



## Protective Role of *Tridax Procumbens* against Adjuvant Induced Arthritis in a Murine Model

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**Abstract:** Rheumatoid arthritis is the inflammatory disease affecting the joints and imposes significant burden among the individuals. Oxidative stress is one of the major mechanisms involved in the progression of arthritis. It is postulated that inflammation and secretion of various inflammatory cytokines also plays pivotal role in rheumatoid arthritis. *Tridax procumbens* (Asteraceae) is one of the potent herb and possess various biological actions. So, the present study was carried out to evaluate the protective role of *Tridax procumbens* in a murine model of complete Freund's adjuvant (CFA) provoked arthritis. The rats were made arthritic by injecting 0.1ml CFA into rat hind paw by intradermal route. *Tridax procumbens* methanolic extract (200 and 400 mg/kg, b.wt) were administered orally daily for 28 days after induction of arthritis. The anti arthritic effect was evaluated by, alterations in paw volume and body weight, changes in hematological parameters, increased lipid peroxidation and decreased antioxidants (SOD, CAT, GPx and GSH) due to arthritis. Treatment with *Tridax procumbens* significantly reduced the paw volume and increased the body weight in arthritic rats. Further, treatment with *Tridax procumbens* significantly increased the RBC and hemoglobin and decreased the WBC and ESR level as compared to the arthritic rats. The antioxidant levels were significantly increased in arthritic rats after treatment with extracts. Thus the anti-arthritic potential of *Tridax procumbens* might be mediated through antioxidant and anti-inflammatory effect of the extracts

**Keywords:** *Tridax procumbens*, arthritis, complete freund's adjuvant, inflammation, antioxidant.

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## 1. INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that damages synovial membrane, leads and prelude to distortion of articular tissue and further erosion of bones as a result of extended inflammation<sup>1</sup>. Globally, it hampers 2-4% population and the prevalence varies based on the ethnic people<sup>2</sup>. RA is a clinical condition encompassing the following events, elevated immune cells such as macrophages in the synovial space, rampant release of free radicals which oxidizes various biomolecules and leads to articular tissue damage and development of disease<sup>3</sup>. The management of arthritis is focused on the following strategies such as blocking of proinflammatory cytokines, inflammatory enzymes like cyclooxygenase and T-Lymphocytes and thus reduces the articular tissue degeneration<sup>4</sup>. The most widely used drug for the management of RA is NSAIDs, corticosteroids, DMARDs and monoclonal antibodies like rituximab and infliximab<sup>5</sup>. Meanwhile, the above mentioned management strategies impose noxious adverse effects such as duodenal ulcer, hepatic damage, immunosuppression and infections<sup>6,7</sup>. Currently mounting Indian medicinal plants have been tested in preclinical models for its anti arthritic effect<sup>8</sup>. In the traditional medicine system, *Tridax procumbens* (*T. procumbens*) is widely employed in the management of inflammation and pain. Previous report displayed the protective effect of ethanolic extract of *T. procumbens* in complete freund's adjuvant (CFA) mediated murine model of arthritis<sup>9</sup>. In this backdrop, the present study was conducted to evaluate the protective effect of the methanolic extract of *T. procumbens* on CFA induced arthritic emphasizing its anti-inflammatory and antioxidant potential.

## 2. MATERIALS AND METHODS

### 2.1 Collection, identification and authentication of *T. procumbens*

The whole plant of *T. procumbens* was collected from the shrubs and sandy places of Khammam, Khammam district, Telangana, India with 16° 45' and 18° 35' N latitude and 79° 47' to 81° 47' E longitude respectively. The collected plant was authenticated by Dr. K.Madhava Chetty, Assistant Professor, Sri Venkateswara University, Chittoor district, Andhra Pradesh. The plant materials were dried and powdered for extraction.

### 2.2 Chemicals and solvents

The entire chemicals used for this experimental study were analytical grade and purchased from S.D. Fine Chemicals, Mumbai. Freund's complete adjuvant (CFA) was procured from Sigma Aldrich, India.

### 2.3 Extraction of plant material

100 gm of powdered *T. procumbens* was extracted with 500 ml of methanol by the maceration process. Then the solvent was removed by filtration and concentrated using a rotary evaporator.

### 2.4 Experimental design

Male albino Wistar rats (160-180 gms) were selected and allowed for unrestricted water consumption. The study was

conducted as per the CPCSEA of Sanzyme health care business, Hyderabad.

### 2.5 CFA induced arthritis

The animals were grouped as follows (n=6),  
Group I: Control rats received 2% gum acacia, p.o.  
Group II: Arthritis was induced by single intradermal injection of 0.1 ml of CFA into the right hind paw.  
Group III: Arthritis rats received methanolic extract of *T. procumbens* suspended in 2% gum acacia (200 mg/kg /day; p.o).  
Group IV: Arthritis rats received methanolic extract of *T. procumbens* suspended in 2% gum acacia (200 mg/kg /day; p.o).  
Group IV: Arthritis rats received Diclofenac Sodium (5 mg/kg /day; p.o).  
The respective groups received the standard drug and plant extracts through an oral route for 28 days. On day 1, the animals of all groups, except the control, received a single intradermal dose of CFA (0.1 ml) into the right hind paw.

### 2.6 Assessment of body weight and paw volume

The hind paw volume was estimated at initial period (Day 0) before CFA intoxication and continued at various time intervals till day 25 with the help of plethysmograph. The paw volume was calculated as a difference between final and initial paw volume. Further, the body weight was measured as a difference between initial (day 0) and final (day 28) body weight.

### 2.7 Blood collection and tissue processing

After the final doses of extract and standard drugs the access to food was restricted overnight and on 29<sup>th</sup> day the animals were anaesthetized using phenobarbital sodium (35mg/kg; i.p) and sacrificed by cervical decapitation. The blood was collected from the jugular vein in heparinized tubes and the serum was separated for the measurement of hematological parameters. The liver tissue was excised, cleaned from adherent tissues, washed in ice cold saline and dried. Then a 100 mg weighted tissue was homogenized (10% w/v) in a chilled Tris-HCl buffer at pH 7.5 and used for the analyses of various biochemical markers.

### 2.8 Estimation of hematological parameters

The levels of RBC, WBC, hemoglobin (Hb) and ESR were evaluated using Hematology analyzer (Sysmex XE-2100, India).

### 2.9 Measurement of lipid peroxidation

The lipid peroxidation (LPO) marker, malondialdehyde (MDA) was measured according to the information mentioned in the kit procured from Kamineni Life Sciences Pvt. Ltd. Hyderabad, India.

### 2.10 Measurement of antioxidants

The hepatic antioxidants catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) were measured instructions according to the instruction mentioned in the kit procured from Kamineni Life Sciences Pvt. Ltd. Hyderabad, India.

### 3. STATISTICAL ANALYSIS

The data were expressed as Mean  $\pm$  SEM. The data were analysed by one way analysis of variance followed Tukey's comparison using SPSS 16.0 v.  $p < 0.05$  was noted as statistically significant.

### 4. RESULTS

#### 4.1 Effect of *T. procumbens* on body weight and paw swelling parameters in CFA induced arthritis

CFA rats showed significant ( $p < 0.05$ ) decrease in body weight on day 8, 16 and 24 as compared to the control group. Meanwhile treatment with *T. procumbens* methanolic extract at the dose of 200 and 400mg/kg significantly ( $p < 0.05$ ) increased the body weight as that of the arthritic rats. The data were shown in Fig 1.

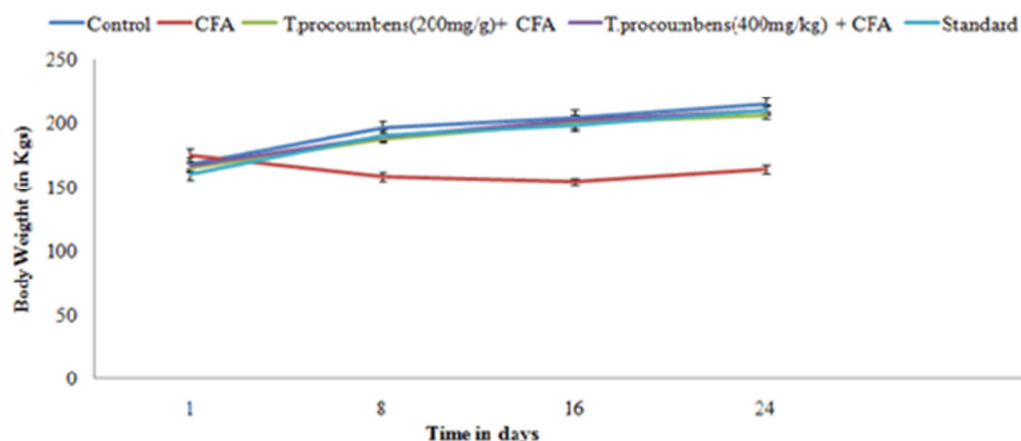


Fig 1. Effect of *T. procumbens* on body weight in CFA induced arthritic rats

CFA rats elicited significant ( $p < 0.05$ ) increase in paw volume in CFA induced arthritic rats, as a sign of inflammation as that of the normal control rats. The paw volumes were measured at day 1, 8, 16 and 24 and there was a gradual elevation in paw volume of arthritic rats. In our study, arthritic insulted

rats treated with *T. procumbens* methanolic extract at the dose of 200 and 400 mg/kg displayed significant reduction in rat paw oedema volume compared to arthritic rats. The results were shown in Table 1.

Table 1. Effect of <i>T. procumbens</i> on paw volume in CFA induced arthritic rats				
Groups	Paw Volume (in mm)			
	Day 1	Day 8	Day 16	Day 24
Control	4.3 $\pm$ 0.1	4.5 $\pm$ 0.2	4.4 $\pm$ 0.1	4.6 $\pm$ 0.1
CFA	7.6 $\pm$ 0.5 <sup>a*</sup>	16.6 $\pm$ 0.2 <sup>a*</sup>	22.5 $\pm$ 0.3 <sup>a*</sup>	25.7 $\pm$ 0.6 <sup>a*</sup>
<i>T. procumbens</i> (200mg/kg) + CFA	6.1 $\pm$ 0.7 <sup>b*</sup>	7.8 $\pm$ 0.4 <sup>b*</sup>	6.9 $\pm$ 0.4 <sup>b*</sup>	5.8 $\pm$ 0.6 <sup>b*</sup>
<i>T. procumbens</i> (400mg/kg) + CFA	5.8 $\pm$ 0.4 <sup>b*</sup>	7.1 $\pm$ 0.5 <sup>b*</sup>	6.2 $\pm$ 0.4 <sup>b*</sup>	4.7 $\pm$ 0.4 <sup>b*</sup>
Diclofenac Sodium (5mg/kg)	5.9 $\pm$ 0.4 <sup>b*</sup>	7.1 $\pm$ 0.3 <sup>b*</sup>	6.2 $\pm$ 0.4 <sup>b*</sup>	4.9 $\pm$ 0.5 <sup>b*</sup>

Values are expressed as mean  $\pm$  SEM; n=6; One-way ANOVA followed by Tukey's test was used to compare between the groups. a- CFA vs Control; b-Extract and standard vs CFA. \*  $p < 0.05$  was statistically significant.

#### 4.2 Effect of *T. procumbens* on hematological parameters in CFA induced arthritis

CFA induced arthritic rats displayed a significant ( $p < 0.05$ ) increase in WBC and ESR as that of the control rats. Further, arthritic rats displayed a significant ( $p < 0.05$ ) decrease in RBC

and Hb as compared to the control rats. Oral intubation of *T. procumbens* methanolic extract at the dose of 200 and 400 mg/kg significantly ( $p < 0.05$ ) restored the altered WBC, ESR, RBC and Hb levels to normal as compared to the arthritis induced rats. The results were shown in Table 2.

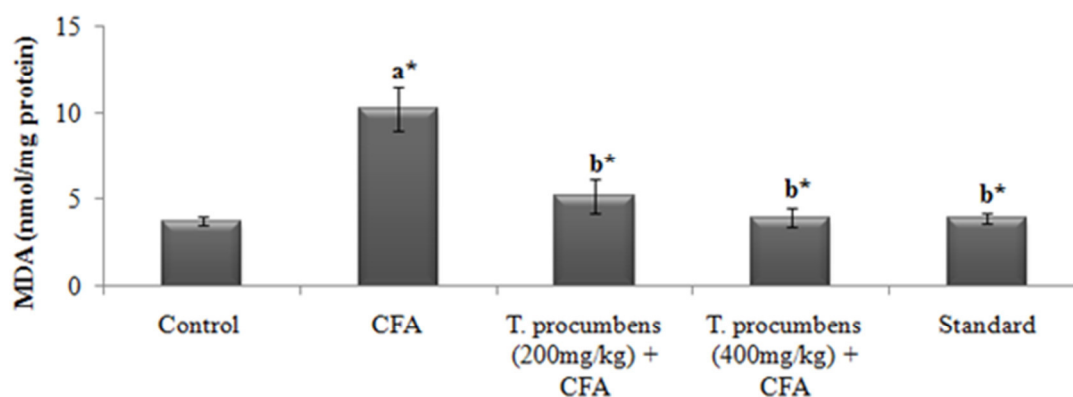
Table 2. Effect of <i>T. procumbens</i> on hematological alteration in CFA induced arthritis				
Groups	WBC( $\times 10^3$ /L)	RBC( $\times 10^6$ /L)	ESR(mm/Hr)	Hb(gm/dl)
Control	5.2 $\pm$ 0.98	7.4 $\pm$ 1.25	3.0 $\pm$ 0.58	14.2 $\pm$ 2.25
CFA	9.4 $\pm$ 1.29 <sup>a*</sup>	4.9 $\pm$ 0.78 <sup>a*</sup>	20.3 $\pm$ 3.56 <sup>a*</sup>	9.3 $\pm$ 1.12 <sup>a*</sup>
<i>T. procumbens</i> (200mg/kg) + CFA	7.6 $\pm$ 1.45 <sup>b*</sup>	6.5 $\pm$ 0.98 <sup>b*</sup>	12.4 $\pm$ 2.85 <sup>b*</sup>	12.2 $\pm$ 3.45 <sup>b*</sup>
<i>T. procumbens</i> (400mg/kg) + CFA	5.9 $\pm$ 1.25 <sup>b*</sup>	7.2 $\pm$ 1.28 <sup>b*</sup>	9.85 $\pm$ 1.28 <sup>b*</sup>	13.9 $\pm$ 3.75 <sup>b*</sup>
Diclofenac Sodium (5mg/kg)	5.56 $\pm$ 1.45 <sup>b*</sup>	7.3 $\pm$ 1.12 <sup>b*</sup>	9.8 $\pm$ 1.78 <sup>b*</sup>	13.8 $\pm$ 2.89 <sup>b*</sup>

Values are expressed as mean  $\pm$  SEM; n=6; One-way ANOVA followed by Tukey's test was used to compare between the groups. a- CFA vs Control; b-Extract and standard vs CFA. \*  $p < 0.05$  was statistically significant.

#### 4.3 Effect of *T. procumbens* on lipid peroxidation in CFA induced arthritis

Further, CFA induced arthritic rats elicited significant ( $p < 0.05$ ) increase in malondialdehyde (MDA) levels as that of

the control rats. Treatment with *T. procumbens* methanolic extract at the dose of 200 and 400mg/kg significantly ( $p < 0.05$ ) reduced the MDA level as compared to the arthritic rats. The results were shown in Fig 2.



MDA: Malondialdehyde. Values are expressed as mean  $\pm$  SEM;  $n=6$ ; One-way ANOVA followed by Tukey's test was used to compare between the groups. a- CFA vs Control; b-Extract and standard vs CFA. \*  $p < 0.05$  was statistically significant

**Fig 2. Effect of *T. procumbens* on lipid peroxidation alteration in CFA induced arthritis**

#### 4.4 Effect of *T. procumbens* on antioxidant levels in CFA induced arthritic rats

In the present study, CFA induced arthritic rats showed significant ( $p < 0.05$ ) decline in the level of antioxidants like

SOD, CAT, GPx and GSH. Administration of *T. procumbens* methanolic extract at the dose of 200 and 400mg/kg significantly ( $p < 0.05$ ) increased the antioxidant level as that of the arthritic rats. The results were shown in Table 3.

Groups	SOD	CAT	GPx	GSH
Control	7.28 $\pm$ 0.76	65.75 $\pm$ 4.12	122.45 $\pm$ 6.76	3.45 $\pm$ 0.05
CFA	3.56 $\pm$ 0.45 <sup>a*</sup>	32.45 $\pm$ 2.56 <sup>a*</sup>	85.76 $\pm$ 6.45 <sup>a*</sup>	1.28 $\pm$ 0.06 <sup>a*</sup>
T. procumbens (200mg/kg) + CFA	5.56 $\pm$ 0.45 <sup>b*</sup>	46.38 $\pm$ 3.78 <sup>b*</sup>	108.12 $\pm$ 5.65 <sup>b*</sup>	2.09 $\pm$ 0.07 <sup>b*</sup>
T. procumbens(400mg/kg) + CFA	7.12 $\pm$ 0.56 <sup>b*</sup>	55.85 $\pm$ 4.12 <sup>b*</sup>	118.28 $\pm$ 6.12 <sup>b*</sup>	3.05 $\pm$ 0.05 <sup>b*</sup>
Diclofenac Sodium (5mg/kg)	7.14 $\pm$ 0.45 <sup>b*</sup>	58.65 $\pm$ 3.45 <sup>b*</sup>	120.56 $\pm$ 6.12 <sup>b*</sup>	3.12 $\pm$ 0.08 <sup>b*</sup>

Values are expressed as mean  $\pm$  SEM;  $n=6$ ; One-way ANOVA followed by Tukey's test was used to compare between the groups. a- CFA vs Control; b-Extract and standard vs CFA. \*  $p < 0.05$  was statistically significant. SOD (units/ mg of protein); CAT (n moles of  $H_2O_2$  decomposed / min / mg of protein); GPx (n moles of GSH oxidized / min / mg of protein); GSH (nmoles/g tissue).

## 5. DISCUSSION

In preclinical studies, CFA mediated arthritis is widely employed since it reflects the human arthritis characterized by joint swelling with the recruitment of inflammatory cells, cartilage erosion and bone damage<sup>11</sup>. Measurement of paw swelling is the easiest and effective method to evaluate the degree of inflammation during arthritis and also to study the efficacy of drugs. The long term inflammation during arthritis triggers the release of inflammatory mediators such as cytokines, interferon and platelet growth derived factor. These noxious mediators are the major factors in the progression of pain, cartilage damage which prelude to marked disability<sup>12</sup>. In the present study treatment with methanolic extract of *T. procumbens* significantly reduced the paw volume which is in line with the earlier reports<sup>9</sup>. Alteration in body weight is generally observed during disease condition and evaluation of this change is essential to assess the efficacy of drugs during treatment<sup>13</sup>. When the progression of arthritis is high there has been a significant change in the CFA induced arthritic rats. Previous studies show that there has been a marked decrease in body weight during arthritis and it might be due to the metabolic changes

in the arthritic rats<sup>14</sup>. Previous study shows that there is a delayed intestinal absorption of  $^{14}C$ -glucose and  $^{14}C$ -leucine during inflammation<sup>14</sup>. Further, it has been shown that treatment with anti-inflammatory agents significantly increased intestinal absorption, as a result of decreased inflammation<sup>15</sup>. The increased body weight during the treatment of *T. procumbens* may be due to the restoration of the absorption capacity of the intestine. During arthritis there has been a significant elevation of WBC as a result of inflammatory response triggered by interleukin- $1\beta$  which inturn increases the level of GM-CSF<sup>11</sup>. In this study, leucocytes' migration to inflamed areas is effectively reduced by *T. procumbens* extract as that of the arthritic rats, which is evident from significant reduction in the WBC count. Erythrocyte Sedimentation Rate (ESR) is used to determine the RBC stability in its suspension state in plasma. ESR reflects the count and size of RBCs corresponding to the concentration of proteins such as fibrinogen, alpha and beta globulins. This elevated ESR rate is an indicative of active or chronic disease condition. Further, acute phase proteins such C-reactive increases the ESR during inflammation and necrotic conditions. In the present study the ESR significantly increased arthritic rats, whereas sedimentation rates were

remarkably counteracted upon treatment with *T. procumbens*<sup>16</sup>. In our study, the CFA induced arthritic rats showed decreased level of RBC count and Hb level<sup>17</sup>. All these symptoms indicate an anaemic condition, which is a common diagnostic feature in patients with chronic arthritis. However, treatment with *T. procumbens* significantly increased the RBC count and Hb level. When the activated phagocytic cells engulf the pathogens or immune complex it generates an oxidative state and induces rampant release of free radicals and destruct the pathogens<sup>18</sup>. Meanwhile, the free radicals like nitric oxide and peroxynitrite also orchestrate a predominant role in the generation of oxidative stress during arthritic condition<sup>19</sup>. The generated ROS in turn enhances the oxidative stress by lipid peroxidation process. It has been shown that ROS mediated lipid peroxidation peroxide the membrane lipids and released the toxic adduct malondialdehyde (MDA) which damages membrane<sup>20</sup>. Previous studies show that MDA level was significantly increased in CFA induced arthritis which is in corroboration with the present study<sup>21</sup>. Treatment with *T. procumbens* reduced the MDA level and thus prevented the membrane damage as a result of lipid peroxidation. In the present study, antioxidants such as SOD, CAT, GPx and GSH were decreased in CFA induced arthritic rats as a result of rampant involvement in the reduction of ROS generated by CFA<sup>22</sup>. Treatment with *T. procumbens* restored the altered antioxidants level to normal and thus prevented the

oxidative stress due to CFA. Previous studies report that centaureidin and procumbenetin are the flavonoids identified in *T. procumbens*<sup>23,24</sup>. Thus the antiarthritic activity exhibited by *T. procumbens* might be due to the presence of these phytoconstituents.

## 6. CONCLUSION

Thus present reveals that arthritic rats reveal decreased in body weight and increase in paw volume as result of inflammation induced by CFA. Further, there was significantly hematological and antioxidants alteration with increase in lipid peroxidation. Treatment with *T. procumbens* extracts showed effective anti arthritic effect through its antioxidant and anti inflammatory mechanism.

## 7. AUTHORS CONTRIBUTION STATEMENT

Tirupathi Rao Y R K V designed the study. Gopal Rao K conducted the study, collected the data and performed the analysis and drafted the manuscript. Satishchandra A edited and improved the manuscript draft.

## 8. CONFLICT OF INTEREST

Conflict of interest declared none.

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