



## Original Article

# Evaluation of anti-inflammatory and anti-arthritic property of ethanolic extract of *Clitoria ternatea*

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## ABSTRACT

**Objective:** *Clitoria ternatea* is a well-known bioactive plant used to treat several inflammatory ailments in Ayurvedic system of medicine in India. The present investigation aimed to determine the anti-inflammatory and anti-arthritic activity of ethanolic extract of *Clitoria ternatea* roots (EECT) in animal models.

**Methods:** The anti-inflammatory activity of the EECT was evaluated by carrageenan and histamine-induced paw edema.

**Results:** EECT showed a significant reduction in mean paw edema volume in both carrageenan and histamine-induced inflammation. The efficacy of EECT in rheumatoid arthritis was tested against Freund's complete adjuvant (CFA) induced arthritic model in Wistar rats. The anti-arthritic effect of EECT was determined by systematic scoring of arthritis symptoms and measuring paw edema. A considerable decrease in paw diameter was observed in the EECT (200 and 400 mg/kg) and diclofenac (10 mg/kg) treated groups after day 7. Diclofenac (10 mg/kg) and EECT (400 mg/kg) showed a significant reduction in paw diameter from day 14 compared with CFA control ( $P < 0.001$ ). The anti-arthritic activity was also confirmed from the altered biochemical, haematological (Hb, RBC and WBC) and anti-oxidant parameters (SOD, MDA, CAT, and GSH). EECT (400 and 200 mg/kg) also showed a marked inhibition of joint destruction.

**Conclusion:** This study provides a pharmacological rationale for the traditional use of *C. ternatea* against inflammation and rheumatoid arthritis in India.

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## 1. Introduction

Inflammation is defined as the local response of living mammalian tissues to injury due to any foreign agent, in order to eliminate the spread of injurious agent, followed by removal of the necrosied cells and tissue (Mohan, 2013). Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disorder that principally attacks the joints producing a nonsuppurative proliferative inflammatory synovitis which often progress to destruction of articular cartilage and ankylosis of the joint. RA may also affect many tissues and organs like skin, heart, lung, blood vessels and muscles. Major pathological lesions are detected in the joints, tendons and less often extra-articular lesions are encountered (Kumar, Abbas, Fausto, & Aster, 2005). About 1% of the world population is affected by RA, women are two to three times more often affected than men (Silman & Pearson, 2002.). Although the exact cause of

RA is unknown, the interaction of genetic and environmental factors is believed to trigger an autoimmune response in the body. The condition is most likely triggered by a combination of factors including genetics (Kochi, Suzuki, & Yamamoto, 2014), age, injury, environmental triggers which includes infections (Fortunato, 1996), smoking and hormones. Common symptom of RA includes, joint pain and swelling, stiffness, fatigue, depression, anaemia, flu like symptoms, such as fever, sweating. Less common symptoms include weight loss, rheumatoid nodules and inflammation of other parts of the body.

*Clitoria ternatea* Linn. is a perennial, twining herb that belongs to the family Fabaceae. *C. ternatea* consists of bioactive compounds such as alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids and phenols. The major phytoconstituents found are the pentacyclic triterpenoids such as taraxerol and taraxerone. Phytochemical investigations of *C. ternatea* have revealed the presence of flavonoid glycosides such as rutin, delphinidin, kaempferol, quercetin and malvidin. *C. ternatea* is being used in traditional medicine for the treatment of severe bronchitis, asthma, indigestion, consti-

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pation, fever, arthritis, eye ailments, sore throat and skin diseases. It is commonly used in the Ayurvedic medicine, as a memory enhancer, anxiolytic, antidepressant, tranquilizing and sedative agent. The root, stem and flower are recommended for the treatment of snakebite and scorpion sting in India. The decoction or powder of root of *C. ternatea* is given in rheumatism, and ear-diseases. Ranaweera et al. assessed the anti-rheumatoid arthritic potential of an aqueous root extract (ARE) of *C. ternatea* using a well-recognised *in vitro* bioassay model: inhibition of heat denaturation of albumin protein, which is claimed to act as an index of anti-arthritic activity and concluded for the first time, that *C. ternatea* has marked antirheumatoid arthritic properties *in vitro* (Ranaweera, Pathirana, Ambalanduwa, Jayakody, & Ratnasooriya, 2014). However, no *in vivo* studies have been performed so far to further validate the anti-inflammatory and anti-arthritic property of *C. ternatea*. Therefore, this study was carried out to evaluate the anti-arthritic and anti-inflammatory property of *C. ternatea*.

## 2. Materials and methods

### 2.1. Plant collection and authentication

The roots of plant *C. ternatea* were collected from Thrissur district of Kerala and authenticated from the Botanical survey of India (BSI), southern circle, Coimbatore, Tamil Nadu. The authentication certificate number is No. BSI/SRC/5/23/2015/Tech/2551.

### 2.2. Extraction of plant materials

Dried and coarse powder of *C. ternatea* roots (800 g) was extracted successively with ethanol in Soxhlet extractor. Extract was concentrated to dryness in rotary evaporator under reduced pressure to yield a dark brown mass of ethanol extract of *C. ternatea* roots (EECT) (Taur & Patil, 2010). The extract was subjected to phytochemical analysis and estimation of phenolic and flavonoid content.

### 2.3. *In vitro* antioxidant studies

#### 2.3.1. DPPH free radical scavenging assay

The antioxidant activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical (Blois method). The solution of DPPH (0.3 mmol/L) in methanol was prepared and 1 mL of this solution was added to 1 mL of various concentrations of sample and the reference compound, quercetin. The sample was shaken vigorously and left to stand in the dark at room temperature for 30 min and then absorbance was measured at 517 nm. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples (Alam, Bristi, & Rafiqzaman, 2013). Percentage inhibition (I %) =  $(\text{Abs control} - \text{Abs sample} / \text{Abs control}) \times 100$

#### 2.3.2. ABTS free radical scavenging assay

ABTS radical scavenging activity of the extract was measured by Rice-Evans method. ABTS solution was diluted with phosphate buffer saline pH 7.4 (PBS) to an absorbance of  $0.70 \pm 0.02$  at 734 nm and equilibrated at 30 °C. After addition of 1 mL of diluted ABTS solution to various concentrations of sample or reference compound, the reaction mixture was incubated for 6 min and then absorbance was measured at 734 nm against a blank. The percentage inhibition was calculated according to the formula:

Percentage inhibition (I %) =  $(\text{Abs control} - \text{Abs sample} / \text{Abs control}) \times 100$ .

### 2.4. Pharmacological study

#### 2.4.1. Animals

Female Wistar rats of 6–8 weeks old and 160–180 g body weight were offered by KMCH College of pharmacy, Coimbatore, India. All rats were housed and maintained under standard conditions of temperature ( $25 \pm 5 \text{ }^\circ\text{C}$ ), relative humidity ( $55 \pm 10\%$ ), and 12/12 h light/dark cycle. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study. Protocols for the study were approved by the Institutional Animal Ethical Committee (IAEC) for Animal Care (Proposal number: KMCRET/M. Pharm/13/2015–16).

#### 2.4.2. Carrageenan-induced paw edema in rats

Wistar albino rats, 150–250 g were divided into four groups with six animals for each. The group II was treated with diclofenac (20 mg/kg) i.p and group III and IV were treated with 200 and 400 mg/kg of EECT respectively and group I served as control. Treatments were given 30 min before the administration of carrageenan. The rats were then challenged with subcutaneous injection of 0.1 mL of 1% solution of carrageenan into the sub plantar region of left paw. The paw volume was measured before (0 h) and after carrageenan injection at 1, 2, 3, 4, 5 and 6 h by plethysmometer (Vetal, Bodhankar, Mohan, & Thakurdesai, 2013; Meshram, Kumar, Rizvi, Tripathi, & Khan, 2016).

#### 2.4.3. Histamine-induced rat paw edema

One hour after the drug treatment, inflammation was induced by injection of 0.1 mL of freshly prepared histamine (1%) in normal saline underneath the plantar tissue of the right hind paw of rats. Paw volume, measured using a plethysmometer before histamine administration and at 90 and 180 min after histamine injection (Sowemimo, Samuel, & Fageyinbo, 2013).

#### 2.4.4. Anti-arthritic activity of EECT

Anti-arthritic activity of EECT was evaluated in arthritis rats model induced by Complete Freund's Adjuvant (CFA). Diclofenac was used as standard drug. The drug was dissolved in 1% Na CMC and administered orally at a dose of 10 mg/kg daily for 28 d. EECT was dissolved in distilled water and administered orally to the rats at a dose of 200 and 400 mg/kg/d. Rats were divided into five groups of six animals for each. Arthritis rat model was established in all the groups except normal control. After induction of arthritis, the animals were treated as follows: Group-I was treated with solvent alone; Group-II was treated with Complete Freund's Adjuvant (CFA) 0.1 mL on the left hind paw; Group-III was treated with CFA + Diclofenac (10 mg/kg), p.o; Group-IV was treated with CFA + EECT (200 mg/kg), p.o. Drug treatment was started on day 1 and continued till 21st day. On day 21, the animals were anaesthetized and radiographs of the adjuvant injected hind paws were taken using X-ray. The animals were then sacrificed and terminal blood collection was done at the end of the study.

#### 2.4.5. Evaluation of anti-arthritic activity

Paw thickness was measured by Digital Vernier on days 1, 3, 5, 7, 14 and 21. The rats were assessed every three days for signs of arthritis for 21 d post CFA, using scoring system developed to evaluate the severity of AA. Paws were examined for severity and loci of Erythema, swelling and indurations using a 5-point scale. 0 = no signs of disease; 1 = signs involving the ankle/wrist; 2 = signs involving the ankle plus tarsal of the hind paw and/or wrist plus carpals of the forepaw; 3 = signs extending to the metatarsals or metacarpals; 4 = severe disease involving the entire hind or fore paw. The maximum arthritic score per rat was set at 16 (4 points  $\times$  4 paws) (Zhang et al., 2009).

#### 2.4.6. Estimation of catalase (CAT)

Hydrogen peroxide (4 mL) and phosphate buffer (5 mL) were added to 1 mL of tissue homogenate and mixed well. From this, 1 mL of solution was mixed with dichromate acetic acid reagent and was allowed to incubate for 30 min at room temperature. The absorbance was measured at 570 nm. The activity of catalase was expressed as  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  consumed /mg protein.

#### 2.4.7. Estimation of superoxide dismutase (SOD)

The 5% of tissue homogenate was mixed with 75 mmol/L Tris-HCl (pH 8.2), 30 mmol/L EDTA, and 2 mmol/L pyrogallol respectively. Then, the absorbance was measured at 420 nm. The percentage of inhibition was calculated depending on the ability of enzyme to inhibit oxidation (Marklund & Marklund, 1974; Li, 2012).

#### 2.4.8. Estimation of reduced glutathione (GSH)

TCA solution (1 mL) was added to 1 mL of the homogenate and centrifuged. The supernatant was collected and the precipitate formed was removed. To 0.5 mL of supernatant 2 mL of DTNB was added, the volume was made up to 3 mL with phosphate buffer. The absorbance was read at 412 nm. The amount of glutathione was expressed as  $\mu\text{g}/\text{mg}$  protein.

#### 2.4.9. Determination of lipid peroxidation (LPO)

The 2 mL of TBA-TCA-HCl reagent (ratio of 1:1:1) was added to 0.1 mL of the sample, mixed well and kept in a boiling water bath for 15 min. The solution was cooled and supernatant was removed. The absorbance was measured at 535 nm against reference blank. The level of lipid peroxidation was given as n moles of MDA formed/mg protein (Ohkawa, Ohishi, & Yagi, 1979).

### 2.5. Radiological analysis

The rats were anesthetized on the 21st day and were subjected to radiographical analysis using X-ray instrument (FUJIFILM, FCR PRIMA II). The instrument was operated at 75 kV peak, 50 mA and 2 s exposure time. Radiological changes were evaluated on the basis of a) joint space narrowing, b) joint space destruction, and c) degree of bone erosion.

### 2.6. Histological analysis

The sections were stained with haematoxylin and eosin and evaluated under light microscope for the presence of hyperplasia of synovium, pannus formation, inflammatory cell infiltration, and bone necrosis (Mehta, Sethiya, Mehta, & Shah, 2012).

### 2.7. Statistical analysis

Data were analyzed by one-way ANOVA followed by Dunnett's/Tukey's multiple comparison test using Graphpad 5.0 software. The values were expressed as Mean  $\pm$  SEM.  $P < 0.05$  was considered to be statistically significant.

## 3. Results

### 3.1. Extractive yield

The percentage yield of the extract was found to be 7.95%. The preliminary phytochemical analysis of the extract revealed the presence of alkaloids, flavonoids, terpenoids and phenolics. The total phenolic content in EECT was found to be 137 mg gallic acid equivalent per gram of extract. The total flavonoid content in EECT

was found to be 27.79 mg/g of extract calculated as quercetin equivalent.

### 3.2. In vitro antioxidant activity

The  $\text{IC}_{50}$  value of EECT and quercetin was found to be 34.71  $\mu\text{g}/\text{mL}$  and 6.040  $\mu\text{g}/\text{mL}$  respectively.  $\text{IC}_{50}$  value of quercetin and EECT was found to be 0.1794  $\mu\text{g}/\text{mL}$  and 3.82  $\mu\text{g}/\text{mL}$  respectively.

### 3.3. Carrageenan-induced paw oedema in rats

A significant anti oedematous activity at both doses (200 and 400 mg/kg) of EECT was observed during the second phase of inflammation. The maximal percentage inhibition was observed at 3rd and 4th h (Table 1). The Potency of EECT was found similar to that of diclofenac (20 mg/kg).

### 3.4. Histamine-induced rat paw oedema method

The two doses of extract (200 mg/kg and 400 mg/kg) exerted a significant inhibition of 41.66% and 54.16% at 90 min and 69.76% and 81.39% at 180 min respectively in the histamine-induced rat paw edema model (Table 2).

### 3.5. Antiarthritic activity of EECT

There was a significant increase in paw diameter of rats of all the groups treated with CFA compared to normal group. A considerable decrease in paw diameter was observed in the EECT (200 and 400 mg/kg) and diclofenac (10 mg/kg) treated groups after day 7. Diclofenac (10 mg/kg) and EECT (400 mg/kg) showed a significant ( $P < 0.001$ ) reduction in paw diameter from day 14 compared to CFA control (Table 3).

### 3.6. Arthritic scoring

The total arthritis score in inflamed paw increased significantly from 9th day onward in the control as well as the treated groups. The Manifestations peaked between days 15 and 17 and maximal scores were recorded on these days. Animals treated with standard drug (diclofenac 10 mg/kg) and EECT at a dose 400 mg/kg showed a significant decrease in arthritic score from 15th day onward till the end of the study (Table 4).

### 3.7. In vivo antioxidant study

A significant ( $P < 0.001$ ) depletion in the GSH, SOD and catalase ( $P < 0.001$ ) and a significant ( $P < 0.001$ ) elevation of LPO were observed in the edematous tissues of arthritic rats. With the treatment of EECT (200 and 400 mg/kg), diclofenac sodium (10 mg/kg) significantly ( $P < 0.01$ ,  $P < 0.05$ ) restored the levels of GSH, SOD and catalase (Table 5).

### 3.8. Histopathology of joints

Histopathological studies of the joint of CFA treated animals showed a significant disruption of synovial lining, dense infiltration of lymphocytes and plasma cells in subsynovial stroma. It also showed a higher degree of bone necrosis compared to the vehicle control group. Diclofenac (10 mg/kg) and EECT (400 mg/kg) treated group showed normal synovial lining, subsynovial stroma and no evidence of inflammatory cells and bone necrosis. Joints of rats treated with low dose of EECT (200 mg/kg) showed moderate infiltration of lymphocytes, plasma cells, eosinophils in subsynovial stroma, pannus formation with mild hyperplasia compared to the

**Table 1**  
Effect of EECT on carrageenan-induced paw oedema in rats (mean ± SEM, n = 6).

Treatments	Mean edema volume (mL) and inhibition (%)				
	1 h	2 h	3 h	4 h	5 h
Carrageenan	0.15 ± 0.003	0.17 ± 0.007	0.42 ± 0.02	0.46 ± 0.01	0.17 ± 0.013
Diclofenac (20 mg/kg)	0.11 ± 0.007** (26.66%)	0.09 ± 0.003*** (47.05%)	0.09 ± 0.02*** (77.57%)	0.03 ± 0.008*** (93.47%)	0.01 ± 0.002*** (94.11%)
EECT (200 mg/kg)	0.14 ± 0.009 <sup>ns</sup> (6.66%)	0.12 ± 0.003*** (29.41%)	0.10 ± 0.009*** (76.19%)	0.06 ± 0.009*** (86.95%)	0.03 ± 0.009*** (82.35%)
EECT (400 mg/kg)	0.12 ± 0.010 <sup>ns</sup> (20%)	0.10 ± 0.009*** (41.17%)	0.09 ± 0.0*** (77.57%)	0.05 ± 0.009*** (89.13%)	0.02 ± 0.004*** (88.23%)

Statistical analysis was done by one-way analysis of variation (ANOVA) followed by Dunnett's test. \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05 and ns- non significant. Standard and test were compared with carrageenan group.

**Table 2**  
Effect of EECT on histamine-induced rat paw oedema in rats (mean ± SEM, n = 6).

Groups	Treatments and doses	Mean edema volume (mL) and inhibition (%)	
		90 min	180 min
I	Histamine 1% (0.1 mL)	0.24 ± 0.018	0.43 ± 0.018
II	Diclofenac (20 mg/kg)	0.07 ± 0.009*** (70.83%)	0.05 ± 0.009*** (88.37%)
III	EECT (200 mg/kg)	0.14 ± 0.003** (41.66%)	0.13 ± 0.003*** (69.76%)
IV	EECT (400 mg/kg)	0.11 ± 0.018*** (54.16%)	0.08 ± 0.012*** (81.39%)

Statistical analysis was done by one-way analysis of variation (ANOVA) followed by Dunnett's test. \*\*\*\*P < 0.001, \*\*\*P < 0.01, \*\*P < 0.05 and ns- non significant. Standard and test were compared with histamine group.

arthritic control. The EECT suppressed joint inflammation in a dose-dependent manner (Fig. 1).

### 3.9. Radiological analysis

The radiological analysis of all the groups were taken and the drug treated groups were compared with arthritic control. Radiographs were analyzed for joint space narrowing, joint space destruction and bone erosion. Increased bone erosion and joint space reduction, and joint deformation were observed in the arthritic control group. The radiological results showed a significant joint space reduction (inter tarsal joints) which clearly indicates the cartilage degeneration, bone erosion and soft tissue swelling and joint deformation in the arthritic control group while the standard and extract treated groups showed no visible sign of joint deformation (Fig. 2). Thus, EECT (400 and 200 mg/kg) and diclofenac (10 mg/kg) showed a marked inhibition of joint destruction.

## 4. Discussion

The present study was carried out to evaluate the anti-inflammatory and anti-arthritic activity of *C. ternatea* based on

**Table 3**  
Effect of EECT on CFA induced arthritis paw diameter (mean ± SEM, n = 6).

Groups	Increase in paw diameter / mm				
	Day 3	Day 5	Day 7	Day 14	Day 21
Normal	0.07 ± 0.009	0.093 ± 0.4	0.09 ± 0.0	0.12 ± 0.0	0.13 ± 0.0
Arthritic control	2.48 ± 0.208***	4.09 ± 0.3***	4.9 ± 0.4***	5.6 ± 0.2***	6.63 ± 0.4***
CFA + Diclofenac (10 mg/kg)	2.03 ± 0.2 <sup>ns</sup>	3.10 ± 0.2 <sup>ns</sup>	3.53 ± 0.5 <sup>ns</sup>	2.86 ± 0.3***	2.91 ± 0.0***
CFA + EECT (200 mg/kg)	2.2 ± 0.3 <sup>ns</sup>	3.800 ± 0.3 <sup>ns</sup>	4.03 ± 0.6 <sup>ns</sup>	3.8 ± 0.3*	4.60 ± 0.4***
CFA + EECT (400 mg/kg)	2.43 ± 0.3 <sup>ns</sup>	3.60 ± 0.4 <sup>ns</sup>	3.93 ± 0.5 <sup>ns</sup>	3.16 ± 0.4***	3.30 ± 0.2***

Statistical analysis was done by one-way analysis of variation (ANOVA) followed by Tukey's multiple comparison test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 and ns- non significant. Arthritic control was compared with normal and the treated groups were compared with arthritic control.

the reports that *C. ternatea* possesses antioxidant activity and anti-inflammatory activity (Mukherjee, Kumar, Kumar, & Heinrich, 2008; Devi, Boominathan, & Mandal, 2003). Rheumatoid arthritis is a chronic inflammatory, autoimmune disease characterized by swelling, pain and injuries of cartilage, synovial membrane, tendons, muscles and bone. Complete Freund's adjuvant (CFA) is a commonly and widely used model to induce a rheumatoid arthritis-like inflammation in rats (Bihani, Rojatkhar, & Bodhankar, 2014; Choudhary, Kumar, Gupta, & Singh, 2014). Freund's complete adjuvant (CFA)-induced arthritis in rats has been employed widely as a model for chronic systemic inflammation and possesses many features in common with human rheumatoid arthritis. CFA consists of inactivated and dried mycobacterium, which effectively stimulates cell mediated immunity and ultimately leads the immunoglobulin production. During the developmental course of AA (Adjuvant Arthritis), there is initially an acute periarticular inflammation characterized by synovial mononuclear cell infiltration after 3–5 d. This is followed by chronic arthritis (secondary lesion) involving inflammation in non-injected sites (collateral paw, ear, nose and tail), synovial hyperplasia and destruction of periarticular bone and cartilage. Secondary lesions start after 12–14 d of induction. An increase in reactive oxygen species (ROS) and Reactive nitrogen species (RNS) is observed in many pathological conditions including arthritis. The production of ROS and RNS is also believed to be caused as a result of interaction between cellular immune system and body's endogenous and /or exogenous antigens that results in the activation of inflammatory signalling pathway like NK-κB and release of pro-inflammatory cytokines and chemokines. Free radical induced oxidative stress is the major cause of rheumatoid arthritis and other inflammatory disorders. Plants are potential source of antioxidant compounds like phenolic acids, poly phenols and flavonoids that scavenge free radicals such as peroxide, hydrogen peroxide of lipid hydroxyl and thus inhibit the oxidative damage that lead to the risk of various degenerative diseases associated with oxidative stress. Therefore, in the present study, the potential of the EECT to serve as an antioxidant was assayed.

To confirm the antioxidant property *C. ternatea*, phenolic and flavonoid content of ethanolic extract of *C. ternatea* was evaluated.

**Table 4**  
Effect of EECT on arthritic score (mean ± SEM, n = 6).

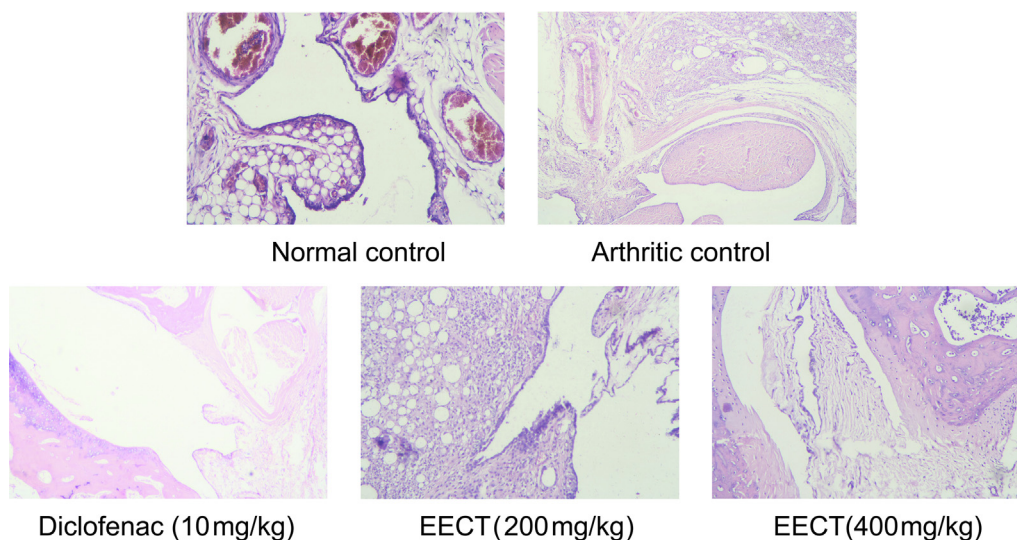
Groups	Arthritic scores									
	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
Control	1.66 ± 0.210	2.33 ± 0.2	3.16 ± 0.6	4.83 ± 0.9	5 ± 0.6	5 ± 0.6	5.17 ± 0.9	5.66 ± 0.8	6 ± 0.894	6 ± 0.8
CFA + Diclofenac (10 mg/kg)	0.93 ± 0.166**	1 ± 0.00**	1.33 ± 0.3*	2.13 ± 0.2*	2.13 ± 0.2*	2.33 ± 0.2*	3.66 ± 0.2 <sup>ns</sup>	3.16 ± 0.6 <sup>ns</sup>	3.16 ± 0.6*	3 ± 0.0**
CFA + EECT (200 mg/kg)	1.66 ± 0.210 <sup>ns</sup>	1.83 ± 0.1 <sup>ns</sup>	2.6 ± 0.5 <sup>ns</sup>	3.16 ± 0.6 <sup>ns</sup>	4.5 ± 1.0 <sup>ns</sup>	4.66 ± 1.0 <sup>ns</sup>	5.15 ± 0.9 <sup>ns</sup>	5 ± 1.6 <sup>ns</sup>	4.83 ± 0.9 <sup>ns</sup>	5 ± 1.0 <sup>ns</sup>
CFA + EECT (400 mg/kg)	1 ± 0.0*	1.16 ± 0.3*	1.5 ± 0.3 <sup>ns</sup>	2 ± 0.3**	2.13 ± 0.3*	2.6 ± 0.5 <sup>ns</sup>	3.5 ± 0.2 <sup>ns</sup>	4.5 ± 1.0 <sup>ns</sup>	4.3 ± 0.3 <sup>ns</sup>	3.5 ± 0.2*

Statistical analysis was done by one-way analysis of variation (ANOVA) followed by Dunnett’s test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 and ns- non significant. Standard and test were compared with control.

**Table 5**  
Effect of EECT on enzymatic and non-enzymatic antioxidant levels in rat paw tissue (mean ± SEM, n = 6).

Groups	Antioxidant enzymes			
	SOD (Unit/mg protein)	CAT (µmol of H2O2 consumed/ mg protein)	GSH (Glutathione µg/mg)	LPO (nmol of MDA/mg protein)
Normal	2.1 ± 0.3044	19.57 ± 1.130	12.98 ± 0.722	9 ± 0.577
Arthritic control	0.89 ± 0.082***	13.55 ± 0.977***	7.70 ± 0.570***	18.8 ± 0.621***
CFA + Diclofenac	1.79 ± 0.178**	18.10 ± 0.822**	12.17 ± 0.703***	10.17 ± 0.703***
CFA + EECT 200 mg/kg	1.26 ± 0.111 <sup>ns</sup>	17.17 ± 0.600*	10.73 ± 0.714*	14.33 ± 0.988**
CFA + EECT 400 mg/kg	1.61 ± 0.054*	17.9 ± 0.616**	11.77 ± 0.744**	12.83 ± 0.945***

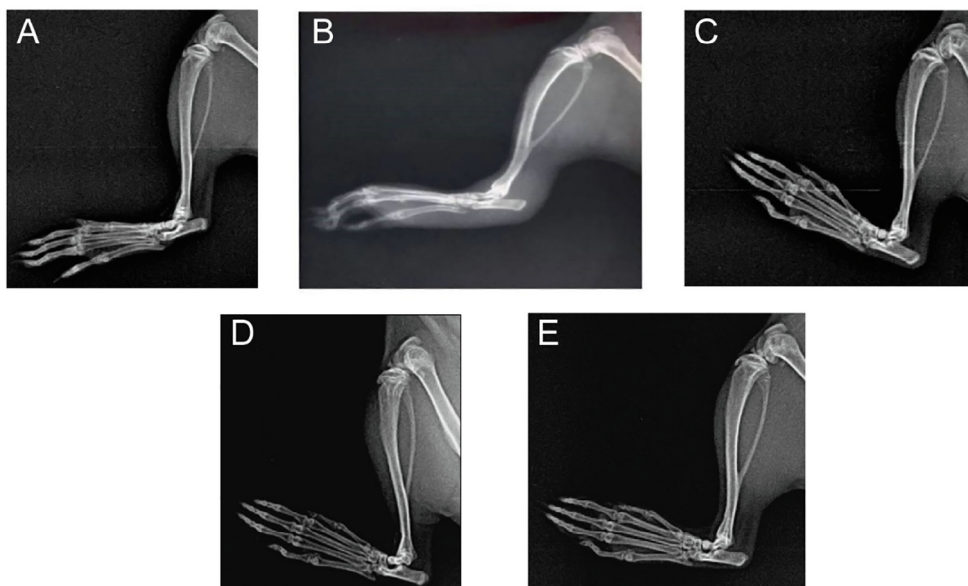
Statistical analysis was done by one-way analysis of variation (ANOVA) followed by Tukey’s multiple comparison test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 and ns- non significant. Arthritic control was compared with normal and the treated groups were compared with arthritic control.



**Fig. 1.** Histopathology of Joints. Normal control group shows normal synovial lining and no signs of inflammation. The arthritic control shows a significant disruption of synovial lining, bone necrosis, dense infiltration of lymphocytes and plasma cells in subsynovial stroma. The synovial lining is normal with no signs of inflammation and bone necrosis in the group treated with diclofenac. A moderate degree of inflammation and infiltration of lymphocytes were observed in the group treated with EECT 200 mg/kg. A normal synovial lining, subsynovial stroma and no evidence of inflammatory cells and bone necrosis was observed in the group treated with EECT 400 mg/kg.

The results indicated the presence of flavonoids and polyphenols in ethanolic extract of *C. ternatea*. The antioxidant potential of ethanolic extract of *C. ternatea* was further confirmed by DPPH and ABTS assay. In addition, *in vivo* antioxidant studies were also carried out to detect the levels of SOD, CAT and GSH. The treatment with ethanolic extract of *C. ternatea* restored the levels of these antioxidants confirming its antioxidant property. Phenols and flavonoids are phytoconstituents present in medicinal plants and these compounds have been reported in a plethora of studies to attenuate inflammation. The above results suggest that the anti-inflammatory activity of the EECT extract could be due to the presence of phenols and flavonoids which in turn contributes to its antioxidant property.

The anti-inflammatory activity of the EECT was evaluated by two experimental models, i.e., carrageenan-induced paw edema and histamine-induced paw edema. Carrageenan-induced paw edema model was used to screen the anti-inflammatory activity of a drug in the acute phase of inflammation. Sub plantar injection of carrageenan into the rat paw produces plasma extravasation and the inflammation characterized by increased tissue water and plasma protein exudation with neutrophil extravasation and metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways. Edema induced by carrageenan is a biphasic event. The first phase (1 h) involves the release of serotonin and histamine and the second phase (>1h) is mediated by prostaglandins. The mean edema volume and percentage inhibi-



**Fig. 2.** Radiological analysis of joints. Normal group (A), control group (B), diclofenac (10 mg/kg) group (C), test (200 mg/kg) group (D), test (400 mg/kg) group (E). An increase in bone erosion and joint space reduction and deformation of joints was observed in the control group. The groups treated with diclofenac, 200 and 400 mg/kg of test substance showed a marked inhibition of bone erosion and deformation of joints.

tion was calculated for 5 h. A significant anti edematous activity of both doses (200 and 400 mg/kg) of EECT was observed during the second phase of inflammation, indicating the inhibition of prostaglandin release. The maximal percentage inhibition was observed at 3rd and 4th hours. The Potency of EECT was found similar to that of diclofenac (20 mg/kg).

The two doses of extract (200 mg/kg and 400 mg/kg) exerted a significant inhibition of 41.66% and 54.16% at 90 min and 69.76% and 81.39% at 180 min respectively in the histamine-induced rat paw edema model. It was observed that the plant extract was capable of inhibiting oedema induced by histamine and the effectiveness for suppression of edema might be due to the ability of extract to inhibit the synthesis, release or action of histamine involved in the inflammation.

The anti-arthritis effect of EECT was determined by systematically scoring arthritis symptoms and by measuring paw edema.

There was a significant increase in paw diameter of rats of all the groups treated with CFA compared to normal group. A considerable decrease in paw diameter was observed in the EECT (200 and 400 mg/kg) and diclofenac (10 mg/kg) treated groups after day 7 (Bhalekar, Upadhaya, & Madgulkar, 2016). Diclofenac (10 mg/kg) and EECT (400 mg/kg) showed significant ( $P < 0.001$ ) reduction in paw diameter from day 14 compared to CFA control.

The rats were assessed every 3 d for signs of arthritis for 21 d post CFA, using a five points scale scoring system, developed to evaluate the severity of arthritis. The total arthritis score in inflamed paw was increased significantly from 9th day onward in the control as well as the treated groups. The manifestations peaked between days 15 and 17 and maximal scores recorded on these days. Animals treated with standard drug (diclofenac 10 mg/kg) and EECT at a dose of 400 mg/kg showed a significant decrease in arthritic score from 15th day onward till the end of the study, i.e. day 21 as compared to control animals.

The production of oxygen free radicals that occurs with the development of arthritis leads to decreased GSH and SOD levels as a consequence of their consumption during oxidative stress and cellular lysis, which is evident by decreased levels of GSH and SOD in arthritic control group. Lipid peroxidation is a critical mechanism of the injury that occurs during rheumatoid arthritis,

which is often measured by analysis of tissue MDA. The large amount of MDA in arthritic control group is consistent with the occurrence of damage mediated by free radicals (Patil, Kandhare, & Bhise, 2012). A significant ( $P < 0.001$ ) depletion in the GSH, SOD and catalase ( $P < 0.001$ ) and a significant ( $P < 0.001$ ) elevation of LPO were observed in the edematous tissues of arthritic rats. With the treatment of EECT (200 and 400 mg/kg), diclofenac sodium (10 mg/kg) restored significantly ( $P < 0.01$ ,  $P < 0.05$ ) the depletion of GSH, SOD and catalase, probably by competing for scavenging of free radicals and prevented the elevation of LPO levels in edematous tissues. It was also observed that the total protein level was significantly lower in the control group and the level was found markedly improved in standard and extract treated groups.

Histopathological studies of the joint of CFA treated animals showed a significant disruption of synovial lining, dense infiltration of lymphocytes and plasma cells in subsynovial stroma. It also showed a higher degree of bone necrosis compared to the vehicle control group. Diclofenac (10 mg/kg) and EECT (400 mg/kg) treated group showed normal synovial lining, subsynovial stroma and no evidence of inflammatory cells and bone necrosis. Joints of rats treated with low dose of EECT (200 mg/kg) showed moderate infiltration of lymphocytes, plasma cells, eosinophils in subsynovial stroma, pannus formation with mild hyperplasia compared to the arthritic control. The EECT suppressed joint inflammation in a dose-dependent manner.

The radiological results showed significant joint space reduction (inter tarsal joints) which clearly indicates the cartilage degeneration, bone erosion and soft tissue swelling and joint deformation in the arthritic control group while the standard and extract treated groups showed no visible sign of joint deformation. Thus, EECT (400 and 200 mg/kg) and diclofenac (10 mg/kg) showed a marked inhibition of joint destruction (histopathological and radiological analysis).

From this study, CT may be considered as a potent anti-inflammatory herb for the treatment of Rheumatoid arthritis, from its significant inhibitory effect on the release of inflammatory mediators (histamine and prostaglandins) and antioxidant property.

## 5. Conclusion

*C. ternatea* is a well-known bioactive plant in Ayurvedic system of medicine. The present investigation was aimed at determining the anti-inflammatory and anti-arthritic activity of *C. ternatea* root extract. This study confirms the ayurvedic claim that the ethanolic extract of *C. ternatea* root possesses significant anti-inflammatory and anti-arthritic activity. The anti-inflammatory and anti-arthritic property of ethanolic extract of *C. ternatea* maybe due to the presence of terpenoids, flavonoids and triterpenoids like taraxerol, taraxerone, rutin, quercetin, delphinidin, kaemferol, and malvidin. However, further studies are required to isolate and identify the possible phytoconstituents of *C. ternatea* responsible for the activity, which would facilitate the future use of isolated phytoconstituents of *C. ternatea* in inflammation related diseases.

## Declaration of competing interest

The authors declare no conflict of interests.

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