

Evaluating Weekly Progression In Arthritic Index, Ankle Diameter And Paw Volume Due To Ethanolic Fenugreek Seed Extract And No Modulators On Adjuvant Induced Rheumatoid Arthritis In Rats

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1. INTRODUCTION

A chronic inflammatory autoimmune illness known as rheumatoid arthritis (RA) causes joint degeneration, impairs joint function and increases mortality [1]. RA has a female preponderance, with a female to male ratio of 3:1, similar to most autoimmune illnesses, despite the fact that the overall frequency of RA among adults approaches 1%. [2]. It is between age group 30 and 50 when the incidence peaks [3]. RA is a multifactorial disease and its occurrence and severity is related to both genetic and environmental factors [4].

Pathophysiology of RA starts with tenacious joint synovial inflammation owing to permeation of actuated T-lymphocytes and macrophages, pannus development and consequent demolition of contiguous bone and cartilage tissue [5]. In the modern and developing world, it can involve multiple organ system leading to inflammation and irreversible joint damage, which causes early mortality, senility, and a reduced quality of life [6]. Macrophages, T and B lymphocytes, fibroblasts, pro-inflammatory cytokines, including interleukin (IL)-6, IL-1, and TNF-, along with COX (cyclooxygenases) and LOX (lipoxygenases), play a significant role in the pathogenesis of RA, which involves the hyperplasia of articular cartilage [7].

Over recent years evidence has emerged linking RA with anti-citrullinated peptide antibody currently measured as anti-cyclic citrullinated peptides (anti-CCP) autoantibodies [8]. Anti-CCP are now recognized to be a valuable biomarker of diagnostic and prognostic significance [9].

The level and scope of inflammation leading to cellular damage are determined by the imbalance between pro and anti-inflammatory cytokines. One of the key mediators in the pathogenesis of RA is thought to be pro-inflammatory cytokines such tumor necrosis factor (TNF) and interleukin 1 (IL-1) [10, 11]. While interleukin is another important inflammatory cytokine in the process of joint deterioration, tumor necrosis factor is one of the primary pro-inflammatory mediators recognized to play a significant role in the pathogenesis of RA that involves induction and continuation of joint inflammation [12].

Now, it has been established that the free radicals, involving reactive oxygen species (ROS) and reactive nitrogen species (RNS), play an important role in RAs inflammatory process [13]. Recently the formation, behavior and scavenging properties of oxygen and nitrogen free radicals in the biological system had received much attention [14]. Fortunately, the ROS production in healthy individuals is low and lipid peroxidation is inhibited by the combined activities of various antioxidants present in the plasma.

While effective drugs are available to treat symptoms and slow the progression of rheumatoid arthritis, they can have serious side effects. For example, non-steroidal anti-inflammatory drugs (NSAIDs) can cause gastrointestinal bleeding or perforation, and disease-modifying drugs can cause severe anemia and even death. Methotrexate can cause cirrhosis of the liver, sulfasalazine can cause thrombocytopenia, chloroquine can cause maculopathy, and leflunomide and etanercept can cause chest infections. Furthermore, these drugs may not provide good symptom control for all patients, and not all patients will find relief [15].

Numerous agent derived from plants have the potential for the treatment of rheumatoid arthritis like *Boswellia Serrata*, *Curcuma longa*, *Zingiber Officinale*, *Withania Somnifera* and *Trigoenella foenum graecum*. *Trigonella foenum graecum* also known as “methi” in Hindi or fenugreek in English belongs to family Fabaceae. Fenugreek is an annual plant, with leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semiarid crop. Its seeds and its leaves are common ingredients in dishes from South Asia.

Various activities are found in seed extract, seed oils, leaves which are their hypoglycemic effect [16-17], immunomodulatory effect [18], digestive effect [19] hypocholesterolemic effect [20], etc. These pharmacological activities of fenugreek show potential for the cure of chronic diseases but its mechanism of action is not well elucidated. Therefore there is need to understand the molecular mechanism of action of fenugreek.

Few studies have previously been conducted to assess the effectiveness of trigonella foenum graecum and mangiferin in the management of rheumatoid arthritis [21-23].

The current study examines the weekly progression of the arthritic index, body weight, and ankle diameter following inoculation of ethanolic fenugreek seed extract and NO modulators in rheumatoid arthritis-induced rats. This study as a whole assessed the protective role of ethanolic fenugreek seed extract and its potentiation by NO modulators is mediated through pro-inflammatory/anti-inflammatory cytokine imbalance and oxidative markers [24].

2. MATERIALS AND METHODS

A. Experimental Animals

For this investigation, 54 male Wistar rats (n = 6, 180-225 g) were used. Rats were bought from the CSIR-Indian Institute of Toxicological Research (IITR), Lucknow, certified breeding facility. The care of the animals were as per Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of KGMU, Lucknow (No. 91/IAEC/2018).

B. Methodology/Experimental Design

Prior to the experiment, animals were acclimatized to the surroundings for a period of 12 days during which they were given a normal pellet diet and water *ad libitum*. The rats were grouped into nine groups of 6 rats each as follows.

| | |
|-------------------|---|
| Group I | Control (normal saline) |
| Group II | Arthritis Group (CFA) |
| Group III | Arthritis Group + Methotrexate (5mg/kg) |
| Group IV | Arthritis Group + Fenugreek Seed Extract (200mg/kg). |
| Group V | Arthritis Group + Fenugreek Seed Extract (400mg/kg). |
| Group VI | Arthritis Group + L- arginine (100mg/kg). |
| Group VII | Arthritis Group + ^N -Nitro-L-arginine methyl ester hydrochloride (L-NAME) (10mg/kg). |
| Group VIII | Arthritis Group + Fenugreek Seed Extract (best dose) + L- arginine (100mg/kg). |
| Group IX | Arthritis Group + Fenugreek Seed Extract (best dose) + L- NAME (10mg/kg). |

CFU=Complete Freund's Adjuvant

C. Induction of Arthritis and Administration of Drugs

On day "0," rats were inoculated with 0.2 ml of Complete Freund's adjuvant (CFA) in the sub-plantar region, as described by Li et al., 2010 (25) and Pal et al., 2016. (26). On day "0," CFA sensitivity was altered by intradermal injection of 0.1 ml Squalene prior to CFA inoculation into a different site in the sub-plantar surface of the right hind paw. Arthritis was induced in the rats of group II to IX by injecting 0.1 ml of Complete Freund's Adjuvant (CFA) intradermally in the footpad of their left hind paw.

DAY 0-On day zero control group was given normal saline while rest group (group II to group IX) was injected with Complete Freund's adjuvant 0.1 ml on the left hindpaw.

DAY 1-14-As it takes 7-14 days for Complete Freund's Adjuvant to induce arthritis in rats, days 1-14 were spent in observation for the appearance of swelling in the limbs as a marker of induction of arthritis.

By day 14, almost all the rats developed arthritis which was suggested by the appearance in their limbs. Grading of arthritis was done in all the rats according to the arthritic index. After this, the pharmacological parameters i.e. paw thickness, ankle diameter, paw volume and body weight were again measured.

Vernier scale was used to assess changes in the diameter and hind paw volume of both ipsilateral (injected) and contralateral (non-injected) paws in experimentally induced arthritis in rats [30]. Ankle diameter was measured by compressing the tibiotarsal joint by vernier calipers till pain was elicited as indicated by squeaking or leg withdrawal. % inhibition of ankle diameter was calculated using the formula: -

$$\% \text{ Inhibition} = \frac{Dc - Dt}{Dc} \times 100$$

Where, Dc is main changes in ankle diameter of arthritis control group and DT is mean changes in ankle diameter of treated group [21-24].

H. Paw Volume

Each hind paw was immersed vertically to the level of the lateral malleolus in the plethysmometer. Mean changes in paw volume were calculated and % inhibition of paw oedema was calculated using the formula: -

$$\% \text{ Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, VC is mean changes in paw volume of control group and VT is mean changes in paw volume of treated groups [21-24].

I. Statistical Analysis

The data was analyzed by two-way ANOVA followed by Newman Keul's post hoc test for in group analysis by Graph Pad Prism6.0 and p value of <0.05 was taken as significant.

3. RESULTS

A. Effect of Fenugreek Seed Extract and NO Modulators on Adjuvant-Induced Changes in Arthritic Index

Significant increase in arthritis index was observed in CFA-inoculated rats during the course of study ($p < 0.001$). Arthritis index of adjuvant group (Group II) on day '0' was zero while it peaks to maximum level of 15.6 ± 1.8 on day 28. Treatment with methotrexate (5mg/kg) significantly decreases arthritis index as compared to adjuvant only group from 3.2 ± 0.8 on day 7 to 4.2 ± 0.73 on day 28 ($p < 0.05$). Treatment with fenugreek seed extract 200 mg/kg alone from decreases arthritis index 11.6 ± 1.93 on day 7 to 3.8 ± 0.73 on day 28, while treatment with fenugreek seed extract 400 mg/kg decreases arthritic index from 12.4 ± 1.4 on day 14 to 4.2 ± 0.8 on day 28 ($p < 0.001$). The group receiving adjuvant and L-arginine has shown maximum increase in arthritic index from 3.8 ± 0.54 on day 7 to 16.2 ± 0.6 on day 28.

Fenugreek seed extract 400 mg/kg and L-arginine 100 mg/kg together also decreased arthritis index, however the reduction was only partial. This group's arthritis score reached a maximum of 11.2 ± 1.2 on day 14 and remained stable until the start of treatment before dropping to 4.2 ± 0.73 on day 28. However, rats treated with fenugreek seed extract 400 mg/kg alone and in conjunction with low doses of NO modulator L-NAME (10 mg/kg) showed the greatest reduction in arthritis index of CFA-inoculated rats ($p < 0.001$). Table 1 provides a summary of these findings [21-24].

Table 1: Arthritis index of control, adjuvant and treatment groups on day 0, 7, 14, 21 and 28 of the study (all values represented here are in mean \pm SEM) (values on day 0, day 6 and 12 represent the value before treatment, and values on day 21 and day 28 are after treatment values; n = 6/group).

| Days | Group-I control | Group II adjuvant | Group III methotrexate | Group IV FSE (200mg/kg) | Group V FSE (400mg/kg) | Group VI adjuvant + L-arginine (100mg/kg) | Group VII adjuvant + L-NAME (10mg/kg) | Group VIII FSE (400 mg/kg) + L-arginine (100mg/kg) | Group IX FSE (400 mg/kg) + L-NAME (10mg/kg) |
|--------|-----------------|-------------------|------------------------|-------------------------|------------------------|---|---------------------------------------|--|---|
| Day 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Day 7 | 0 | 5.4 ± 0.6^a | 3.2 ± 0.8^a | 3.6 ± 0.6^a | 4.8 ± 0.73^a | 3.8 ± 0.54 | 4.4 ± 0.24 | 4.4 ± 1.03^a | 5.6 ± 1.28^a |
| Day 14 | 0 | 14.8 ± 1.2^a | 13.6 ± 1.47^a | 11.6 ± 1.93^a | 12.4 ± 1.4^a | 14.2 ± 1.3 | 12.8 ± 0.8 | 11.2 ± 1.2^a | 14.8 ± 1.2^a |
| Day 21 | 0 | 15.2 ± 1.5^b | 9.2 ± 0.8^b | 7.6 ± 0.97^b | 6.2 ± 1.14^b | 15.8 ± 1.4 | 10.6 ± 0.6 | 11.2 ± 1.2^b | 4.2 ± 0.73^b |
| Day 28 | 0 | 15.6 ± 1.8^c | 4.2 ± 0.73^c | 3.8 ± 0.73^c | 4.2 ± 0.8^c | 16.2 ± 0.6 | 9.4 ± 0.4 | 8.4 ± 0.9^c | 1.8 ± 0.4^c |

* $p < 0.05$ compared to day (0); ^a $p < 0.01$ compared to day (6); ^b $p < 0.05$ compared to day (14); ^c $p < 0.05$ compared to day (14).

B. Effect of Fenugreek Seed Extract and its Interactions with NO Modulators on Adjuvant-Induced Changes in Ankle Diameter

Significant increase in the ankle diameter of inoculated rats was observed due to marked inflammation in ankle joints. In adjuvant group ankle diameter increases from 2.3 ± 0.33 mm on day 0 to 12 ± 1.1 mm on day 28. Peak ankle diameter of methotrexate 5 mg/kg was 6.20 ± 0.37 mm on day 14 and then it decreased to 2.8 ± 0.2 mm on day 28. Ankle diameter of fenugreek seed extracts (FSE) 200mg/kg decreases from 6.6 ± 0.7 mm on day 14 to 3.8 ± 0.37 on day 28. Maximum change in ankle diameter was observed in group receiving FSE 400 mg/kg. Ankle diameter of FSE 400 mg/kg group reached a maximum value of 6.8 ± 0.37 mm on day 14 of the study and at the end of the study it was 2.4 ± 0.25 mm. Changes in ankle diameter of other groups were not as marked as the fenugreek seed extract 400 mg/kg group [21-24].

Ankle diameter of rats treated with combination of fenugreek seed extract 400 mg/kg and L-arginine 100 mg/kg decreases from 6.6 ± 0.25 mm on day 14 to 3.8 ± 0.2 mm on day 28. Maximum decrease in ankle diameter was observed in fenugreek seed extract 400 mg/kg and L-NAME 10 mg/kg combination. Ankle diameter of fenugreek seed extract 400 mg/kg and L-NAME 10 mg/kg reached a maximum of 7.20 ± 0.4 on day 14 and decreases to 3 ± 0.31 at the end of the study. These results are summarized in table 2 [21-24].

Table. 2: Ankle diameter of left hind paw (in millimeter) on day 0, 7, 14, 21 and 28 of the study (all values represented here are in mean \pm SEM) (values on day 0, day 6 and 12 represent the value before treatment, and values on day 21 and day 28 are after treatment values; n = 6/group).

| Days | Group II adjuvant | Group III methotrexate | Group IV FSE (200mg/kg) | Group V FSE (400mg/kg) | Group VI adjuvant + L-arginine (100mg/kg) | Group VII adjuvant + L-NAME (10mg/kg) | Group VIII FSE (400 mg/kg) + L-arginine (100mg/kg) | Group IX FSE (400 mg/kg) + L-NAME (10mg/kg) |
|--------|-----------------------|------------------------|-------------------------|------------------------|---|---------------------------------------|--|---|
| Day 0 | 2.3±0.33 | 2.4±0.24 | 2.6±0.25 | 2.2±0.2 | 2.2±0.4 | 2.1±0.6 | 2.8±0.2 | 2.4±0.24 |
| Day 7 | 4.6±0.33 [*] | 4.2±0.37 | 3.8±0.37 | 3.6±0.25 | 3.8±0.4 | 3.6±0.24 | 4.4±0.25 | 4.6±0.25 |
| Day 14 | 6.3±0.330 | 6.2±0.37 [*] | 6.6±0.7 [*] | 6.8±0.37 [*] | 7.4±0.34 [*] | 6.8±0.21 [*] | 6.6±0.25 [*] | 7.2±0.37 [*] |
| Day 21 | 10.6±0.67 | 4.4±0.25 | 5.2±0.37 | 3.4±0.25 | 10.8±0.46 | 4.6±0.62 | 5.2±0.37 | 4.4±0.25 |
| Day 28 | 12.0±1.1 [*] | 2.8±0.20 ^b | 3.8±0.37 ^b | 2.4±0.25 ^b | 12.6±0.64 [*] | 4.0±0.24 ^b | 3.8±0.20 ^b | 3.0±0.31 ^b |

* $p < 0.05$ compared to day (0); ^a $p < 0.01$ compared to day (0); ^b $p < 0.05$ compared to day (14);

C. Effect of Fenugreek Seed Extract (FSE) and Nitric Oxide (NO) modulators on adjuvant-induced changes in paw volume

CFA inoculation significantly increases paw volume of rats. Changes in paw volume of left hind paw were more significant as compared to right hind paw. Change in paw volume of adjuvant group was $45.31 \pm 13.6\%$ on day 7, which increases to 98.43% on day 28 ($p < 0.05$). Significant decrease in paw volume was observed in group receiving methotrexate 5mg/kg as compared to adjuvant only group. Paw volume of this group decreased from 54.9 ± 6.1 on day 7 to 23.0 ± 3.6 on day 28 [21-24].

Treatment with fenugreek seed extract (FSE) 200mg/kg reduced paw volume from $48.8 \pm 1.6\%$ on day 7 to $27.6 \pm 2.6\%$ on day 28 while the group with FSE 400 mg/kg alone showed more reduction in the paw volume from 15.9 ± 3.2 on day 7 to 41.63 ± 5.8 on day 28 as compared to adjuvant group. Treatment with adjuvant with L-arginine showed slight decrease in paw volume from $52.64 \pm 4.2\%$ on day 7 to 32.12 ± 1.6 on day 28. Treatment with FSE 400 mg/kg and its combination with L-NAME show maximum decrease in paw volume from 39.09 ± 4.1 on day 6 to 20.31 ± 4.4 on day 24 ($p < 0.01$). Combination of fenugreek seed extract 400 mg/kg with L-arginine 100mg/kg reduces the paw volume from $55.18 \pm 6.2\%$ day 7 to $32.28 \pm 1.7\%$ on day 28 but to a lesser extent than the combination of fenugreek seed extract 400 mg/kg and L-NAME 10 mg/kg. These results are summarized in table 3[21-24].

Table. 3: Percentage change in paw volume on day 0, 7, 14, 21 and 28 of the study (all values represented here are in mean \pm SEM) (values on day 7 and 14 represent the value before treatment, and values on day 21 and day 28 are after treatment values; n = 6/group).

| Days | Group II adjuvant | Group III methotrexate | Group IV FSE (200mg/kg) | Group V FSE (400mg/kg) | Group VI adjuvant + L-arginine (100mg/kg) | Group VII adjuvant + L-NAME (10mg/kg) | Group VIII FSE (400 mg/kg) + L-arginine (100mg/kg) | Group IX FSE (400 mg/kg) + L-NAME (10mg/kg) |
|--------|-----------------------|------------------------|-------------------------|------------------------|---|---------------------------------------|--|---|
| Day 7 | 45.3±13.6 | 54.9±6.1 | 48.8±1.6 | 41.63±5.8 | 52.64±4.2 | 52.4±5.4 | 55.18±6.2 | 39.09±4.1 |
| Day 14 | 63.2±6.3 [*] | 69.4±10.5 [*] | 63.6±4.6 [*] | 74.0±5.3 [*] | 56.84±3.2 [*] | 67.6±8.7 [*] | 70.68±2.5 [*] | 84.09±8.1 [*] |
| Day 21 | 81.7±6.1 [*] | 54.3±3.1 [*] | 40.9±3.7 [*] | 31.7±2.8 [*] | 51.76±6.4 [*] | 56.7±6.2 [*] | 52.19±3.6 [*] | 46.88±2.6 [*] |
| Day 28 | 98.4±6.5 [*] | 23.0±3.6 ^b | 27.6±2.6 ^b | 15.9±3.2 ^b | 32.12±1.6 ^b | 26.8±4.2 ^b | 32.28±1.7 ^b | 20.31±4.4 ^b |

* $p < 0.05$ compared to day (0); ^a $p < 0.01$ compared to day (0); ^b $p < 0.05$ compared to day (14).

4. DISCUSSION

The present study was conducted to explore the anti-arthritic and anti-inflammatory effect of ethanolic extract of *Trigonella foenum graecum* (fenugreek) seed and its interaction with NO modulators, which was compared with the standard drug methotrexate in experimental arthritis in rats.

An immune system imbalance that results from a complex interaction of genetic, environmental, and immunological variables causes rheumatoid arthritis. Adjuvant-induced arthritis in rats is a well-known model that has been validated for studying the inflammatory process [31], the pathophysiology of RA and other autoimmune disorders, and the identification of prospective treatment targets [32]. Animal models of RA are routinely induced with complete Freund's adjuvant (CFA) [33].

The findings of this study demonstrate that fenugreek has potential for both anti-inflammatory and anti-arthritic effects. After treatment with fenugreek seed extract alone or in conjunction with NO modulators in an experimental arthritic model, there was a noticeably marked suppression of inflammation and paw edoema, a decline in arthritis index, a reduction in ankle diameter, and an increase in body weight.

As mentioned in material and methods section, rheumatoid arthritis was induced in 9 groups of rats i.e. Group II to Group IX by an intradermal injection of 0.1ml of Complete Freund's Adjuvant in footpad of their left hind paw. This is now a well documented method of producing an experimental model of rheumatoid arthritis, being supported by many recent studies [34].

In terms of clinical and serological alterations, as well as the involvement of inflammatory mediators in the arthritic aetiology, adjuvant-induced arthritis is strikingly comparable to human RA. An indicator of several medications' anti-arthritic activity is the measurement of paw edema. The release of several mediators, including the cytokines GM-CSF, interferons, and PGDF, is what causes this persistent inflammation [21-24, 35].

During the duration of the investigation, CFA-inoculated rats showed a significant increase in arthritis index (p 0.05). This can be explained by the fact that the CFA's mycobacterial components continued to induce cytokines, which caused a reliable onset and progression of easily measurable polyarticular inflammation, noticeable bone resorption and periosteal bone proliferation, synovial proliferation, and cartilage destruction, all of which worsened the arthritic condition.

When compared to the adjuvant-only group, methotrexate treatment (5 mg/kg) significantly reduces the arthritis index (p 0.05). Treatment with fenugreek seed extracts (200–400 mg/kg), either alone or in conjunction with nitric oxide modulators, significantly lowers the arthritis index (p 0.05). However, FSE 400 mg/kg alone and in conjunction with a low dose of the NO modulator L-NAME (10 mg/kg) caused the greatest drop in the arthritis index of CFA-inoculated rats (p 0.05), which may be related to its anti-inflammatory effects. L-NAME exerts its anti-inflammatory effect when administered in low doses by blocking the NO-NOS pathway, which causes inflammation by activating inflammatory mediators including cyclooxygenase [36]. After receiving CFA, rats' ankle diameters significantly increased (p 0.05), likely as a result of the ankle joints' noticeable inflammation. The arthritic parameter of ankle diameter is protected by methotrexate (5 mg/kg) treatment (p 0.05). The increase in ankle diameter of experimentally induced arthritic rats is considerably reduced (p 0.0001) by treatment with fenugreek seed extracts (200-400 mg/kg) alone and in combination with FSE (400 mg/kg) mg/kg and L-NAME 10 mg/kg.

The volume of rats' paws increases dramatically after CFA vaccination. When compared to the non-CFA inoculated right hind paw, the left hind paw showed more significant changes in paw volume. After treatment with methotrexate (5 mg/kg), paw volume decreased significantly when compared to the adjuvant-only group (p 0.05). Fenugreek seed extract (200-400 mg/kg) treatment alone and in combination with low-dose NO modulators reversed the CFA-induced change in paw volume in a dose-dependent manner. The combination of FSE 400 mg/kg and L-NAME results in the greatest decrease in paw (p 0.01). Pal et.al. , while studying the effect of theophylline on adjuvant induced rheumatoid arthritis found that theophylline (10 and 20mg/kg) significantly decreased adjuvant induced increased arthritic-index, paw volume and ankle diameter (p<0.05 in all parameters) compared to only adjuvant control group. It also reversed adjuvant induced slight decrease in body weight to normalcy. I- Arginine 100mg/kg + theophylline 20mg/kg suppressed TNF- α and elevates IL-10 level as well as reversed adjuvant-induced elevated arthritic parameters as compared to only adjuvant and prednisone group (p<0.001). Synovial TNF- α level of adjuvant only group was several fold higher than its serum level. Treatment with theophylline 20mg/kg significantly reduces synovial TNF- α level as compared to adjuvant only group. Theophylline 20mg/kg +L-NAME 10mg/kg significantly reversed these adjuvant-induced changes in immunological, histopathological and arthritis parameters (p<0.05) [37].

Trigonella foenum graecum ethanol extract can significantly suppress the development and progression of CFA-induced arthritis in rats. FSE treatment with NO inhibitors may have greater therapeutic potential in the treatment of inflammatory diseases such as RA. FSE may be used clinically in the future to target the NO signaling pathway in RA [21-24].

5. CONCLUSION

Keeping in view the results obtained in the present study, the following conclusions may be drawn regarding the potential effectiveness of ethanolic extract of *Trigonella foenum graecum* seed and its combination with NO modulators.

- Complete Freund's adjuvant (CFA) was used to induce arthritis in rats and changes in inflammatory marker, arthritic index and oxidative stress markers induced arthritis in the rats as compared to control group.
- An effective and dose dependent anti-arthritic effect of fenugreek seed extract in two different doses i.e. 200 and 400 mg/kg was clearly evident.

Higher doses of ethanolic fenugreek seed extract i.e. 400 mg/kg were more effective than its lower dose i.e. 200 mg/kg against the CFA induced elevation of ankle diameter, paw volume and arthritic index.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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