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Original Research Article

Evaluation of Anti-arthritic and Analgesic Activity of *Celastruspaniculatus* in Different Animal Models

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ABSTRACT

The goal of the management of arthritis is to alleviate pain, joint swelling and inflammations. Most commonly prescribed nonsteroidal anti-inflammatory agents (NSAIDs) have associated side effects on human health. The search for a novel agent is therefore essential. The study evaluated the anti-arthritic and analgesic activity of *Celastruspaniculatus*ethanol extract in animal models. Arthritis was induced using formalin (0.1 mL 2% v/v), while hyperalgesia by acetic acid and eddy's hot plate in Wistar rats. The extracts, at 250 mg/kg and 500 mg/kg was administered to the animals for ten days. Changes in paw thickness, paw oedema volume and C-reactive protein levels were recorded. Histopathological analysis of knee joint was performed. The study results revealed that the extracts of *Celastruspaniculatus*significantly ($p< 0.05$) inhibited the increase in parameters of arthritis, inflammation and pain in a dose-dependent manner (the maximum effect was observed in 500 mg/kg body weight) and restored the normal architecture of the knee joint tissues. The study confirms the anti-arthritic and analgesic activity of *Celastruspaniculatus*.

Keywords: *Celastruspaniculatus*, formalin, anti-arthritic, C-reactive protein.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects mainly ankle joints, leading to its destruction. It is characterized by pain, swelling and synovial inflammations. This debilitating disorder can lead to cartilage destruction and result in disability.[1] The associated disability and poor quality of life increases morbidity and mortality in rheumatoid arthritis compared with the general population.[2] Cardiovascular disease comorbid condition in rheumatoid arthritis increases the mortality rate by 50% higher than the general population.[3] Globally, Rheumatoid arthritis accounts for approximately 0.5% to 1% of the population. Women are three times more prone compared to men. In women, rheumatoid arthritis most commonly begins between the ages of 30 and 60 years.[4-7] Perfect diagnosis of rheumatoid arthritis may be difficult during its progression. Early use of disease-modifying anti-rheumatic drugs and biologics has improved outcomes but requires close monitoring of disease course and adverse events.[8]
Prophylaxis with non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids has been beneficial in reducing pain and joint stiffness but has failed to restrict disease progression.[9,10] The mechanism of chronic and painful inflammatory arthritis is not well documented.[11] However, several experimentally induced methods of pain have explained that the tolerance levels are lower in RA patients compared to healthy subjects.[12,13] Generally prescribed painkillers are accompanied by toxicities problems even at therapeutic doses.
Despite enhanced drug development in the last few decades, there is still the need to source for new and novel analgesic molecules of plants origin that could be used in the management of RA. Therefore, a new alternative therapy for treating painful arthritic conditions with fewer side effects is highly recommended and investigated globally.
Since ancient time application of natural remedies in treating inflammatory and painful conditions begins with Ayurvedic treatment and has spread to the European and various systems of traditional medicines. Herbal medicine has been known to be used in different conventional medical practices to manage inflammatory diseases. Folk medicine and ecological awareness suggest that they usually cost less than synthetic drugs and undesirable side effects are less frequent.[2]*Celastruspaniculatus* (CP), 'Tree of life", also known as '*Jyothishmati'* in Sanskrit, belong to the family *Celastraceae*, a traditional medicinal plant used forcenturies as a memory enhancer, anti-inflammatory, analgesic, sedative andantiepileptic agent.[14] It is a large, woody, climbing shrub, distributed almost all over India up to an altitude of 2000 m and cultivated wildly in China, Taiwan, Cambodia, Thailand, Vietnam, Indonesia, Malaysia, Nepal, Australia, including the Pacific Islands.[3,15] Different parts of *Celastruspaniculatus* have been employed in the treatment of various medical conditions.[16] The plant's root has been used to treat menstrual pain and promote fertility,and the root powder for pneumonia and rheumatism.[17,18] The leaves and roots poultice are used as a paste to treat headaches.[19] The powdered mixture of the root and stem bark of CP is used in treating malaria.[20] The decoction of leaves is applied externally on swellings and fractures.[17] Singh *et al* have reported the effect of the seed oil in treating gout and rheumatism.[21] The present study investigated the anti-arthritic and analgesic activity of the whole plant of *Celastruspaniculatus* extracts in Wistar rats.

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Materials and Methods

Chemicals
Ethanol and formaldehyde (Qualigens Fine Chemicals from Crescent Chemicals, Hyderabad), diclofenac sodium injection IP [Mfg.Lic.no:DD/L/713; Batch no: 172010SP Mfg dt:02/2017; Exp dt:07/2018 (Apollo Pharmacy, Hyderabad, India, Pentazocine and

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Aspirin were used as standard controls for the study. Other chemicals and reagents used for the analysis were of analytical grade.

Plant collection and extraction

The whole plant of *Celastruspaniculatus* was collected and identified on 17th January 2017 by Dr. K. MadhavaChetty, Professor from Department of Botany and Plant Taxonomy, Sri Venkateshwara University, Tirupati, India. A voucher (1124) specimen was deposited in the Department.

Extraction process

The dried powder (300 g) of the whole plant of *Celastruspaniculatus* was loaded onto a Soxhlet evaporator and extracted with 220 mL of 70% ethanol at 70°C for 72 hrs. The extract was concentrated to dryness at 50°C and stored in an airtight glass container at 4-8°C until further use.[22]

Preliminary Phytochemical screening

The ethanol extract was subjected to qualitative analysis for the various phytoconstituents: Alkaloids with Dragendorff's reagent, flavonoids with alkaline reagent (sodium hydroxide) and lead acetate, tannins with 1% gelatin solution, phenolic compounds with ferric chloride solution, glycosides with legal test and froth test for saponins.[23]

Experimental animals

Healthy adult male Wistar rats weighing between 150-180 g and female mice (20-25 g) were used for the experiments. All the animals were obtained from Sainath Agency (282/PO/Bt/S/2000 CPCSEA) Hyderabad, Telangana, India. Ethical approval (No. IAEC/ SWCP/2017/001) for this study was obtained from the Institutional Animal Ethics Committee on 6th May 2017. The study was carried out with the guidelines stipulated by the Committee for Control and Supervision of Experiments on Animals. The animals were housed in clean cages with husk bedding and maintained at 24°C ± 2°C under 12 hrs light/dark cycle and fed *ad libitum* with standard pellets diet (supplied by Pranav Agro Industries Ltd. Sangli. India) and had free access to water. Before use, the animals were acclimatized to laboratory conditions for two weeks and cared for according to the criteria outlined in CPCSEA 102 guidelines 2003, Government of India.

Acute oral toxicity studies

Healthy female mice (20-25 g) were subjected to acute oral toxicity studies, according to the fixed-dose procedure described in the OECD guideline 423, with ethanol extract of the whole plant. Animals were observed individually after dosing at regular time interval for the first 4 hours followed by periodic monitoring for next 24 hours, and then daily for 14 days. Changes in respiration, skin colour, eyes, central nervous system-related activities, and other behavioural patterns were also noted (OECD, 2001).

Formalin induced Arthritis

Experimental design

Animals were divided into five groups of six animals each (n=6). The baseline paw thickness was measured by a verniercalliper and paw volume by plethysmograph on day 0 of the experiment.

Group 1- Normal control received vehicle (1 ml/kg, saline p.o.)

Group 2 -Arthritic control group was administered 0.1 mL of 2% v/v formalin only.

Group 3 - Standard drug diclofenac sodium (10 mg/kg, i.p.)

Group 4- Ethanol extract of *Celastrus Paniculatus* (EECP) (250 mg/kg p.o)

Group 5- Ethanol extract of *Celastrus Paniculatus* (EECP) (500 mg/kg p.o)

Thirty minutes after oral administration of vehicle/drugs, arthritis was induced by subplantar administration of 0.1 mL formalin (2% v/v) into all the animals' right hind paws.[24] This was designated as Day 1. The same dosing schedule was observed for the next 9 days. On Day 3 the animals were again injected with formalin (0.1 mL 2% v/v) into the same paw.[25] The changes in paw thickness and paw volume was recorded on days 0, 2, 4, 6, 8 and 10, 30 minutes after administering the respective vehicle/drug treatment. The body weight changes were recorded every day by weighing balance. Arthritis was assessed by measuring the mean increase in paw thickness and oedema volume over 10 days.

The percentage inhibition of right paw oedema was calculated from the following formula:

% inhibition of edema = (1-Vt)/Vc x 100

Where, V_C = Paw edema volume of control group
V_t = Paw edema volume of the test group

Assessment of Arthritis

The progression of formalin-induced arthritis was evaluated by measuring the following parameters on 0, 2, 4, 6, 8, and 10days after injection.

Paw volume

The swelling in the hind paw from the ankle was measured periodically on the days mentioned above using a plethysmograph. The oedema component of arthritis was estimated by calculating the difference between day 0 paw volume and paw volumes at various time points.[26]

Paw thickness

The oedema component of arthritis was estimated by calculating the difference between day 0 paw thicknesses and paw thicknesses at various time points using a vernier calliper.[27]

Arthritis score

Rats were scored for arthritis (arthritis index) daily by a set of visual criteria.[28]

The following scoring system was used:

No change = 0

Swelling and erythema = 1

Mild swelling = 2

Gross swelling = 3

Gross swelling and deformity = 4

Experimental method for the evaluation of Analgesic Activity

The analgesic activity of the extracts was evaluated using the acetic acid-induced writhing test and Eddy's Hot Plate method.

Writhing test

The writhing test was performed on Swiss mice as described by Chao.[29]The animals were divided into four groups containing six in each. Group, I received vehicle (1 mL normal saline). Standard drug, aspirin was administered to Group II animals at a dose of 30 mg/kg *p.o.* Group III and IV received EECP p.o. at a dose of 250, 500 mg/kg body weight, respectively. Thirty minutes after administering vehicle, standard drug and EECP, writhings were induced by intraperitoneal injection of 0.1 mL of 0.6% v/v of acetic acid solution. Numbers of writhes were counted over 20 minutes after acetic acid injection in all the groups. The same treatment schedule as per the assigned groups was continued for ten days. On the last day of the study (Day 10), the same test procedure was repeated. The percentage inhibition in writhes was calculated from the formula:

$$\text{Percent protection} = 100 - \left(\frac{X_t}{X_c}\right) \times 100$$

Where, X_t= Average number of writhes in treated group
x_c = Average number of writhes in control group

Hot plate test

The Hot plate test was carried out on the Swiss albino mice using a hot plate analgesiometer (IITC, USA). Animals were accustomed twice to the hot plate in advance. The response was defined as licking or biting a paw or jumping (where all four paws leave the plate). The

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time gap in seconds between the baseline and reaction was recorded as the response latency. To prevent tissue damage, a cut off time of 15 seconds was determined. Animals were divided into four groups containing six in each. Group I received vehicle (1 mL normal saline), Group II received the standard drug, pentazocine IP. (17 mg/kg body weight), Group III and IV received EECP orally, at a dose of 250 and 500 mg/kg, respectively. The animals were placed on the hot plate maintained at 55 ± 0.5°C. Latency time until either licking or jumping occurs was recorded periodically at 0, 20, 60, 90, and 120 min after administration of vehicle, standard and EECP.

Histopathology
On day 10 of the experiment, all the animals were anaesthetized under light ether anesthesia and sacrificed by cervical decapitation. Then, the right hind (arthritis induced) limb was removed just distal to the knee, washed with saline and stored in 10% formalin. The knee joints were washed in running water, decalcified by placing in formic acid and dehydrated with alcohol and at last cleared in xylene. Then the tissues were embedded in molten paraffin wax. Then hard paraffin wax was melted and poured into square-shaped blocks. The knee joints were then placed into the liquid paraffin quickly and allowed to cool. The blocks were cut using microtome to get sections of thickness 10 µm. The section was dried completely before staining. Eosin an acidic stain and hematoxylin a basic stain was used for staining and observed under an electronic microscope for histopathological changes.[24]

Biochemical Estimation
On the last day of the study, blood was collected by retro-orbital plexus for evaluating C-reactive protein (CRP) levels in plain tubes. The serum was separated, and samples were analyzed for CRP by an enzyme-linked immunosorbent assay kit.[30,31]

Statistical Analysis
The data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. The results were expressed as mean ±standard error of the mean. The level of significance was set at $p<0.05$. All statistical tests were carried out using Prism 6.0 (Graph Pad Prism®, San Diego CA, USA) statistical software.

Results and Discussion

The percentage yield of extract
The percentage yield of extract was found to be 18%.

Preliminary phytochemical analysis
Phytochemical screening results of the ethanol extract of CP revealed the presence of alkaloids, glycosides, tannins, flavonoids, saponins, proteins, terpenoids, and phenolic compounds in the plant sample.

Acute oral toxicity study
No changes in the behavioural pattern or any signs of toxicity or mortality were observed after 2 weeks. The extract was safe up to a maximum tested dose of 2000 mg/kg body weight. The doses selected for the study were 250 mg/kg (low dose) and 500 mg/kg (higher dose).

Anti-arthritic activity
Formalin induced arthritis model in rodents is a widely accepted method for demonstrating swelling of joints, as it produces similar effects noticed in human arthritis. Several compounds, especially of plant origin, have been extensively studied to treat the arthritic condition. Measurement of hind paw swelling is a simple and reliable procedure for determining the degree of inflammation and the beneficial effect of drugs.[1] Progression of chronic swelling in joints involving inflammatory cells causes erosion of joint cartilage and finally destroying bones. Release of mediators such as cytokines (IL-1B and TNF-alpha) and platelet-derived growth factor (PGDF) in response to inflammation and injury to tissues lead to pain, bone, and cartilage disruption promote severe disability.[32-34] In our study, we observed an increase in paw thickness, oedema volume of the right-hand paw of animals on the administration of 0.1 mL of 2% v/v formalin compared to normal control, with a significant increase in C-reactive protein levels.

Effect of EECP on Paw oedema volume in formalin-induced arthritis (FIA) Rats
The animals treated with formalin showed a significant increase in paw oedema. A significant decrease was observed in paw oedema volume after treatment with both the standard drug and EECP extracts. The 500 mg/kg extract showed better inhibition ($p<0.01$) of paw volume in the experimental animals compared to the 250 mg/kg dose during the study period. The percentage inhibition in paw oedema volume was comparable at 500 mg/kg dose of extract to the standard drug (Table 1).

Effect of EECP on Paw thickness in FIA rats
There was an increase in paw thickness recorded in all the groups of animals throughout the treatment with formalin except the normal control group. Though there was a decrease in the paw thickness in all the treatment groups, statistical significance was only observed in standard ($p<0.001$) and both doses of the extract on day 10 of the study. The ethanol extract of CP at 500 mg/kg dose produced a better and more significant ($p<0.001$) response than 250 mg/kg (Table 2).

Effect of EECP arthritis score in FIA rats
A marked increase in arthritis score was observed after induction of arthritis in the control group. Conversely, a pronounced significant decrease ($p<0.001$) was observed in the scores in treatment groups compared to arthritic control (Table 3).

Histopathology of Knee Joints
Histopathological examination of arthritic control animals exhibited inflammation of periosteum indicating induction of arthritis of synovial joints compared to the normal control group (Figure 1A and C). We observed regeneration of cartilage and new bone formation with mild changes in the hypertrophied synovial lining in the animals treated with 500 mg/kg of EECP groups (Figure 1E) compared to positive control. These findings prove the anti-arthritic potential of CP extracts. Several rheumatologists based on clinical manifestations suggested that disability in patients can be controlled by early diagnosis of the condition. Studies show that determining the rheumatoid factor and anti-cyclic citrullinated peptide (anti-ccp) can be helpful in the diagnosis of RA.[35-37]

Effect of EECP on Serum C-reactive protein levels in FIA rats
C-reactive protein is valued as a potent biomarker for increased risk of rheumatoid arthritis.It is regarded as an acute-phase protein and recognized as an exclusive marker in prognosis and diagnosis of various inflammatory, degenerative and neoplastic diseases.[38]CRP is treated as a sensitive marker of systemic inflammation and is elevated in patients with RA[39,40]. In their study, Masi et al.show that there was increased CRP levels during the early phase of the disease.[41] Several other published reports explain the association of elevated levels of CRP and inflammatory diseases causing tissue damage with more preference to RA patients.[42] In our previous study, we reported enhanced CRP levels in all animals treated with formalin, indicating the induction of inflammatory arthritis.[43] Similarly, the current study showed a marked increase in C-reactive protein levels in the arthritic control and test groups compared to normal, which proves the presence of inflammation. However, upon ten days of treatment with both doses of ethanol extract of CP and diclofenac sodium, a significant ($p<0.001$) reduction in CRP levels was recorded compared to control animals. There was a significant mean percentage change in all the treatment groups compared to arthritic control ($p<0.01$). The results reflect the association of CRP and RA (Table 4) (Figure 2).

Analgesic Activity
Effect of EECP on Acetic acid-induced Writhing test Model on Day 1 and Day 10
Persisting pain in knee joints in inflammatory arthritis needs medical advice and rheumatologic care. Global statistics show that in patients

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with a complaint of RA, approximately 90% reported pain as the main reason for consulting a rheumatologist.[44-46] The American College of Rheumatology Pain Management Task Force has proclaimed that "pain is probably the most important patient-reported outcome in rheumatology".[46] The essential requirement for healthcare practitioners is to develop treatment strategies and to study the potential of new pharmacological interventions that can benefit patients at each stage of the disease progression.[47,48]

The acetic acid-induced writhing test is typically used to evaluate the effect of drugs and chemicals through peripheral analgesic action. The endpoint of the procedure includes constriction of the abdomen, turning of the trunk (twist) and extension of hind limbs as a writhing reaction. The abdominal constrictions produced after administration of acetic acid is related to the sensitization of nociceptive receptors to prostaglandins.[49] Figure 3 shows acetic acid-induced writing response on days 1 and 10. The data clearly indicates that the number of abdominal stretching correlated with the intensity of acetic acid-induced nociception in the untreated group. Whereas aspirin and 500 mg/kg EECP treated animals showed highly significant inhibition in the number of writhings compared to normal control (p<0.001).

Furthermore, enhanced effect was observed in inhibition of writhings in all the treatment groups after ten days of continued dosing. (EECP at 500 mg/kg dose showed the maximum percentage of inhibition compared to standard and 250 mg/kg (Figure 4). These results confirm the analgesic effect of CP and explain that the extracts may be acting by inhibiting the release of prostaglandins and sympathomimetic mediators like PGE2 and PGE2α. The response is thought to be mediated by peritoneal acid-sensing ion channels and the prostaglandins pathway.[50]

Effect of EECP on Results of Eddy's Hot Plate test on Day 1and 10

Thermally induced nociception tests indicate narcotic involvement, i.e., central analgesic action. The hot plate method is usually used to study centrally acting analgesics that elevate the pain threshold in mice towards heat. The study revealed an increase in pain response in mice when subjected to Eddy's hot plate test on day 1. The standard drug, pentazocine (17 mg/kg) and EECP (250 and 500 mg/kg), produced significant pain inhibition compared to normal control in the hot plate method. At 500 mg/kg, the extract showed maximum analgesic effect as indicated by a marked increase in reaction time at first 30 minutes and continued effect at the last 120 minutes. The increase in reaction time indicates the potent analgesic effect of the standard drug and the extracts. Ten days of continued dosing further increased pain latency in all the treatment groups at all-time points. However, 500 mg/kg EECP showed the maximum nociceptive responses to thermal stimuli (Tables 5 and 6).

Table 1: Mean changes in paw edema volume and percent inhibition in FIA rats

Group	Day 2	Day 6	Day 10	% Inhibition
Normal control 1 mL of saline	0.18 ± 0.03	0.20 ± 0.03	0.18 ± 0.03	-
Arthritic control 0.1 mL of 2% formalin	3.88 ± 0.13	3.90 ± 0.15	3.96 ± 0.17	-
Standard control 10 mg/kg, IP	2.66 ± 0.13 †,§	2.68 ± 0.17 †,§,	3.20 ± 0.08 †,§	27.85 ± 8.95†,§
EE of CP 250 mg/kg, p.o	4.01 ± 0.14 NS	3.95 ± 0.16 NS	3.51 ± 0.09 *,†	20.93 ± 7.33*,†
EE of CP 500 mg/kg, p.o	.23 ± 0.08 *,†	3.13 ± 0.06 *,†	3.23 ± 0.08 †,§	27.18 ± 7.98 †,§

All values expressed as Mean ±SEM. EE CP- Ethanol extract of *CelastrusPaniculatus*
*-p<0.05 compared to arthritic control, †-p<0.01 compared to Normal control
§ -p<0.001compared to Arthritic control, NS-Non-significant

Table 2: Effect of treatments on Paw thickness in FIA rats

Group	Day 2	Day 6	Day 10	% Inhibition
Normal control 1 mL of saline	0.16 ± 0.33	0.20 ± 0.03	0.18 ± 0.04	-
Arthritic control 0.1 mL of 2% formalin	0.68 ± 0.06	3.90 ± 0.15	0.78 ± 0.06	-
Standard control 10 mg/kg, IP	0.51 ± 0.07 NS	2.68 ± 0.17 †,§,	0.26 ± 0.03 †,§	59.64 ± 14.50 †,§
EE of CP 250 mg/kg, p.o	0.71 ± 0.60 NS	3.95 ± 0.16 NS	0.43 ± 0.04 †,§	35.11 ± 18.05 †,§
EE of CP 500 mg/kg, p.o	. 0.55 ± 0.04 NS	3.13 ± 0.06 *,†	0.35 ± 0.04 †,§	47.54 ± 16.47 †,§

All values expressed as Mean ±SEM. EE CP- Ethanol extract of *CelastrusPaniculatus*
†-p<0.01 compared to Normal control, §-p<0.001compared to Arthritic control
NS-Non-significant

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Table 3: Effect of treatments on arthritis score in FIA rats

Group	Day 2	Day 10	% Inhibition
Normal control 1 mL of saline	0.0	0.0	-
Arthritic control 0.1 mL of 2% formalin	3.83 ± 0.16	4.00 ± 0.0	-
Standard control 10 mg/kg, IP	1.33 ± 0.21†,§	1.333 ± 0.21†,§	66.67 ± 12.91 †,§
EE of CP 250 mg/kg, p.o	2.33 ± 0.21 †, §	1.833 ± 0.30 †,§	54.17 ± 18.82 †,§
EE of CP 500 mg/kg, p.o	. 1.33 ± 0.20 †,§	.167 ± 0.16 †,§	70.83 ± 10.21 †,§

All values expressed as Mean ±SEM. EE CP- Ethanol extract of *CelastrusPaniculatus*
†-p<0.01 compared to Normal control, §-p<0.001compared to Arthritic control

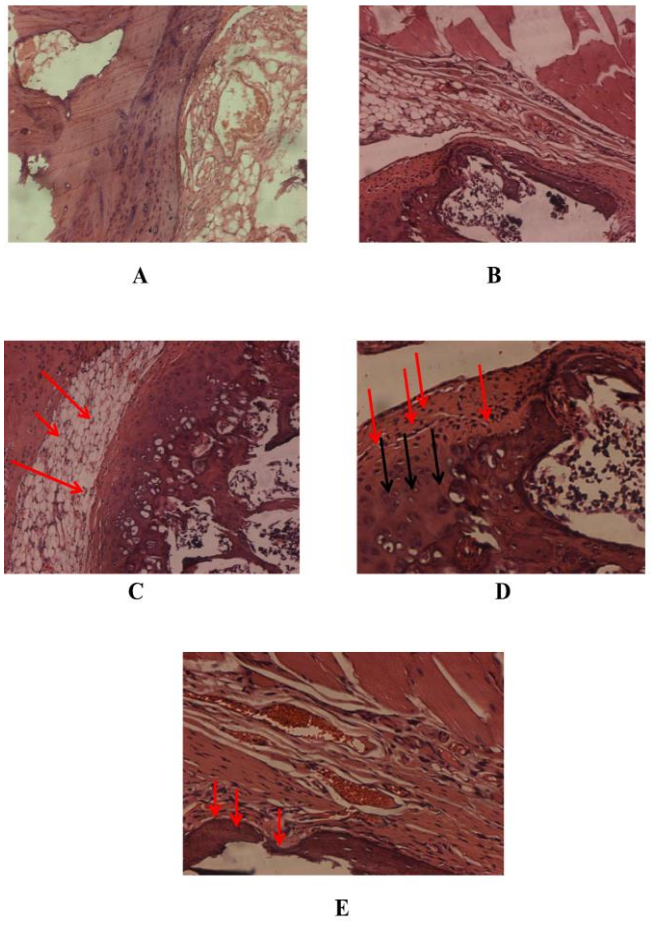

Figure 1: Photomicrographs of knee joint tissues.
(**A)Normal Control group:** These rats showed normal Periosteum of bone and the osteocytes appeared normal, active proliferation of Periosteum with collagenous is seen. **(B) Standard Control group:** Mild inflammation noticed between the Periosteum and bone, Mild thickening or proliferation of connective tissue in the Periosteum. Mild thickening or proliferation of connective tissue in the Periosteum, Mild inflammation noticed in the muscular layer. **(C) Arthritic Control group:** Inflammation of Periosteum with infiltration of inflammatory cells and angiogenesis or new blood vessel formation noticed. Inflammation of Periosteum with infiltration of inflammatory cells. Fatty degenerative changes near the Periosteum layer of the joint. **(D) Ethanol Extract of CP 250 mg/kg group**. Moderate proliferation of osteoblast along with thick fibrous connective tissue replacing the inflamed region of joints. Mild degenerative changes were noticed near the periosteum layer of joints. **(E) Ethanol Extract of CP 500 mg/kg group:** Inflammatory region of Periosteum layer replaced with connective tissue. Moderate angiogenesis was noticed.

Table 4: Effect on Serum C-reactive protein levels in FIA rats

Groups	Day 10
Normal control of 0.5 mL Saline	3.00 ± 0.57
Arthritic control of 0.1 mL 2% v/v formalin IP	46.50 ± 4.31
Diclofenac sodium IP	20.33 ± 0.76§
Ethanol extract of CP 250 mg/kg BW p.o	31.33 ± 0.49§
Ethanol extract of CP 500 mg/kg BW p.o	27.50 ± 0.76§

All values are expressed as Mean ± SEM. CP - *Celastrus Paniculatus*, § - p < 0.001 compared to Arthritic control.

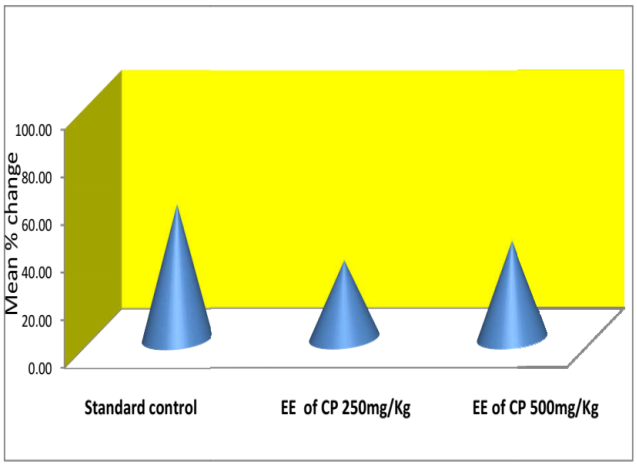

Figure 2: Mean Percent change in C- reactive protein Levels compared to Arthritic Control. EE--Ethanol extract of *CelastrusPaniculatus*, p<0.001 compared to Arthritic control.

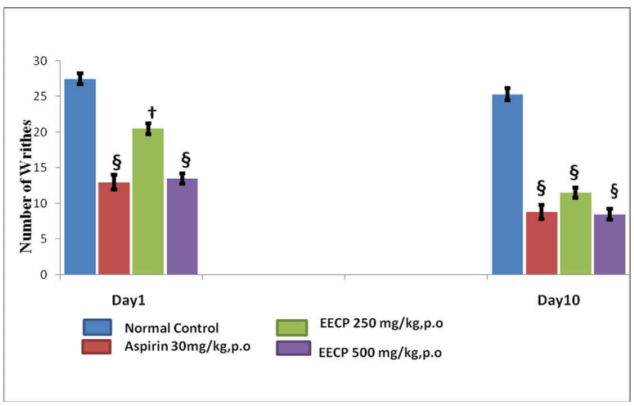

Figure 3: Acetic acid induced Writhing Test Response on Day 1 and Day 10.

All values expressed as Mean ± SEM. EECP-Ethanol extract of *CelastrusPaniculatus*. †-p<0.01 compared to normal control, §-p<0.001compared to Normal control

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Table 5: Results of Eddy's Hot Plate test on Day 1

Groups	0 min	30 min	60 min	120 min
Normal control	4.833 ± 0.30	4.8334 ± 0.30	5.000 ± 0.57	5.833 ± 0.60
Pentazocine17 mg/Kg BW IP	7.500 ± 0.76*	11.33 ± 0.88§	16.17 ± 1.01§	20.50 ± 0.76§
Ethanol extract of CP 250 mg /kg BW p.o	6.500 ± 0.75^{NS}	9.333 ± 0.88§,†	14.33 ± 1.11§	18.50 ± 0.75§
Ethanol extract of CP 500 mg/kg BW p.o	7.000 ± 0.57^{NS}	8.500 ± 0.76§,*	12.50 ± 0.99§	16.00 ± 0.85§,†

All values expressed as Mean ± SEM, CP-*Celastrus Paniculatus*
§-p<0.001compared to Arthritic control, †-p<0.01 compared to normal control
*-p<0.05 compared to Arthritic control, NS-Nonsignificant

Table 6: Results of Eddy's Hot Plate test on Day 10

Groups	0 min	30 min	60 min	120 min
Normal control	4.632 ± 0.30	4.8332 ± 0.30	5.833 ± 0.60	5.167 ± 0.30
Pentazocine 17 mg/kg BW IP	7.566 ± 0.76§	11.02 ± 0.80§,†	16.17 ± 0.60§,†	16.50 ± 0.76§,†
Ethanol extract of CP 250 mg/kg BW p.o	6.520 ± 0.75§,†	9.342 ± 0.82 §,†	13.00 ± 0.73§,†	14.33 ± 0.76§,†
Ethanol extract of CP 500 mg/kg BW p.o	7.050 ± 0.56§,†	10.8500 ± 0.76 §,†	11.83 ± 0.65§,†	12.50 ± 0.84§,†

All values expressed as Mean ± SEM CP-*Celastrus Paniculatus*, §-p<0.001compared to Arthritic control, †-p<0.01 compared to normal control

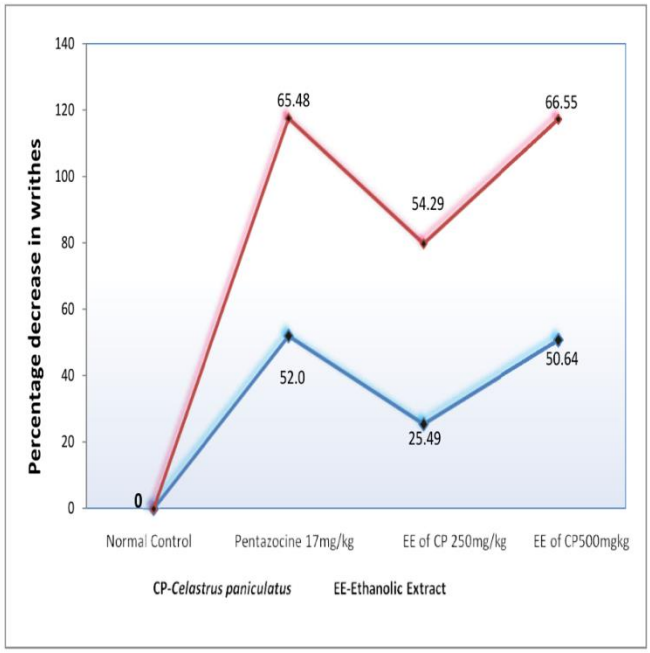

Figure 4: Percentage protection against Acetic acid induced Writhing test on Day 1 and Day10

Conclusion

The observations from the study suggest that the ethanol extract of *Celastruspaniculatus* possess antiarthritic and analgesic effects.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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