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Evaluation of antiarthritic activity of ethanolic extract of *Derris brevipes* (*benth.*) *Baker* leaves

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ABSTRACT

Background: *Derris brevipes* is a common medicinal plant used in the traditional system of medicine as an anti-arthritic agent. **Aims and Objectives:** The present study was to evaluate the anti-arthritic activity of ethanolic extract of *D. brevipes* (EEDB) leaves, belonging to the family Leguminosae. **Materials and Methods:** The dried leaves were collected and extracted using 95% ethanol, and the extract was subjected to a preliminary phytochemical screening. *In vivo* activities were evaluated in collagen (chicken sternal collagen)-induced arthritis (CIA) in Sprague–Dawley rats. Prednisolone was used as the standard, and EEDB (at doses of 300 mg/kg and 600 mg/kg) was administered through the oral route. Body weight measurement, arthritic score, histological score, and radiology score assessments were carried out. **Results:** Preliminary phytochemical screening of leaf extract from *D. brevipes* showed the presence of components such as steroids, triterpenoids, and flavonoids. *In vivo* tests on CIA rats given the extract showed that it could lower the arthritic score, the paw volume, the radiological score, and the histological score. **Conclusion:** From this study, it is concluded that the *D. brevipes* leaf extract possesses antiarthritic activity.

Key words: Musculoskeletal and joint diseases; Arthritis; Rheumatoid arthritis; Collagen induced arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic auto-immune disease that is associated with pain, stiffness, swelling, and limited function of the involved joints. A plethora of drugs is available for the treatment of RA, such as non-steroidal anti-inflammatory agents, disease-modifying antirheumatic drugs (non-biological and biological agents), and corticosteroids. However, they have been linked to a slew of side effects, including GI ulceration and bleeding, myelosuppression, osteoporosis, hypertension, hepatotoxicity, and others. The newly introduced biological agents are known for some severe adverse effects such as multiorgan failure, life-threatening infections, increased risk of malignancies, and immunogenic reactions.

These limitations have necessitated the pursuit of novel therapeutic agents, which facilitated the exploration of indigenous herbs and concoctions that were used in traditional systems of medicine for joint disorders. Ayurveda offers a variety of herbal products that have been used in the treatment of patients with chronic inflammatory disorders for several decades. The presence of many biologically active phytochemicals, such as triterpenoids, flavonoids, alkaloids, steroids, tannins, and glycosides, in various plant extracts may be responsible for their pharmacological properties like anti-inflammatory and anti-arthritic activity.[1]

Folklorically, *Derris brevipes* has been used as an antifertility and antiarthritic agent. The plant belongs to the family

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Leguminosae and is widely distributed in the Western Ghats of India. Literature supports that the *D. brevipes* extract has sterols, triterpenoids, and flavonoids as contents.[2,3]

The acute toxicity studies with *D. brevipes* root powder confirmed that it is safe in doses up to 6000 mg/kg body weight in mice.[4] Various studies on *D. brevipes* have proved its utility as an antifertility,[4] anticancer,[5] antimutagenic,[6] antioxidant,[7] and antibacterial agent.[8]

A study on ethanolic extract of *D. brevipes* (EEDB) and *Derris indica* on carbon tetrachloride-induced oxidative stress in Wistar rats indicated their ability to activate antioxidant enzymes that catalyze the action of anti-oxidants and free radical scavengers.[7] Telekone and Khan isolated and structurally elucidated beta-sitosterol from a methanol extract of aerial parts of *D. brevipes* (benth.) *Baker*.[3] Liz et al., investigated the anti-inflammatory effects of ß-sitosterol in activated neutrophils from mice.[9] ß-sitosterols were shown to have anti-inflammatory properties, as they inhibited pro-inflammatory mediators in the mouse air pouch model. This anti-inflammatory effect appeared to be mediated by the calcium uptake in activated neutrophils in a time- and dose-dependent manner through L-type voltage-dependent calcium channels, intracellular calcium, PI3K activity, and microtubule modulation.[9]

Based on the literature evidence available and considering the folklore usage of *D. brevipes*, an attempt was made in this study to scientifically establish its potential as an anti-arthritis drug, which could prove to be beneficial in the treatment of RA.

Aims and objectives

The study was aimed to evaluate the anti-arthritic activity of ethanolic extract of Derris brevipes (Benth.) Baker leaves in Sprague-Dawley rats.

MATERIALS AND METHODS

Plant collection and authentication

The leaves of *D. brevipes* (Benth.) *Baker* was obtained from the Ernakulum district of Kerala, India, during February and authenticated by the Department of Botany, C.M.S. College, Kottayam, Kerala, India. A voucher specimen was preserved at the herbarium with voucher number 260.

Preparation of suspensions

Leaves were dried in the shade for 2 weeks and coarsely powdered. About 100 g of leaf powder in a porous bag made of muslin cloth was placed in a round bottom flask under reflux at a temperature of 60°C. The solvent used for extraction was ethanol (rectified spirit), and it was extracted for 24 h. After extraction, it was filtered, and the mark obtained was again extracted twice using the same procedure. Ethanol was distilled out, and the extract was concentrated to obtain a sticky mass. The percentage yield was calculated from the formula: Percentage yield = [practical yield/theoretical yield] × 100.

Selection of animals

Twenty-four healthy female Sprague-Dawley rats weighing between 150 and 170 g were purchased from the College of Veterinary and Animal Sciences, Mannuthy, Thrissur, and were maintained in Animal house at the University College of Pharmacy, Cheruvandoor campus, Ettumanoor, Kottayam. The animals were housed in polypropylene cages in a room where a congenial temperature of 27±1°C, 30–60% relative humidity, and 12-h light and dark cycles were maintained. The animals were allowed to acclimatize to the environment in 4 groups for 14 days. They were fed a standard pellet diet collected from Hindustan Lever Limited, Bangalore, and water *ad libitum*. All procedures and experiments were conducted during the daytime according to the specifications of the Indian National Science Academy. The experiment was initiated after obtaining approval from the Institutional Animal Ethical Committee at the University College of Pharmacy, Cheruvandoor (with IAEC number 008/MPH/UCP/CVR/13 dated December 09, 2013).

Preliminary phytochemical screening

The EEDB was subjected to qualitative tests for the identification of various phytoconstituents.[10] Mayer's Test, Wagner's test, Hager's test, and Dragendr off's Test for alkaloids; Legal's test and Baljet Test for glycosides; aqueous sodium hydroxide test and Shinoda test for flavonoids; Molisch's test, Fehling's Test and Benedict's test for carbohydrates; Millon's test, Biuret test and Ninhydrin test for proteins and amino acids; Salkowski test for terpenoids; Foam test for saponins were done.

In vivo screening for anti-arthritic activity

In vivo screening for anti-arthritic activity was done using collagen-induced arthritis (CIA) in Sprague Dawley rats by observing the following parameters: arthritic scores, paw volume, body mass, radiologic scores, and histologic scores: CIA is an experimental auto-immune disease that can be elicited in susceptible strains of rodents and non-human primates by immunization with type II collagen, the major constituent protein of articular cartilage.[11] CIA shares both immunological and pathological features with human RA;[12,13], therefore, it has been used extensively as a model to study the pathogenesis of RA and for testing therapeutics.

Preparation of emulsion

Chicken sternal collagen type II was dissolved in 0.1 M acetic acid at a concentration of 2 mg/mL and kept

overnight at 40°C. To make the inducing agent, this solution was added dropwise to an equal volume of chilled Incomplete Freund's adjuvant and stored on ice before use. The glassware used in this process was prechilled.[11]

Induction of arthritis

On day 1, each rat received a total of 0.5 mg of collagen in 0.5 mL equally divided into five sites (the base of the tail, and the region above each limb). It was followed by a booster injection given on day 7, for which the concentration of the emulsion was the same as that of the primary immunization. 0.1 mL of booster emulsion was given at the base of the tail, a different location from the first injection site. All injections were given through the intra-dermal route.[11,14,15]

Grouping

Animals were divided into four groups, consisting of six animals each (Table 1).

The vehicle and the drug or extract were administered orally using an intra-gastric tube from day 20 to day 40 after the primary immunization with emulsion. After euthanasia by cervical dislocation, the synovial tissues were taken on day 41 from each rat for biochemical examination. On day 41, samples of the rats' knee joints were collected for histological examination.

In-vivo parameters

Arthritic scores

Every week, beginning the day after the collagen emulsion injection, the incidence and severity of arthritis were assessed using a scoring system (Table 2). The severity of inflammation in each limb was evaluated every week for degree of inflammation, extent of erythema and edema of the periarticular tissues, and enlargement, distortion, or ankylosis of the joints.[16] The total arthritic scores were calculated from the sum of scores for four limbs, with a maximum possible score of 16 for each rat.

Paw volume

The effect of the reference and test drugs on the inflammation was quantified by the difference in paw volume.[17] The inflammatory reaction was measured using a mercury plethysmometer on days 1, 14, 21, 28, 35, and 40 from the day of the CIA injection.

Body mass

From the first to the 40th day after CIA injection, body weight was measured using a digital weighing scale.[17]

Radiological scores

At the end of the experiment, rats were anesthetized by giving intra-peritoneal phenobarbitone sodium at a dose of 35 mg/kg. Anesthetized rats were placed in a radiographic box at 107 cm from the X-ray source. Radiographic analysis of arthritic hind paws was performed using an X-ray machine. A blind independent assessment of the radiological score was performed by an observer. The severity of arthritis in each rat was determined according to a radiological score ranging from 0 (normal) to 3 (maximum) (Table 3).[16]

Histological scores

Paws from rats were amputated and fixed in Blouin's fluid; subsequently, the specimens were decalcified with 10% ethylene diamine tetra-acetic acid for 7 days, dehydrated, and embedded in paraffin blocks. Sections of ankle joints (5 μm thick) were cut and stained with hematoxylin and eosin before being mounted on a glass slide for microscopic examination. Grading of cellular infiltration, synovial hyperplasia, pannus formation, joint space narrowing, and cartilage and bone erosion of the ankle joints was blindly investigated by an independent pathologist using a semi-quantitative scale from 0 (normal), 1 (mild changes), 2 (moderate changes), 3 (severe changes), and 4 (very severe changes). Histopathological scores were combined and expressed as the sum for both ankle joints, with a maximum histological score of eight for each histological parameter in a rat.[18]

Statistical analysis

Results were analyzed using one-way analysis of variance followed by Dunnet's multiple comparison tests using Graph Pad Prism (version 5).

RESULTS

Extraction and preliminary phytochemical screening

The percentage yield obtained after the ethanolic extraction of dried leaves of *D. brevipes* was found to be 12.33% w/w. The preliminary phytochemical screening showed the

Table 1: Protocol for the *in-vivo* study

S. No.	Group	Treatment	Number of animals	Dose
Group I	Positive control	Vehicle (CMC)	6	-
Group II	Standard	Prednisolone	6	10 mg/kg
Group III	Test (low dose)	EEDB	6	300 mg/kg
Group IV	Test (high dose)	EEDB	6	600 mg/kg

EEDB: Ethanolic extract of *Derris brevipes*

presence of carbohydrates, phenols, steroids, triterpenoids, alkaloids, and flavonoids.

In vivo screening: CIA in rats

Arthritic index

The arthritic index of animals in all groups was recorded on days 1, 14, 21, 28, 35, and 40. Following the injections of collagen emulsion, rats developed arthritis beginning from the 8th day onwards. After the onset of treatment, the EEDB-treated rats showed a marked reduction in their arthritic score.

Figure 1 shows the arthritic scores in the control and treatment groups. As a result of inflammation induced by collagen emulsion, the arthritic score was increased in vehicle-treated rats. The low (EEDB 300 mg/kg) and high dose (EEDB 600 mg/kg) groups showed a significant reduction (P<0.001) in the arthritic score at day 40 when compared to the control groups. The EEDB 600 mg/kg treated rats showed a dose-dependent reduction in arthritic score compared to the EEDB 300 mg/kg treated groups by reducing soft tissue swelling, erythema, edema, and ankylosis of the joints. Prednisolone reduces the arthritic score significantly when compared to the control group.

Table 2: Grading scale for arthritic index

Score	Description
0	No inflammation
1	Unequivocal inflammation of one joint of the paw
2	Unequivocal inflammation of at least two joints of the paw or moderate inflammation of one joint
3	Severe inflammation of one or more joint
4	Maximum inflammation of one or more joint in the paw

Table 3: Grading scale for radiological score

Score	Description
0	Normal or no tissue swelling or no bone damage
1	Tissue swelling and edema
2	Joint erosion
3	Bone erosion and osteophyte formation

Paw volume

Paw volume is measured on days 1, 14, 18, 22, 30, 36, and 40 (Table 4). CIA in rats showed soft tissue swelling and edema around the ankle joint, as observed by an increase in paw volume after the onset of inflammation.

On days 36 and 40, it was clear that treatment groups had a significant reduction (P<0.001) in paw volume. On the final day of treatment, the EEDB-treated group (600 mg/kg) improved similarly to the prednisolone-treated group. EEDB 600 mg/kg showed significantly higher improvement than the group treated with EEDB 300 mg/kg.

Body weight

During the arthritis phase, loss of body weight was observed in the control group. The prednisolone and EEDB treatment groups had significant increases in body weight during the study period. The data are represented in Figure 2.

It was observed that, after the onset of inflammation, body weight decreased. When the treatment was initiated, EEDB 300 and EEDB 600 and prednisolone-treated groups exhibited a steady increase in the body weight until the end of the study (Figure 2). On the 40th day, it was found that the EEDB-treated groups showed a significant increase in body weight (P<0.001). Weight gain was more pronounced in the EEDB 600 mg/kg group than in the prednisolone-treated group.

Radiological evaluation

Radiographs provide a measure of damage to the joints of the rat paws. Radiographs of rat paws were recorded on

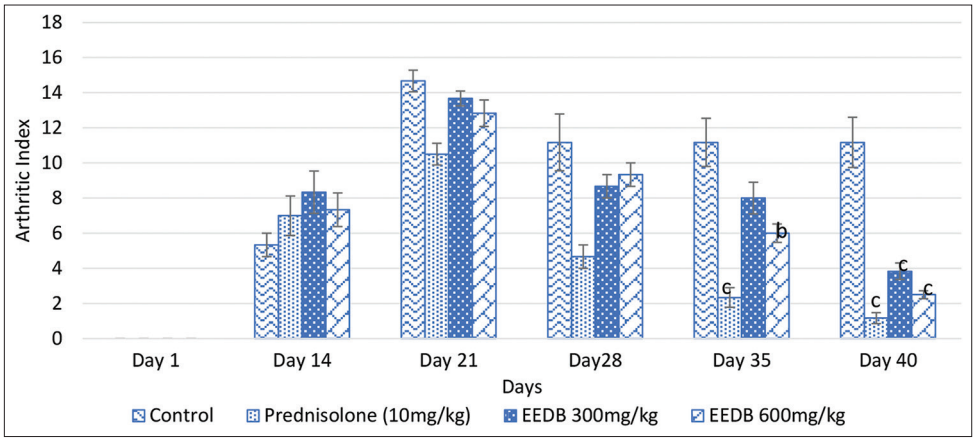

Figure 1: Effect of ethanolic extract of *Derris brevipes* on arthritic index in collagen induced arthritis in Sprague-Dawley rats. Each bar represents the mean score±SEM, n=6; (a) P<0.05, (b) P<0.01, (c) P<0.001 when compared to control

Table 4: Effect of ethanolic extract of *Derris brevipes* leaves on paw volume in collagen induced arthritis in rats

Day	Group I (control)	Group II (prednisolone)	Group III (EEDB: 300 mg/kg)	Group IV (EEDB: 600 mg/kg)
Day 1	7.66±0.21	7.33±0.21	7.5±0.223	7.5±0.2236
Day 14	9.6±0.55	11.3±0.8	11.0±1.033	11.5±1.50
Day 18	12.8±1.07	14.67±1.08	16.17±0.401	16.67±0.4944
Day 22	13.8±0.47	12.8±0.79	14.83±0.477	15.5±0.5627
Day 30	14.3±0.61	10.5±0.42***	13.67±0.2108	13.17±0.4014
Day 36	13.0±0.44	8.83±0.16***	11.33±0.6146*	10.33±0.4216***
Day 40	13.3±0.33	7.83±0.3***	9.667±0.2108***	8.667±0.21***

*P<0.05, ***P<0.001 when compared with control. EEDB: Ethanolic extract of *Derris brevipes*

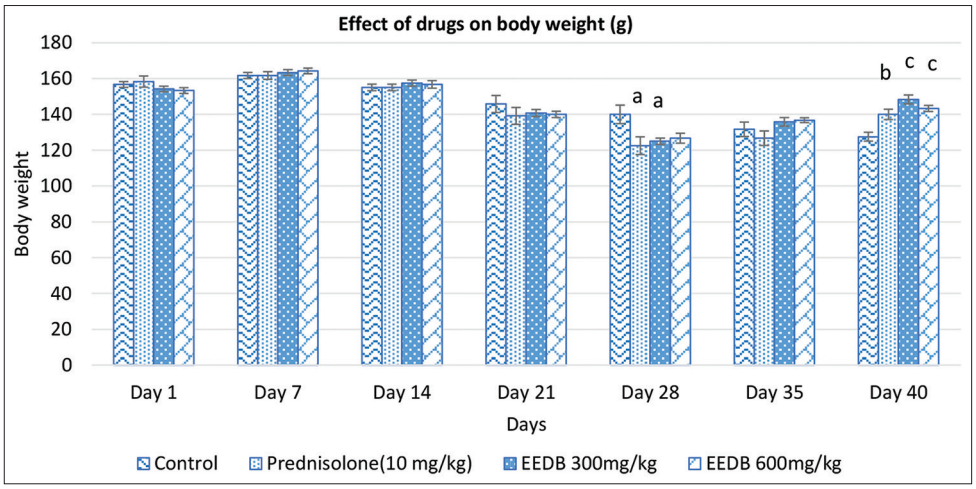

Figure 2: Effect of ethanolic extract of *Derris brevipes* on the body weight of collagen induced arthritis rats. Each bar represents the mean score ±SEM, n=6; (a) P<0.05, (b) P<0.01, (c) P<0.001 when compared to control

day 40. The radiological score was assessed by a veterinary pathologist. The data obtained are tabulated in Table 5.

Radiographic images showed severe soft-tissue swelling and edema, joint erosion, and bone erosion in the ankle joints of CIA rats in the control group. In contrast, the EEDB and prednisolone groups had significantly (P<0.001) less joint and bone erosion of the ankle joints in comparison with the control group, as revealed by radiological scores. EEDB groups had decreased bone loss and edema in joints, which suggests its potential to reduce bone degradation in CIA.

Histopathological evaluation

Histopathology of ankle joints was taken for scoring (Figure 3). Histological examination of the ankle joint of the control group showed marked cellular infiltration, synovial hyperplasia, pannus formation, joint space narrowing, cartilage destruction, and erosion of bone with a score of 4, suggesting very severe changes. The treatment of CIA with EEDB 300 mg/kg exhibited severe changes (score 3) in synovial infiltration and hyperplasia. EEDB 600 mg/kg treated rats showed moderate changes in synovial hyperplasia and cellular infiltration, and the score is 2. Treatment with prednisolone ameliorated these arthritic changes with a score of 1.

DISCUSSION

RA is a chronic and debilitating autoimmune disease that is characterized by progressive and symmetrical inflammation of the joints with subsequent erosive destruction of the cartilage and bones. CIA is a well-demonstrated model of RA with similar histopathology characteristics resembling human disease.[11]

Compared with vehicle-treated rats, arthritic rats treated with EEDB, and prednisolone showed significantly fewer clinical signs and movement limitations than control rats. The alteration in the synthesis of pro-inflammatory cytokines, prostaglandins, leukotrienes, and matrix metalloproteinases causes fluid accumulation in the synovium. This results in an increase in the arthritic score due to damage to joints and bones in CIA rats.[19]

The determination of paw volume is a simple, sensitive, and quick procedure for evaluating the degree of inflammation and the effectiveness of the treatment. A significant increase in the paw volume after intra-dermal administration of collagen reflects the status of arthritis. The chronic inflammation in CIA and RA involves the release of several mediators such as cytokines, granulocyte-macrophage

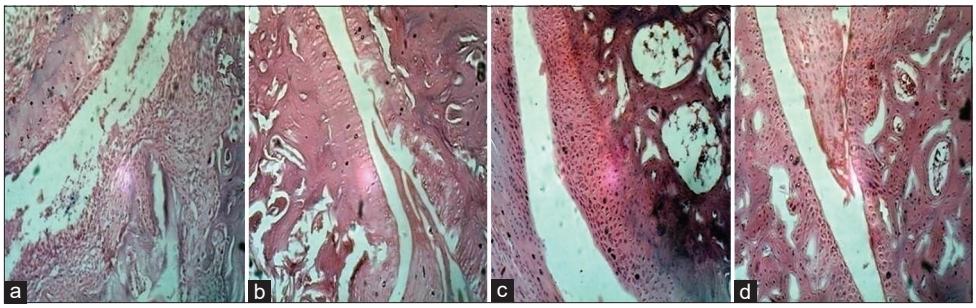

Figure 3: Histological image of ankle joint of collagen induced arthritis rats. (a) Control group: showed very severe changes in histology (b) prednisolone group: Mild changes in histology (c) ethanolic extract of *Derris brevipes* (EEDB) 300 group: Severe changes in histology (d) EEDB 600 group: Moderate changes in histology

Table 5: Effect of EEDB on radiological score in collagen induced arthritis in Sprague-Dawley rats

Groups (n=6)	Score (mean±SEM)
Control	3.0±0.0
Prednisolone	1.66±0.21***
EEDB 300	1.83±0.16***

*P<0.05,***P<0.001 when compared with control. EEDB: Ethanolic extract of *Derris brevipes*

colony-stimulating factor, interferon, and platelet-derived growth factor. These mediators are responsible for the pain along with swelling of limbs and joints, bone deformation, and disability of joint functions.[20,21] Treatment with EEDB decreased the paw volume, which can be due to the inhibition of the release of inflammatory mediators, indicating its anti-arthritic potential in RA.

Changes in body weight have also been used to assess the course of the disease and the response to the treatment of experimental drugs.[22] As the incidence and severity of CIA increased, a change in the body weight occurred during the disease. The weight loss and loss of lean body mass, known as rheumatoid cachexia, are thought to be the result of cytokine-driven hypermetabolism. The loss of lean body mass is associated with decreased physical activity, muscle strength, and endurance in performing activities of daily living.[16]

Radiographs are required to confirm proper disease remission and to accurately assess disease status.[23] Reduction in bone configuration and increased bone resorption are the causes of bone loss in CIA. Radiological studies showed that vehicle-treated CIA rats experienced a significant progression in joint space narrowing, joint erosion, and bone erosion. This caused a significant reduction in joint movement. Tumor necrosis factor-alpha and Interleukin-1 beta contribute to the changes in radiology in CIA.[24] Hence, it is postulated that EEDB is capable of reducing joint and bone erosion by modulating these mediators.

CIA rats treated with EEDB 600 mg/kg showed a significant reduction in bone erosion, cellular infiltration and synovial hyperplasia, and pannus formation, which is comparable with the reference standard drug. A change in synovial activity may reflect a clinical or radiological response to treatment, as suggested by Pettit et al.[24]

Limitations of the study

The study used a collagen-induced arthritis (CIA) animal model to evaluate the effects of Derris brevipes leaf extract. However, the complicated pathophysiology and disease pathways seen in individuals with rheumatoid arthritis may not be adequately replicated in this model. The study does not include a direct comparison of Derris brevipes leaf extract with other standard antiarthritic treatments or other natural remedies. Such a comparison would help assess the relative effectiveness and potential advantages of the extract compared to existing therapeutic options. The study primarily focuses on short-term effects and acute changes in disease markers. However, rheumatoid arthritis is a chronic condition that requires long-term management. Further research into the long-term benefits and safety profile of Derris brevipes leaf extract is warranted.

CONCLUSION

This study showed antiarthritic activity of EEDB (benth.) *Baker* leaves" in *in vivo* model. The extract shows the presence of anti-inflammatory phytoconstituents on *in vitro* phytochemical screening. The *in vivo* analysis showed the capacity to reduce paw volume, arthritic score, bone, and cartilage erosion on X-ray, and reverse CIA-induced body weight reduction in a dose-dependent manner. The action may be due to the inhibition of the release of inflammatory mediators. From the above findings, we concluded that the leaf extract of *D. brevipes* possesses protective action against CIA. This study provides persuasive clues to defend the efficacy of *D. brevipes* in RA and justifies the significance of subsequent clinical trials in RA.

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