WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 2, 778-789. Research Article ISSN 2277–7105

# ANTIARTHRITIC ACTIVITY OF ETHANOLIC EXTRACT OF FICUS RELIGIOSA LEAVES IN FCA INDUCED ARTHRITIS IN RATS

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Article Received on
25 Nov. 2017,
Revised on 15 Dec. 2017,
Accepted on 04 Jan. 2018
DOI: 10.20959/wjpr20182-10661

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## ABSTRACT

The present study was carried out to evaluate the antiarthritic activity of ethanolic extract of *Ficus religiosa* leaves on FCA induced arthritis in rats. The crude ethanolic extract was administered orally at a dose 100, 200 and 400 mg/kg, body weight from day 13 to day 21. Arthritis was assessed by various parameters such as body weight, arthritic score, paw volume and ankle diameter on day 0, 3, 6, 9, 12, 15, 18 and 21. At the end of study, animals were anesthetised and blood was collected for the estimation of various haematological parameters such as haemoglobin content, total RBA, WBC, ESR count, CRP level and TNF alpha level. The result indicated that at a dose of 200 and 400 mg/kg b.w., EtFR protect the rats against primary and secondary lesions, body weight, paw swelling and haematological perturbations induced by FCA. There was a significant increase in body weight, reduction in arthritic score, paw volume and ankle diameter in extract treated animals. Above findings were confirmed by haematological results as, significant improvement in the levels of Hb and RBC count, and suppressed WBC count, ESR count, CRP and TNF alpha levels found in EtFR administered arthritic group. Our finding showed a significant antiarthritic activity of *Ficus religiosa* leaves against FCA induced arthritis in rats.

**KEYWORDS:** *Ficus religiosa*, Rheumatoid arthritis, FCA.

## 1. INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common chronic, autoimmune, progressive, systemic inflammatory polyarticular joint disease characterized by symmetric, synovitis and also may be extraarticular involvement.[1] Currently available treatments in the market are used to modify the progression of disease[2-3] but these treatments are associated with several severe side effects.[4-5] Despite extensive use of current treatment, most RA patients are suffering from declined functional ability because of deformity and disability. Now a day, researchers have taken efforts to investigate newer agents with minimum side effects. Medicinal plants play a important role in the development of plant therapeutic agents.

*Ficus religiosa* Linn is a widely deciduous tree from the family Moraceae found in many tropical area of India, commonly known as '*Pimpala*' or '*Pipal*' tree.[6-8] In folk medicine, its bark, fruits, leaves, latex, seeds and roots have been widely used as medicine purposes.[9] The plant was reported to have wide spectrum of activities such as anticancer[10], antioxidant[10], antidiabetic[11], antimicrobial[12], anticonvulsant[13], anthelmintic[14], antiulcer[15], antiasthmatic[16] and antiamnesic.[17] *Ficus religiosa* plant have traditional claim for use in arthritic disorder. No pharmacological study has been carried out on evaluation of its antiarthritic activity. So present study was carried out to evaluate antiarthritic effect of ethanolic extract of *Ficus religiosa* leaves in freund's complete adjuvent (FCA) induced arthritis in rat.

## 2. MATERIALS AND METHODS

### 2.1 Collection and authentication of plant material

Fresh leaves of *Ficus religiosa* was collected from local area of Ahmedabad district, Gujarat, India in the month of September and were authenticated by Dr. A. Benniamin, Scientist D, Botanical Survey of India, Pune, Maharashtra, India. Authentication number is: (BSI/WC/Tech./2015/JOB-1).

### 2.2 Preparation of leaf extract

Coarsely powdered form of dried *Ficus religiosa* leaves (1000 g) was subjected to successive extraction in a soxhlet apparatus for 72 h using 5 litre of ethanol. Obtained extract was evaporated at room temperature (45-50 ºC). The dried extract was weighed and the percentage yield of the extracts was calculated as follow:

$$\%\ \text{of extractive yield (w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried leaves powder}} \times 100$$

The yield of ethanol extract was found to be 8.2.

### 2.3 Preliminary phytochemical studies

Preliminary qualitative phytochemical screening were done for the presence of different group of chemicals i.e. alkaloids, flavonoids, saponins, tannins, sterols, carbohydrates and glycosides.[18]

### 2.4 Animals

Adult male Wistar rats, weighing 180 - 220 g and albino mice weighing 25-30 g of either sex were used and acclimatized to laboratory condition for one week. All animals were housed in well ventilated polypropylene cages at 12:12 h light/dark schedule at 25±2ºC and 55-65% relative humidity. The rats were fed with commercial pelleted rats chow and water *ad libitum* as a standard diet. Institutional Animal Ethics Committee approved the experimental protocol in accordance with the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

### 2.5 Acute oral toxicity of the extract

The mice were divided into 5 groups of 10 animals each. The mice were fasted for 6 h and access only water *ad libitum* before experimental study. Gr. I received only vehicle (distilled water). Gr. II, III, IV & V received different doses of ethanolic extract of *Ficus religiosa* (EtFR) i.e. 1000, 2000, 3000 and 4000 mg/kg respectively. All the doses and vehicle were administered orally. The animals were observed for 72 h for mortality.[19]

### 2.6 Induction of arthritis

***Freund's complete adjuvant induced arthritis***

The rats were divided into 5 groups of 6 animals each i.e. Gr I: vehicle control; Gr II: Dexamethasone (5 mg/kg, po); Gr III, IV and V received oral administration of ethanolic extract of *Ficus religiosa* (EtFR) at a dose 100, 200 and 400 mg/kg respectively. RA was induced by a single intradermal injection using 15 guage needle of 0.1 mL of freund's complete adjuvant (FCA, Sigma) containing 1.0 mg dry heat-killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil into sub planter region of the left hind paw of rats.[20] FCA produced a pronounced local oedema after a few hours with a progressive increase reaching its maximum on 13$^{th}$ day of FCA injection.

Treatment with EtFR and dexamethasone were started from day 13 to day 21. After the injection of FCA, the body weight, arthritic score, paw volume and ankle diameter for all animal groups were measured at 0, 3, 6, 9, 12, 15, 18 and 21 day. Body weight was measured regularly by using digital balance. Animals were scored regularly by two investigators who were blind to the treatment. Each paw was graded according to the severity, extent of erythema, swelling of periarticular soft tissues, and the enlargement and distortion of the joints. Clinical score ranged from 0 (no sign) to 4 (severe lesions), yielding a maximum score of 16 per animal.[21] Paw volume and ankle diameter was measured by using Plethysmometer (7140-UGO Basile, Italy)[22] and vernier caliper[23] respectively.

At the end of experiment, blood was withdrawn from ratino bulbar venous plexus under light anesthesia (pentobarbitone sodium at a dose 40 mg/kg of body weight of animals, ip) with the help of a glass capillary. The serum separated from the blood was collected for further biochemical assays. Haemoglobin content was estimated by the method of Drabkin and Austin. Red blood cell (RBC) and white blood cell counts (WBC) were estimated according to the method of Chesbrough and Mc Arthur in an improved Neubauer chamber. Estimation of erythrocyte sedimentation rate (ESR) was followed by the method of Westergren. C-reactive protein (CRP) level was estimated using the ELISA kit obtained from Alpha Diagnostics Intl., USA.[24]

Plasma tumour necrosis factor-*α (TNF-α)* concentration was determined with an ELISA commercial kit (Rat TNF α ELISA kit, Sigma Aldrich, St. Louis, USA). At the end of the experiment, samples of blood (0.5 mL) were drawn from a tail vessel. The blood was collected in polyethylene tubes having 25 µL of heparin solution (4000 IU). The plasma samples obtained after centrifugation for 10 min at 3000 *g* and 4ºC were frozen at –80ºC until assay. In brief, 100 µL of standard, sample and control were added to each well of the coated microplate. After 3 h of incubation at 24ºC the microplate was decanted and the liquid discarded. Then, 100 µL of biotinylated anti-TNF-α antibody was added to each well. After 45 min of incubation at 24°C and a further elimination of the liquid from the wells, 100 µL of Streptavidin– horseradish peroxidase conjugate was added. After incubation for a further 45 min and a washing of the wells, 100 µL of chromogen was added. The absorbance of each well was read spectrophotometrically at 450 nm. TNF-α values were expressed as pg/mL.

### 2.7 Statistical analysis

All the values were expressed as mean ± SE. Statistical evaluation of the data was done by one-way ANOVA (between control and drug treatments) followed by Dunnett's t-test for multiple comparisons and two-way ANOVA followed by Bonferroni's multiple comparison test, with the level of significance chosen at P <0.001 using Graph-Pad Prism 5, San Diego, CA software.

### 2.8 RESULTS AND DISCUSSION

Rheumatoid arthritis is a chronic autoimmune inflammatory polyarticular joint disease and it affects several parts of joints including cartilage, synovium, tendon and muscles.[25-26] In the present study, Wistar rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints. Freund's complete adjuvant induced models are extensively used to study the pathogenesis of rheumatoid arthritis for testing therapeutics. These experimental models share several clinical and pathological features with RA. *Ficus religiosa* significantly reduces the signs of pain, inflammation and other symptoms of RA. Therefore further the antiarthritic activity of *Ficus religiosa* was evaluated by FCA induced arthritis in rats.

From the acute oral toxicity study, it was found that ethanolic extract of *Ficus religiosa* at different dose levels were safe up to 4000 mg/kg. Therefore, we have taken 400 mg/kg as the therapeutic dose and made variations by taking 100 mg/kg as lower dose and 400 mg/kg as higher dose for pharmacological evaluation.

Observations such as body weight, arthritic score, paw volume, ankle diameter and haematological parameters were recorded after the induction of arthritis by FCA. FCA injection on the rat hind paw causes pronounced swelling and hyperalgesia without involvement of contra lateral paw. This is associated with the accumulation of leukocytes in the arthritic joint fluid. This response is considered as primary response. The mediators of chronic inflammation are responsible for pain, severe destruction of bone and cartilage that can lead to severe disability. FCA treated rats found progressive decrease in body weight till day 12, and no marked differences were seen between them. This weight loss was significantly ($p<0.001$) reduced from day 15 to day 21 while receiving EtFR (200 and 400 mg/kg) in FCA induced arthritis in rats (Figure 1). In arthritic animals, the loss of body weight could be due to reduced absorption of glucose and leucine in rat intestine in arthritic condition.[27] After inoculation of FCA, animals begun to show progression of clinical inflammation from day 3. The time for the development and progression of disease was

assessed by mean arthritic severity score. The mean arthritic severity score in FCA treated animal was progressive from day 12 to 18 and achieved values of about 10. Administration of EtFR (200 and 400 mg/kg) attenuated mean arthritic severity score significantly ($p<0.001$) from day 15 to day 21 as compared with vehicle treated animals in FCA induced arthritis in rats (Figure 2). Paw swelling is one of the major factors for determination of quick, simple, sensitive and therapeutic effects of drugs.[28] The initial inflammatory response was developed within 3 to 5 days considered as primary lesion. Secondary lesions occur after 11 to 12 days after inoculation of FCA. Oral administration of EtFR (200 and 400 mg/kg) significantly ($p<0.001$) reduced the paw swelling from day 15 to day 21 as compared with vehicle treated animals in FCA induced arthritis in rats (Figure 3). The change in paw volume has been found to associate with an increase in granulocyte and monocytes. Paw thickness (ankle diameter) are also used for assessment of RA. Ankle diameter significantly ($p<0.001$) decreased with the treatment of EtFR (200 and 400 mg/kg) as compared to vehicle treated rats in FCA induced arthritis in rats (Figure 4).

In the present study, the reduction in haemoglobin and RBC count, and increased WBC count are common feature of microbial infectious inflammatory diseases.[29-30] So in arthritic group, there was decrease in haemoglobin and red blood cells count and; increased in total leukocyte number. In the present study, administration of EtFR (200 and 400 mg/kg) significantly improved the haemoglobin and RBC count, and significantly suppressed WBC count as compared to vehicle treated animals in FCA induced arthritis in rats. These findings suggest *Ficus religiosa* may have beneficial effect for joint preservation.

Erythrocyte sedimentation rate (ESR) in the FCA treated animals showed high value compared to drug treated animals. ESR is strogly related with the ability of red cells to aggregate into olderly stacts or rouleaux. Proteins are believed to affect the repellent surface charges on red cells and cause them to aggregate into rouleaux and hence the sedimentation rate increases.[31] Chronic administration with EtFR (200 and 400 mg/kg) significantly ($p<0.001$) decrease the ESR level in FCA induced arthritis in rats.

C-reactive protein (CRP) is a prototype hepatically derived inflammatory biomarkers. During inflammatory process, CRP level increases due to increased concentration of IL-6 in plasma which is produced by macrophages[32] as well as adipocytes.[33-34] Increased level of CRP in FCA treated group was significantly ($p<0.001$) decreased with the treatment of EtFR (200 and 400 mg/kg).

Chronic inflammation involves the release of number of mediators like IL-1B, TNF alpha, interferon and prostaglandins. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability.[35] Chronic oral administration of EtFR (200 and 400 mg/kg) significantly suppressed the levels of TNF alpha as compared to vehicle treated animals in FCA induced arthritis in rats (Table 1).

From the present investigation, it can be concluded that ethanolic extract of *Ficus religiosa* possesses dose dependent significant antiarthritic activity. This activity may attributed due to the phytochemical constituents present in *ficus religiosa* named alkaloids, flavonoids, tannins, phenols and glycosides.[36] The leaves of *Ficus religiosa* contains some constituents such as campestrol, stigmasterol, isofucosterol, α-amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tryosine, methionine, valine, isoleucine, leucine, n-nonacosane, n-hentricontanen, hexa-cosanol and n-octacosan.[37-38] In view of the present work, the potential activity of various ingredients in *Ficus religiosa* acting synergistically and working in concert for overall antiarthritic activity. Extensive phytochemical studies are required to identify the active principle and which may enable us to elucidate exact mechanism of action.

**Table 1: Effect of ethanolic extract of *Ficus religiosa* on change in biochemical parameter in FCA induced arthritis in rats.**

| ↓ Treatment Parameters → | Change in Biochemical parameters in FCA induced arthritic animals | | | | | |
|---|---|---|---|---|---|---|
| | **Hb** | **RBC** | **WBC** | **ESR** | **CRP** | **TNF α** |
| Vehicle | 7.36±0.25 | 3.27±0.25 | 19.47±0.29 | 11.12±0.24 | 427.11±1.52 | 368.92±9.85 |
| Std | 11.96±0.29 *** | 4.35±0.48 *** | 11.92±0.35 *** | 8.43±0.24 *** | 262.17±3.57 *** | 252.17±8.42 *** |
| EtFR (100 mg/kg) | 7.47±0.35 | 3.35±0.12 | 17.34±0.28 | 10.92±0.12 | 401.97±1.12 | 352.32±9.41 |
| EtFR (200 mg/kg) | 9.30±0.27 *** | 3.86±0.19 *** | 14.62±0.74 *** | 9.36±0.18 *** | 344.12±7.46 *** | 305.82±0.72 *** |
| EtFR (400 mg/kg) | 10.37±0.49 *** | 3.99±0.28 *** | 12.03±0.27 *** | 8.53±0.52 *** | 297.12±12.14 *** | 262.21±4.62 *** |

Data was expressed as means ± S.E.M and analysed by one way ANOVA followed by Dunnett's test, n=6, ***p<0.001.

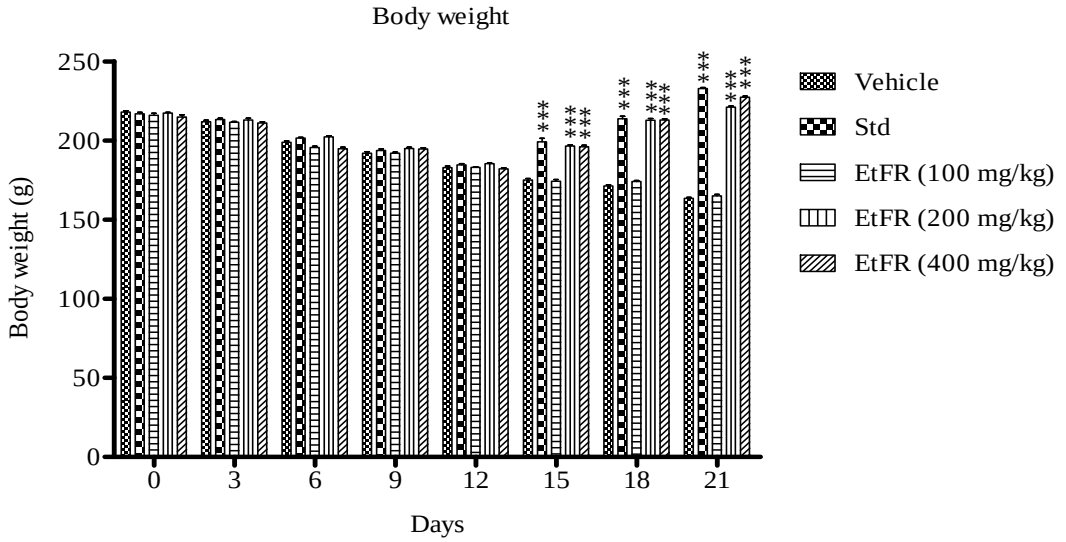


**Figure 1: Effect of ethanolic extract of *Ficus religiosa* on change in body weight in FCA induced arthritis in rats.**

Values are Mean±SE from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

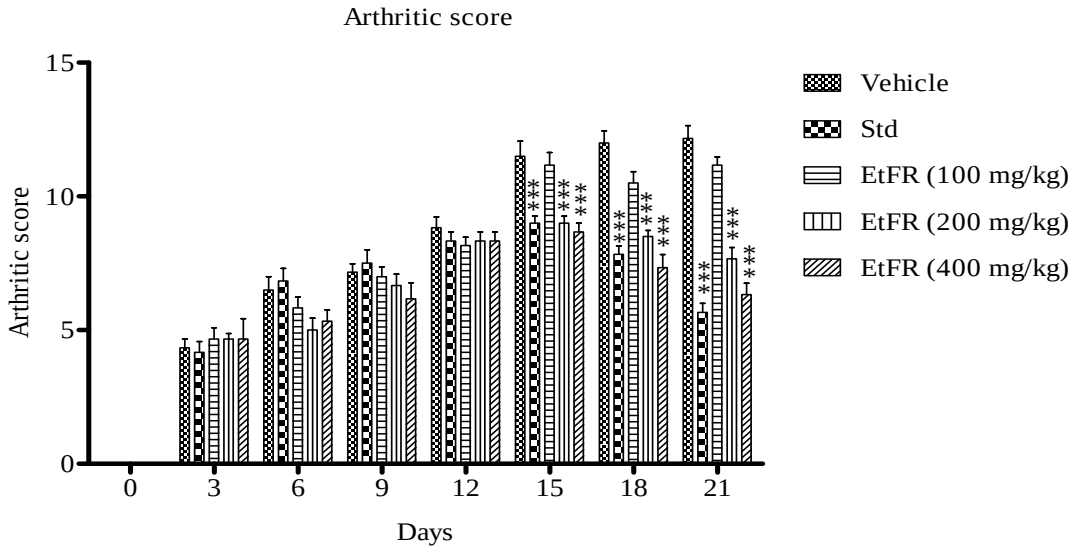


**Figure 2: Effect of ethanolic extract of *Ficus religiosa* on change in arthritic score in FCA induced arthritis in rats**

Values are Mean±SE from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

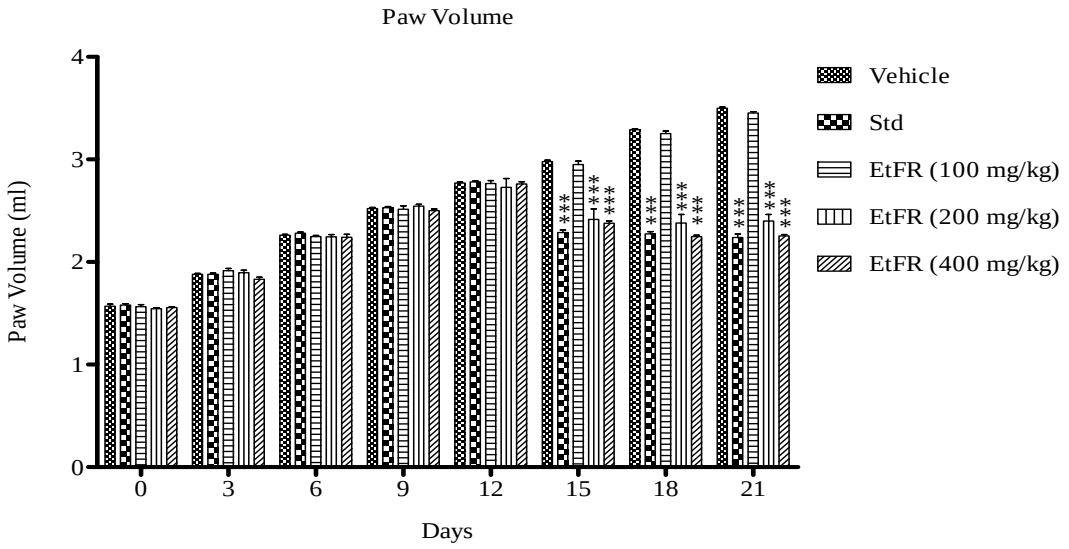


**Figure 3: Effect of ethanolic extract of *Ficus religiosa* on change in injected paw volume in FCA induced arthritis in rats.**

Values are Mean±SE from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

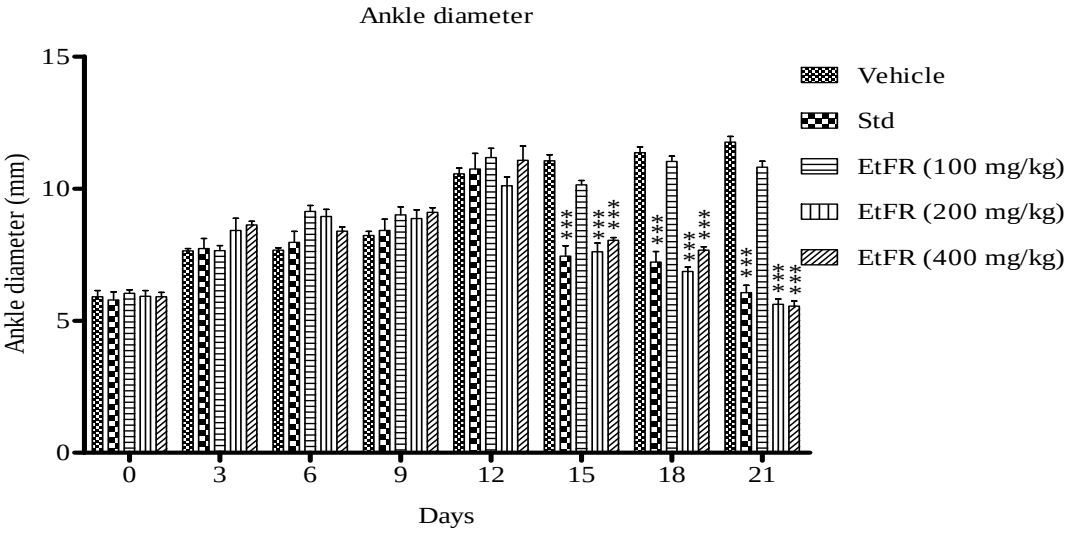


**Figure 4: Effect of ethanolic extract of *Ficus religiosa* on change in ankle diameter in FCA induced arthritis in rats.**

Values are Mean±SE from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

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