Anti-arthritic activity of ethanolic extract of *Tridax procumbens* (Linn.) in Sprague Dawley rats

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ABSTRACT

Objective: To determine the anti-arthritic effect of whole plant ethanolic extract of *Tridax procumbens* (Asteraceae) in female Sprague Dawley (SD) rats using the Freund's Complete Adjuvant (FCA) model. **Materials and Methods:** The plant was collected from different regions of Madurai District, Tamil Nadu, and the phytoconstituents were identified through chemical tests. Ethanol (95%) was used to obtain the whole plant extraction through Soxhlet extractor. Female SD rats were used for anti-arthritic screening. Arthritis was induced using FCA, and the anti-arthritic effect of the ethanolic extract of *T. procumbens* was studied at doses of 250 and 500 mg/kg. The effects were compared with those of indomethacin (10 mg/kg). At the end of the study, the liver enzyme levels were determined and a radiological examination was carried out. **Result:** The preliminary phytochemical analysis of the ethanolic extract of *T. procumbens* indicated the presence of alkaloids, tannins, flavonoids and saponins. *T. procumbens* at 250 and 500 mg/kg significantly inhibited the FCA-induced arthritis in the rats. This was manifested by as a decrease in the paw volume. The arthritic control animals exhibited a significant decrease in body weight compared with control animals without arthritis. *T. procumbens* animals showed dose dependent reduction in decrees in body weight and arthritis. At the same time, *T. procumbens* significantly altered the biochemical and haematological changes induced by FCA ($P<0.05$). The anti-arthritic effect of *T. procumbens* was comparable with that of indomethacin. **Conclusion:** The whole plant extract of *T. procumbens* showed significant anti-arthritic activity against FCA-induced arthritis in female SD rats.

Key words: Arthritis, Freund's complete adjuvant, *T. procumbens*

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INTRODUCTION

Arthritis is an inflammatory disorder involving damage to one or more joints. There are over a hundred different forms of arthritis, of which osteoarthritis, rheumatoid arthritis and psoriatic arthritis are the most common.[1] The treatment of any systemic disorder with allopathic drugs causes moderate to severe adverse events that could cause death. Hence alternative systems of medicine are being explored to treat diseases. In the Ayurvedic system of medicine, which is also called the Indian system of medicine, herbs are used for treatment of diseases. But scientific evidence for treating disorders with herbs is poorly documented.[2] In the Indian system of medicine, *Tridax procumbens* is commonly used as an anti-inflammatory and analgesic agent. The analgesic, antipyretic, hypotensive and anti-bacterial effects of *T. procumbens* have been reported.[3-5] The anti-inflammatory and anti-arthritic properties of *T. procumbens* have not been studied.

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T. procumbens inhibited the growth of *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus*, and it showed possible immunomodulatory properties.[6] Rheumatoid arthritis can be screened using the adjuvant arthritis, rat type II collagen arthritis, mouse type II collagen arthritis and antigen arthritis models. The adjuvant arthritis (Freund's Complete Adjuvant [FCA]) model is one of the models most commonly used in inducing chronic and sub-chronic inflammation/pain.[7-9] Hence the present study was carried out to study the anti-arthritic property of *T. procumbens* using the FCA model in rats.

Collection and identification of plants

T. procumbens (Linn.) (Asteraceae) was collected from

different regions of Madurai District, Tamil Nadu, in July 2011. The plant was taxonomically authenticated by the Botanist of Agricultural College and Research Institute, Madurai, Tamil Nadu. The plant parts were dried in shade for 4-5 days and powdered.

Extraction

The whole plant powder was packed in a Soxhlet extractor and extracted with 95% ethanol at 50°C. Extraction was carried out for 72 hours. The extract was filtered, and the filtrate was concentrated to a dry mass. The yield was found to be 6% W/V. The extract was stored in desiccators at room temperature until analysis.[9,10]

Phytochemical analysis

The ethanolic extract (500 mg) was dissolved in 100 ml of its own mother solvent and used for phytochemical screening. The Salkowski reaction, Liebermann's reaction and the Libermann-Burchard reaction (for detecting the presence of sterols); Dragendroff's reaction, Mayer's test, Wagner's test and Hager's test (alkaloids); the ferric chloride solution test, lead acetate test and bromine water test (tannins); the Keller-Kiliani test, Baljet's test, and Legal test (glycosides); and the Shinoda test and lead acetate test (flavonoids, phenolic compounds and saponins) were carried out.

Selection of *T. procumbens* dose for pharmacological studies

T. procumbens doses were selected form previously published reports (i.e., 250 and 500 mg/kg).[11-13] The extract was suspended in 0.5% w/w carboxymethyl cellulose (CMC) and administered orally.

Animals

Healthy 8-week-old female Sprague Dawley (SD) rats (180-200 g) were obtained from the National Institute of Nutrition, Hyderabad, and maintained in animal house of Department of Pharmacology, Ultra College of Pharmacy, Madurai, India. The rats were housed at 23 ± 2°C and 50-65% humidity under a 12:12 ± 1 hour light-dark cycle. The animals were fed water and rat pellets (Hindustan Lever Ltd., Bangalore, India) *ad libitum*. The study was approved by the Institute Animal Ethics Committee (1516/PO/a/11/CPCSEA), and all the animal experiments were carried out according to CPCSEA guidelines.

Anti-arthritis screening of *T. procumbens* extract

The rats were divided into five groups of five animals each as follows.

- Group-1: Vehicle control
- Group-2: Arthritis control
- Group-3: *T. procumbens* 250 mg/kg/day, p.o.
- Group-4: *T. procumbens* 500 mg/kg/day, p.o.
- Group-5: Indomethacin 10 mg/kg/day, p.o.

The method described by Newbould was employed with some modifications.[14] Adjuvant arthritis was induced by subcutaneous injection of FCA (0.1 ml) (Difco Labs, Chennai) into the subplantar tissue of the right hind paw of each rat. The test groups consisted of FCA-injected rats challenged with their respective doses of the test drug administered orally 24 hours before FCA injection. The vehicle control rats were injected with 0.1 ml of liquid paraffin (Incomplete Freund's Adjuvant) only. The drug treatments were continued for 20 days after inducing arthritis.

The swelling in the injected paw and the contralateral hind paw were monitored daily using a mercury displacement plethysmometer. The increase in the extent of erythema and oedema of the tissue shows the severity of the inflammation. The differences between the experimental groups and the arthritis control group were statistically analyzed. The change in body weight was also recorded daily.[15,16]

Biochemical parameters

At the end of the study, blood samples were withdrawn from all groups through retro-orbital plexus puncture, and the biochemical parameters were analyzed.[17] Haematological parameters such as the hemoglobin (Hb) level, the red blood cell (RBC) count, the white blood cell (WBC) count and the erythrocyte sedimentation rate (ESR) were estimated manually. Liver markers such as SGOT and SGPT were analyzed using an auto-analyzer (Vital Scientific N.V., the Netherlands). The liver enzyme levels were estimated using Lab Kit enzymatic kits.

Radiographic analysis

At the end of the study, the animals were anaesthetized using diethyl ether, and digital x-rays were taken for radiographic analysis of the knee joints. X-rays were taken of the knee joints for confirmation and evaluation of the severity of arthritis in FCA-induced rats.[12,13]

Statistical analysis

The mean ± SEM values were calculated for each group. Statistical differences among the groups were determined using one-way ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ was considered to be significant.

RESULTS

The preliminary phytochemical analysis of the ethanolic extract of *T. procumbens* showed the presence of alkaloids, tannins, flavonoids and saponins.

The arthritic control animals exhibited a significant decrease in body weight compared with the control group.

The results showed that indomethacin at 10 mg/kg and *T. procumbens* at 250 mg/kg and 500 mg/kg ameliorate the weight loss that occurs during arthritis [Table 1].

The latency of the arthritic secondary response ended after a few days and was characterized by joint swelling and nodule events on the 7th day. Administration of *T. procumbens* (250 mg/kg) significantly ($P < 0.01$) protected against joint swelling in paws in rats with induced arthritis compared with the arthritis control group. But a significant reduction was observed from day 11 to day 13 in the *T. procumbens*-treated (250 mg/kg) group. However, the effects of *T. procumbens* treatment at 500 mg/kg were found to be significant ($P < 0.001$) from the initial stage of the secondary response and were maintained throughout the experiment. They were significant ($P < 0.01$) 15-19 days after FCA injection compared with the group treated with the reference standard, indomethacin at 10 mg/kg [Table 2].

As shown in Table 3, elevated SGOT and SGPT levels and reduced RBC, ESR and Hb levels were observed in the arthritic controls compared with the normal controls. Administration of the ethanolic extract of *T. procumbens* to arthritic rats (Group-3, Group-4, Group-5) enhanced the Hb and RBC levels compared with the arthritic control group.

Clinical analysis of rheumatoid arthritis allows therapeutic monitoring, which remains the standard method for evaluating the progress of the disease. The loss of articular cartilage leads to diminished joint spaces, which may be brought about through a variety of pathological mechanisms. The normal knee bone structure of normal control animals may be seen in Figure 1. In contrast, the arthritis control animals exhibit a loss of articular cartilage, severe soft tissue swelling and reduced joint spaces. The degree of bone resorption, diminution of joint spaces and swelling of tissue were markedly reduced in *T. procumbens* at 500 mg/kg. The lower dose of *T. procumbens* (250 mg/kg) produced similar results. The standard drug, indomethacin, also produced fractional tibial epiphysis, and the appearance of the femoral

Table 1: Effect of ethanolic extract of *T. procumbens* on rodent growth

Group (n=5 in each group)	Body weight (g)		Increase in body weight (%)
	Initial	Final	
Normal control	179±1.62	190.23±2.89	6.27
Arthritis control	183.30±1.66	190.23±2.49	3.78
T. procumbens, 250 mg/kg	171.80±1.05	181.33±0.35	5.54
T. procumbens, 500 mg/kg	179±1.52	188.46±7.39	5.28
Indomethacin	160.13±0.18	172.16±1.30	7.51

Table 2: Anti-arthritic activity of ethanolic extract of *T. procumbens* compared with indomethacin in injected paw (swelling volume [ml]±SEM)

Treatment	Post-insult time of assay (days)										
	1	3	5	7	9	11	13	15	17	19	21
Normal control	0.12± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.11± 0.00	0.10± 0.00
Arthritic control	0.74± 0.00	0.85± 0.00	1.05± 0.00###	1.19± 0.00###	0.93± 0.00###	0.85± 0.01###	0.85± 0.01###	0.86± 0.01###	0.86± 0.01###	0.88± 0.01###	0.91± 0.01###
T. procumbens, 250 mg/kg	0.72± 0.01	0.74± 0.00	0.91± 0.01***	0.88± 0.01***	0.89± 0.00*	0.70± 0.00***	0.66± 0.01***	0.69± 0.01***	0.73± 0.01***	0.67± 0.00***	0.75± 0.01***
T. procumbens, 500 mg/kg	0.71± 0.01	0.79± 0.00	0.93± 0.01***	0.86± 0.01***	0.83± 0.00***	0.80± 0.00***	0.68± 0.00***	0.74± 0.01***	0.53± 0.01***	0.51± 0.01***	0.70± 0.01***
Indomethacin, 10 mg/kg	0.64± 0.00	0.74± 0.00	0.85± 0.00***	0.79± 0.00***	0.79± 0.00***	0.67± 0.00***	0.73± 0.00***	0.74± 0.00***	0.63± 0.00***	0.62± 0.01***	0.57± 0.01***

Values are expressed as mean±SEM; n=5 rats in each group; ***P<0.001 compared with arthritic control; ###P<0.001 compared with normal control (Repeated measures ANOVA followed by Bonferroni test.)

Table 3: Effect of the ethanolic extract of *T. procumbens* on biochemical and hematological parameters

Group	Biochemical parameter		Haematological parameter			
	SGOT (U/L)	SGPbT (U/L)	WBC (cells/cu.mm)	RBC (millions/cu.mm)	ESR (mm/hr)	Hb (gm/dl)
Normal control	105.26±0.13	55.68±0.72	7.31±0.06	4.90±0.05	3.27±0.20	13.05±0.24
Arthritic control	232.68±1.98	158.72±1.72	7.67±0.10##	3.65±0.23###	7.18±0.16###	8.87±0.203###
T. procumbens, 250 mg/kg	181.86±2.13***	127.86±3.39***	7.25±0.03	4.81±0.22	5.31±0.15***	9.57±0.115***
T. procumbens, 500 mg/kg	149.91±2.67***	112.64±1.19***	7.29±0.01	4.57±0.14	4.79±0.13***	10.38±0.31***
Indomethacin, 10 mg/kg	126.25±0.77***	93.02±1.62***	7.36±0.01	4.58±0.04	4.15±0.12**	12.14±0.23

Values are expressed as mean±SEM, n = 5 rats in each group, ***P<0.001, **P<0.01 compared with arthritic control, ###P<0.001, ##P<0.01 compared with normal control (Repeated measures ANOVA followed by Bonferroni test); WBC=White Blood Cell; RBC= The Red Blood Cell; HB=Hemoglobin; ESR=Erythrocyte Sedimentation Rate

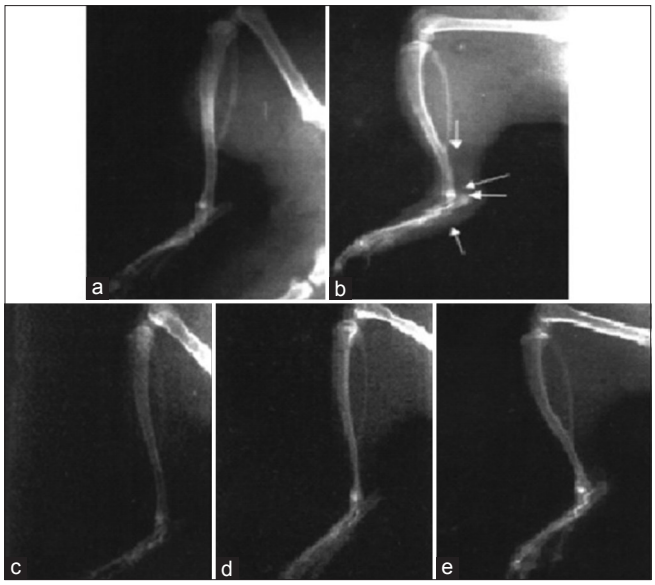

Figure 1: Effect of ethanolic extract of *T. procumbens* (TP) on arthritis (a) normal control animal showing the normal knee bone structure (b) animal with FCA-induced arthritis (c) animal with FCA-induced arthritis treated with *T. procumbens,* 250 mg/kg (d) animal with FCA-induced arthritis treated with *T. procumbens,* 500 mg/kg, showing reduced joint spaces and decreased paw oedema (e) animal with FCA-induced arthritis treated with indomethacin, 10 mg/kg, showing fractional tibial epiphysis

condyle was normal, with no soft tissue swelling [Figure 1]. The animals treated with *T. procumbens* (500 mg/kg) and indomethacin showed a significant decrease in paw oedema volume and increased joint spaces compared with the arthritic control group.

DISCUSSION

T. procumbens at 250 and 500 mg/kg displayed significant anti-arthritic activity, and the activity was comparable with that of indomethacin. *T. procumbens* significantly increased the body weight of animals compared with the arthritic controls. The anti-arthritic activity of *T. procumbens* is comparable with that of the standard drug indomethacin.

There is increasing evidence that lysosomal enzymes play an important role in the development of acute and chronic inflammations.[18] Most of the anti-inflammatory drugs exert their beneficial effects by inhibiting the release of these enzymes, which is one of the inflammatory processes, or by stabilizing the lysosomal membrane. So we can assume that our drug extract acts by inhibiting the lysosomal enzymes or stabilizing the membrane.

The arthritic control animals showed marked increases in the levels of the liver markers, whereas *T. procumbens* inhibited the increase in liver markers induced by FCA. Previous studies found that FCA also alters the biochemical and oxidative parameters.[19] FCA-induced arthritis is used to study the pathogenesis of rheumatoid arthritis for testing therapeutics.[20] One of the reasons for the wide utilization of this model is a strong correlation between the efficacy of therapeutic agents in this model and in rheumatoid arthritis in humans, and this model is characterized by a very rapid erosive disease. Bacterial peptidoglycan and muramyl dipeptide are responsible for the induction of adjuvant arthritis.[9,10]

Changes in body weight have also been used to assess the course of the disease and the response to therapy using anti-inflammatory drugs. Adjuvant arthritis is characterized by reduced body weight, and the weight loss is associated with an increased production of pro-inflammatory cytokines such as TNF-α and interleukin-α.

In the present study, the arthritic rats exhibited a reduced RBC count, reduced Hb level and increased ESR level.[21] All these symptoms indicate an anaemic condition, which is a common diagnostic feature in patients with chronic arthritis. The ESR is an estimate of the suspension stability of RBCs in plasma.[15] It is related to the number and size of the red blood cells and to the relative concentration of plasma proteins, especially fibrinogen and β globulins. An increase in the ESR is an indication of active but obscure disease processes. The acute phase proteins in ESR produce inflammation similar to that produced by an injection, injury, surgery or tissue necrosis.[20] The treatment with *T. procumbens* extract improved the RBC count, Hb level and ESR to a near-normal level, indicating significant recovery from the anaemic condition and arthritic progress, thus establishing that the extract has a significant role in arthritic conditions.

WBCs are a major component of the body's immune system. Indications for a WBC count include infections and inflammatory disease.[6,22] In arthritic conditions there is a mild to moderate rise in the WBC count due to a release of IL-IB. IL-IB increases the production of both granulocyte colony stimulating factor and macrophage colony stimulating factor.[9,23] The WBC count was increased in the arthritic group. Migration of leukocytes produces a significant decrease in the WBC count. Apart from prostaglandin, other cyclooxygenase products and various cells involved in inflammatory changes and free radical activities have all been implicated in the development of rat adjuvant arthritis.[10]

The radiographic analysis of the knee joint in the arthritic and drug-treated animals further supported and confirmed the potent dose-dependent anti-arthritic effect of the ethanolic extract of *T. procumbens* ethanolic extract, which suppresses

pathological changes, such as pannus formation and bone destruction.[24] The anti-arthritic effect of *T. procumbens* is comparable with that of indomethacin, and this action may be due to inhibition of the enzyme cyclooxygenase. However, further studies are required to confirm the effect of *T. procumbens* on cyclooxygenase inhibition.

Our phytochemical investigation revealed the presence of flavonoids. Sterols are known to inhibit articular swelling, reduce the arthritis index and down regulate the content of IL-IB and TNF-α in inflamed tissues of arthritic rats.[25,26]

CONCLUSION

The ethanolic whole plant extract of *T. procumbens* exerts an anti-arthritic activity by significantly altering the pathogenesis during FCA-induced arthritis in female SD rats without exerting any side effects.

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