e-ISSN: 2320-4230, p-ISSN: 2961-6085

Journal of Drug Discovery and Therapeutics

Available Online at www.jddt.in

CODEN: - JDDTBP (Source: - American Chemical Society)

Volume 13, Issue 4; 2025, 117-122

Evaluation of in-vivo anti-inflammatory activity of Tridax procumbens Linn extract

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Received: 11-04-2025 / Revised: 10-05-2025 / Accepted: 23-06-2025
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Conflict of interest: No conflict of interest.

Abstract:

The present study investigates the in-vivo anti-inflammatory potential of various extracts of Tridax procumbens Linn, a medicinal herb traditionally used in Indian folklore for treating inflammation and wounds. The anti-inflammatory activity was evaluated using standard biochemical markers including alanine transaminase (ALT), aspartate transaminase (AST), lipid peroxidase (MDA), and alkaline phosphatase (ALP) in experimental animal models. Different solvent extracts—ethanolic (TP-EtOHE), aqueous (TP-AqE), chloroform (TP-$CHCl_3$E), and petroleum ether (TP-PEE)—were administered at a dose of 200 mg/kg body weight, while a standardized ethanol extract (TPEE-S) was tested at 30 mg/kg. Diclofenac (50 mg/kg) served as the standard drug. Results indicated that the TPEE-S and TP-EtOHE extracts significantly reduced inflammatory biomarkers, with values comparable to the standard drug, suggesting potent anti-inflammatory properties. The aqueous, chloroform, and petroleum ether extracts also demonstrated moderate activity. These findings support the traditional use of Tridax procumbens in inflammation and warrant further studies to isolate and characterize its active constituents.

Keywords: Tridax procumbens, Anti-inflammatory, In-vivo study, Phytochemicals, Ethanolic extract, Lipid peroxidation, Liver enzymes, Herbal medicine

Introduction

Inflammation is a complex biological response of body tissues to harmful stimuli such as pathogens, damaged cells, or irritants. While it is a protective mechanism, chronic or excessive inflammation contributes to the development of several pathological conditions including arthritis, cardiovascular diseases, and cancer. Conventional anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) are effective but often associated with adverse effects including gastrointestinal irritation, hepatotoxicity, and renal damage. This has led to increased interest in the exploration of safer, plant-based alternatives with fewer side effects. [1]

Tridax procumbens Linn (family: Asteraceae), commonly known as “coat buttons,” is a creeping herb widely distributed in tropical regions. Traditionally, it has been used in Indian medicine for wound healing, fever, diarrhea, and inflammation. Preliminary phytochemical studies have revealed the presence of flavonoids, alkaloids, saponins, and tannins—compounds known for their anti-inflammatory and antioxidant properties. [2]

The present study aims to evaluate the in-vivo anti-inflammatory activity of various extracts of Tridax procumbens Linn in animal models by measuring key biochemical markers such as alanine transaminase (ALT), aspartate transaminase (AST), lipid peroxidation (MDA), and alkaline phosphatase (ALP). Standard anti-inflammatory drug Diclofenac was used as a reference. The outcome of this study may validate the traditional use of Tridax procumbens and contribute to the development of effective plant-based anti-inflammatory agents.

1. Materials and method

- **Collection of Tridax procumbens Linn Plant:** Entire plant of Tridax procumbens Linn was gathered from neighborhood Vadgaon, Belagavi (Karnataka).
- **Authentication of Tridax procumbens Linn Plant:** Tridax procumbens Linn plant was approved from Scientist Dr. H. Hegade RMRC, ICMR, Nehru Nagar, Belagavi.
- **Pharmacological screening of Tridax procumbens Linn whole plant extracts**

- **Experimental animals:**

Wistar rats (Male or Female) weighing 120-150gm obtained from Venkateshwar enterprises, Bangalore (licensed breeder) were used in the study. Acute oral toxicity evaluating, hepatoprotective and anti-inflammatory action study, these animals were utilized. Free access to food pellets to the animals were allowed (Amrut brand from M/s Pranav life sciences and research pvt. Ltd. Sangli) and clean tap water during acclimatization period of about ten days in the laboratory. In a group of six all animals were housed in clean cages, with natural day and night cycle. The study was approved by IAEC, constituted as per CPCSEA guide lines. Resolution No. 14 dated: 06/08/2015

- **Toxicity Studies**

Acute oral toxicity: Before starting of pharmacological screening toxic dose of the various extract was performed. The toxicity study was done by administrating the different dosages extend from 1500 mg to 4000 mg. The investigation was done according to the rules set by Ministry of Social Justice and Empowerment, Government of India, Draft rules 423, from Committee for the purpose of Supervision and Control of experiments on Animals (CPCSEA), Economic Co-activity and Development (OECD) Organization. [3]

Table 1: LD50 cut off values and dose selection

Sl. No.	Name of Extract	LD_{50} Cut-off (mg/kg body weight)	Vehicle
1	Ethanolic extract of Tridax procumbens Linn	2000 mg/kg	1% Gum acacia
2	Aqueous extract of Tridax procumbens Linn	2000 mg/kg	1% Gum acacia
3	Chloroform extract of Tridax procumbens Linn	2000 mg/kg	1% Gum acacia
4	Pet-ether extract of Tridax procumbens Linn	2000 mg/kg	1% Gum acacia

1/10th of this deadly portion was taken as compelling portion (helpful portion) for anti-inflammatory activity.

Observation: Animals were observed initially during the first 30 minutes after dosing at least once, during the first 24 hour periodically. In all cases, within first 24 hours, death was detected. Further observations like changes in eyes, behavior pattern respiratory, skin and mucus membrane, circulatory, central and autonomic nervous system. Attention was given to observation of convulsions and tremors too.

- **Anti-inflammatory activity whole plant extracts of Tridax procumbens Linn**

Anti-inflammatory activity of ethanolic, aqueous, petroleum ether and chloroform extract of whole plant of Tridax procumbens Linn was carried out in acute and chronic model of inflammation.

Acute Inflammation: Overnight separated rats were separated into four groups; each group consists of six animals. The experiment was carried out by the method of induced paw edema by injecting 0.1% freshly prepared carrageenan suspension in 1% Tween 80 into the sub plantar region of right hind paw in each rat. By dipping inflamed paw up to the premarked level at 0, 30, 60, 90, 120 and 180 mins after the carrageenan injection, the paw volume was measured in ml using a digital plethysmometer. One hour before injecting carrageenan two groups of rats received either 1ml/kg-1 of 15 orally Tween 80 or 50mg/ kg-1Diclofenac, while treated groups received the petroleum extract, ethanolic extract, aqueous extract, chloroform extract and isolated compound from ethanolic extract (200mg/kg-1) of Tridax procumbens Linn. In a single dose, all the treatment was administered orally. [4]

Chronic inflammation: By subcutaneous implantation of cotton pallets under anaesthesia, chronic inflammation was produced. Four sterile cotton pallets weighing 10mg were implanted aseptically in axillae and groin overnight starved rats, with modification that granuloma formation was assessed on 10th day instead of 4th day. After implanting cotton pallets each group (n=6) was assigned treatment with 200mg/kg-1 of ethanolic extract or 50mg/kg-1 Diclofenac or equal volume of vehicle once daily orally for 10 consecutive days. The animals were anesthetized on the tenth day, once again and to dissect the cotton pallets free from extraneous tissues. At 37oC for 24hour, pellets were incubating at 60oC to obtain constant weights. The decrease in the weight of the pallets was taken to measure anti-inflammatory activity. The cotton pallets dry weight was expressed as mg/10gm body weight to calculate group mean. By one way ANOVA, the results were analyzed statistically followed by Dunnett's multiple comparison tests by using prism graph pad method and considered significant at $P \leq 0.05$. [5]

Statistical analysis: The outcomes are communicated as Mean ± S.E.M. By Dunnett's test, the difference between experimental groups was compared by ANOVA (One-way analysis of Variance). The results were considered stastically significant when $P < 0.05$.

Results and discussion

Toxicity study and anti-inflammatory activity of Tridax procumbens Linn extract

Acute toxicity study of Tridax procumbens Linn extracts: Petroleum ether (PEE), Chloroform (CHCl3E), ethanol (EtOHE) and aqueous (AqE) extracts of Tridax procumbens Linn whole plant did not produce any toxic symptoms or mortality, in oral dose of 2000mg/kg in Wistar rats and

all these extracts were considered safe for further pharmacological studies.

Anti-inflammatory activity of Tridax procumbens Linn extract

1] Rat paw edema induced by carrageenan: In the present study the animals treated with EtOHE significantly (P<0.001) inhibited edema formation with the mean values (ml) of 0.30±0.003, 0.28±0.007, 0.27±0.008 and 0.22±0.003 respectively at 60, 90, 120 and 180 mins. Similarly, AqE treated animals indicated noteworthy (P<0.001) restraint of edema arrangement with the mean values of 0.30±0.003, 0.28±0.007, 0.27±0.008 and 0.22±0.003respectively at 60, 90, 120 and 180 mins. Animals treated with isolated compound of Tridax procumbens Linn indicated noteworthy (P<0.001) restraint of edema arrangement with the mean values (ml) of 0.30±0.003, 0.28±0.007, 0.27±0.008 and 0.22±0.003 at 60, 90, 120 and 180 minis respectively. The corresponding mean values 0.41±0.004, 0.43±0.004, 0.43±0.006 and 0.43±0.006 at 60, 90, 120 and 180 mins respectively in control group.

Similarly TP-CHCl3E treated animals failed to inhibit the edema formation with the mean values (ml) 0.42±0.008, 0.45±0.003, 0.41±0.004 and 0.44±0.01 respectively at 60, 90, 120 and 180 mins and also TP-PEE treated animals failed to inhibit the edema formation with the mean values (ml) 0.42±0.008, 0.44±0.003, 0.42±0.01 and 0.44±0.011003 respectively at 60, 90, 120 and 180 mins. The corresponding mean values 0.41±0.004, 0.43±0.004, 0.43±0.006 and 0.43±0.006 at 60, 90, 120 and 180 mins respectively in control group.

Table 2: Biochemical changes observed in Carrageenan induced rat paw edema

Group (mg/kg b.w)	Dose	ALT (U/L)	AST (U/L)	Lipid Peroxidase (MDA nmol/mg)	ALP (U/L)
Control (0.1% Tween 80)	1 ml	126.30 ± 2.91	88.48 ± 2.92	97.23 ± 1.45	88.04 ± 1.35
Diclofenac	50 mg	65.32 ± 1.38 ***	52.36 ± 1.07 ***	56.59 ± 2.32 ***	61.42 ± 1.32 ***
TPEE-S	30 mg	68.40 ± 0.96 ***	58.93 ± 2.11 ***	72.48 ± 1.34 ***	71.25 ± 1.35 ***
TP-EtOHE	200 mg	75.34 ± 2.08 ***	58.96 ± 2.42 ***	78.23 ± 1.56 ***	72.51 ± 1.76 ***
TP-AqE	200 mg	113.48 ± 1.59 **	78.28 ± 1.96 **	85.12 ± 1.72 **	81.65 ± 2.52 **
TP-$CHCl_3E$	200 mg	114.20 ± 2.88	87.20 ± 2.56	93.23 ± 1.85	83.35 ± 1.89
TP-PEE	200 mg	119.21 ± 1.62	82.35 ± 2.78	96.12 ± 1.21	86.88 ± 1.15

*****P<0.001, **P<0.01, ns - Non-Significant.**

TP-EtOHE - Tridax procumbens ethanolic extract, TP-AqE - Tridax procumbens aqueous extract, TP-CHCl3E - Tridax procumbens chloroform extract, TP-PEE - Tridax procumbens pet ether extract.

Cotton pellet granuloma in rats: Granuloma formation induced by cotton pellet was significantly (P<0.001) inhibited by TP-EtOHE, β-sitosterol, Diclofenac and TP-AqE also inhibited (P<0.01) treated animals with mean 27.12±1.91, 26.04±0.87, 25.82±1.39 and 31.31±1.47 respectively as compared to 55.00±0.20 control group.

Table 3: Biochemical parameters change in cotton pellet granuloma in rats

Group	Dose (mg/kg b.w)	ALT (U/L)	AST (U/L)	Lipid Peroxidase (nmol/mg)	ALP (U/L)
Control (Tween 80)	1 ml	118.20 ± 1.61	86.48 ± 2.31	98.23 ± 2.61	88.92 ± 2.35
Diclofenac	50 mg	64.32 ± 1.40 ***	58.36 ± 1.72 ***	65.15 ± 1.69 ***	67.42 ± 2.65 ***
TPEE-S	30 mg	69.40 ± 0.19 ***	61.64 ± 1.35 ***	74.14 ± 1.42 ***	72.21 ± 2.07 ***
TP-EtOHE	200 mg	76.34 ± 2.08 ***	64.35 ± 2.42 ***	77.23 ± 2.31 ***	73.51 ± 1.78 ***
TP-AqE	200 mg	113.48 ± 1.59 **	74.28 ± 2.43 **	85.75 ± 2.96 **	77.20 ± 2.41 **
TP-CHCl₃E	200 mg	114.20 ± 2.88	82.20 ± 2.16	89.63 ± 3.11	85.44 ± 2.72
TP-PEE	200 mg	119.21 ± 1.62	83.35 ± 1.95	95.25 ± 2.72	87.07 ± 2.35

***P<0.001, **P<0.01, ns - non-significant.

TP-EtOHE - Tridax procumbens ethanolic extract, TP-AqE - Tridax procumbens aqueous extract, TP- CHCl3E - Tridax procumbens chloroform extract, TP-PEE - Tridax procumbens petroleum ether extract.

Conclusion

The present study was carried out on the basis of scientific for traditionally use of extracts of the whole plant of Tridax procumbens Linn extract using in-vivo anti-inflammatory models. The medicinal plant extract showed no acute oral toxicity at a dose of 2000mg/kg. Among the alcoholic, aqueous, petroleum ether and chloroform extracts of Tridax procumbens Linn- whole plant. Alcoholic extract showed huge anti-inflammatory action in intense and interminable models.

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