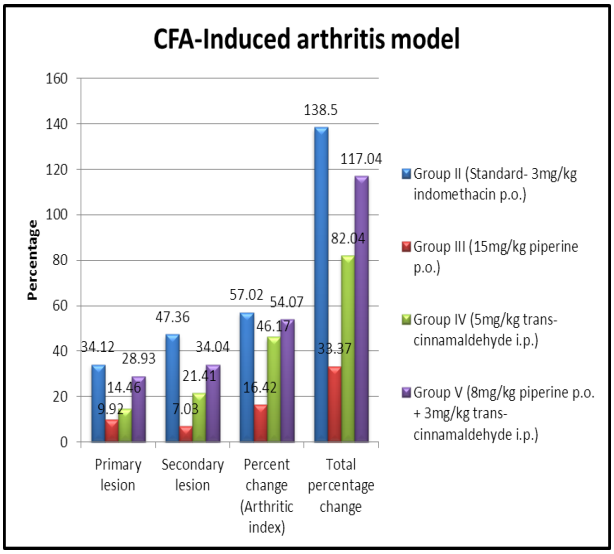

Figure 1: Graph of total percentage change in CFA - induced arthritis model.

Table 8: Measurement of body weight of CFA - induced rats from different groups in comparison with the Normal rats.

TREATMENT GROUPS	Measurement of body weight (g)			
	Day 0	Day 7	Day 14	Day 21
Group of Normal rats	180.83 ± 0.4014	196.16 ± 0.3073	233.16 ± 0.5426	272.83 ± 0.9458
Group I (Induction control- 2ml distilled water)	180.83 ± 0.3073 ns	192.16 ± 0.7032 ****	220.33 ± 0.4216 ****	238.66 ± 0.4216 ****
Group II (Standard- 3mg/kg indomethacin p.o.)	180.00 ± 0.9309 ns	198.16 ± 0.4773 ns	226.5 ± 0.4282 ****	265.83 ± 1.138 ****
Group III (15mg/kg piperine p.o.)	179.83 ± 1.815 ns	191.66 ± 0.4944 ****	223.00 ± 0.7303 ****	242.66 ± 0.7601 ****
Group IV (5mg/kg trans-cinnamaldehyde i.p.)	181.16 ± 1.041 ns	193.5 ± 0.4282 **	223.83 ± 0.4773 ****	248.16 ± 0.8333 ****
Group V (8mg/kg piperine p.o. + 3mg/kg trans-cinnamaldehyde i.p.)	180.50 ± 0.4282 ns	195.33 ± 0.4216 ns	226.16 ± 0.6009 ****	253.16 ± 0.7923 ****

Values are expressed as "mean ± SEM", N=6, One way ANOVA followed by Dunnett's multiple comparison test. ns: not significant, **P<0.01, ****P<0.0001 Vs Group of Normal rats.

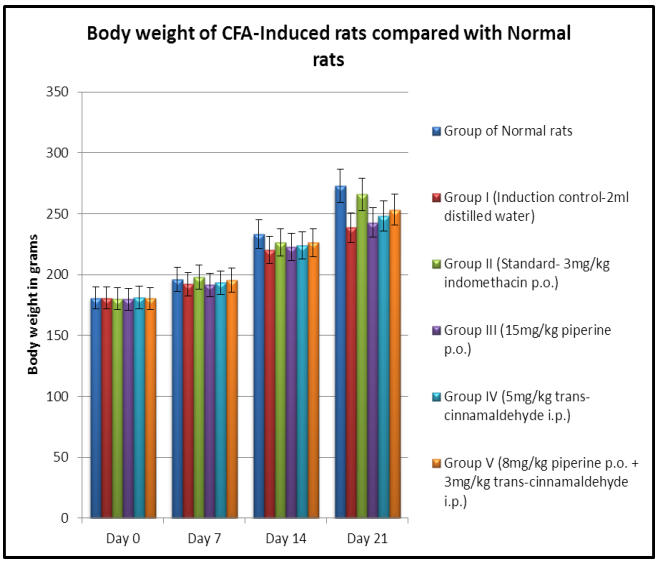

Figure 2: Graph of body weight of CFA - induced rats from different groups in comparison with the Normal rats.

The average gain in the body weight of rats on day 21 was compared with the initial body weight in each group. The rats in the CFA-induced group I gained less body weight and statistically significant as compared with the group of normal rats. Similar effect on the average gain in body weight was clearly apparent in rats of group III, which were treated with 15mg/kg piperine phytoconstituent.

Histopathological Examination:

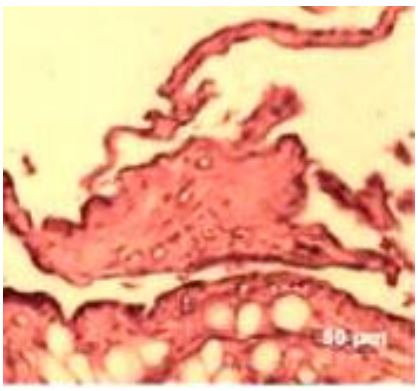

Figure 3a: Normal

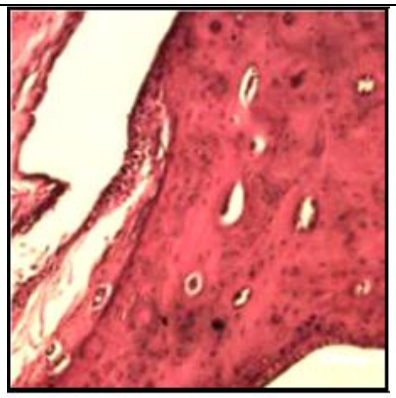

Figure 3b: Arthritic Control

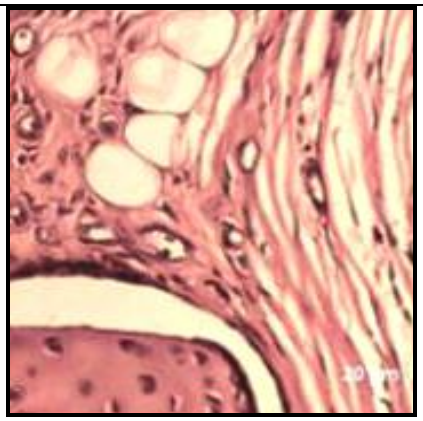

Figure 3c: Indomethacin Treated

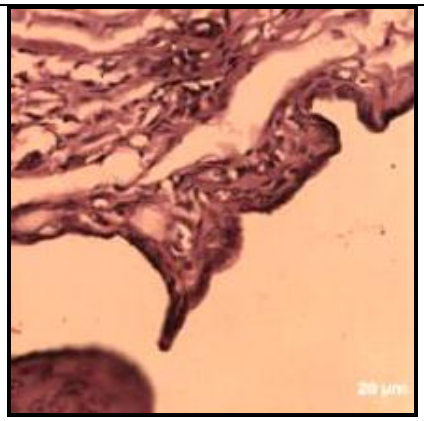

Figure 3d: Piperine Treated

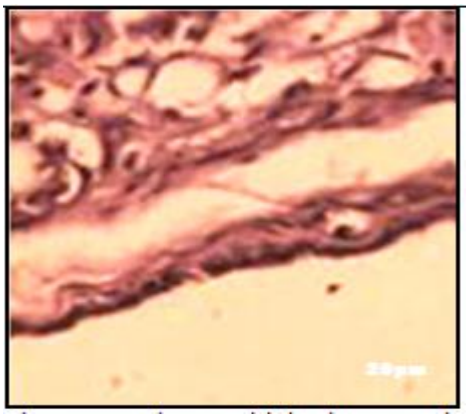

Figure 3e: Cinnamaldehyde Treated

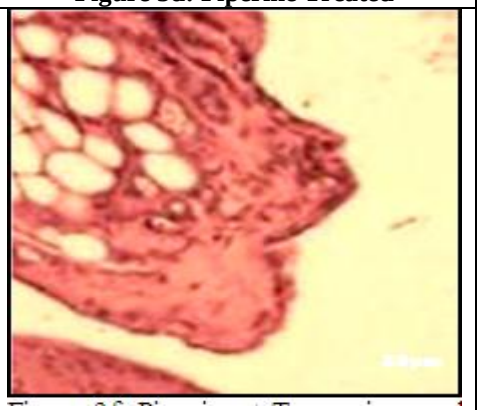

Figure 3f: Piperine + Trans-cinnamaldehyde Treated

Figure 3: Histopathological analysis of hind ankle joints of normal and experimental rats.

Figure 3a showed absence of mononuclear cell infiltration in the synovium and normal articular cartilage. The ankle joint of arthritic control rat (Figure 3b) showed marked synovial hyperplasia, mononuclear cell infiltration in the synovial membrane and cartilage erosion. The rats treated with indomethacin (Figure 3c) and piperine + trans-cinnamaldehyde combination (Figure 3f) showed reduced cellular infiltration and slight synovial hyperplasia. And, the rats treated with piperine (Figure 3d) and trans-cinnamaldehyde (Figure 3e) showed synovial hyperplasia along with moderate and slight infiltration of inflammatory leukocyte, largely neutrophils, respectively.

DISCUSSION

Rheumatoid arthritis involves an aberrant immune response that results in synovial inflammation and destruction of joints. The outcome is chronic inflammation that causes pain and destruction of cartilage and bone.

Injection of CFA into the paw of experimental animal induces inflammation as primary lesion in first 3-5 days. Secondary lesions occurs after an interval of nearly 11-12 days, which are characterized by inflammation of the non-injected sites. The secondary lesions are due to the activation of the immune system by Freund's adjuvant, where the proliferative T-cells are the precursor of pro-inflammatory mediators. The inhibition of the increase in hind paw volume on day 3 is associated with inhibition of neutrophil infiltration. The (piperine + trans-cinnamaldehyde) combination significantly reduced swelling of CFA-injected paw and also inhibited the inflammation of non-injected sites, the results of which were comparable with standard drug indomethacin. The (piperine + trans-cinnamaldehyde) combination inhibited the activation and proliferation of T-cells thereby inhibiting the secondary lesions. Thus, the combination of piperine and trans-cinnamaldehyde limit the inflammatory mediators and the immune system, explaining us rationale behind its anti-arthritic activity.

Histopathological studies support and justify the anti-arthritic effect of (piperine + trans-cinnamladehyde) combination. The TS of the ankle joint of arthritic control rats gave evidence for edema formation, extensive mononuclear cell infiltration and degeneration with erosion of cartilage. The TS of ankle joint of indomethacin treated rat and combination treated rat revealed normal articular cartilage with slight cellular infiltration and insignificant synovial hyperplasia.

CONCLUSION

On the basis of discussion and findings, it can be easily concluded that the combination of trans-cinnamaldehyde and piperine is effective in experimentally induced arthritis model. The current experimental data reveals that there was a significant decrease in the primary lesion

as evident on day 3 was observed in the treatment groups (i.e. Group II, IV and V), except for Group III which was treated with piperine, as compared to arthritic control Group I. With regards to the secondary lesion, similar results were obtained, there was a significant decrease in paw volume of non-injected paw on day 11 in the treatment groups except for the piperine treated Group III.

And finally regarding body weight changes in each group, the control Group I and Group III (piperine) group showed less gain in weight when compared to normal rats. Whereas in Group V (piperine + trans-cinnamaldehyde) and Group II (indomethacin) treated group there was fair gain in weight as compared to normal rats which exhibits the tolerability of the drug during disease treatment.

Thus all put together the experiment confirm that combination treated group significantly reduced paw volume with less affecting the body weight in rats. The inhibition of the increase in hind paw volume may be associated with inhibition of neutrophil infiltration, synovial hyperplasia and cartilage erosion, which is supported by the histopathological reports in the research study.

This finding justifies the usefulness of phytoconstituents trans-cinnamaldehyde and piperine in the pharmacotherapy of the discussed auto-immune inflammatory disease like rheumatoid arthritis. It is concluded that the phytoconstituents possess significant anti-arthritic activity, which is comparable to indomethacin. But, further definitive studies are necessary to ascertain the mechanisms behind its anti-arthritic action.

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