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Assessment of the Anti-inflammatory and Analgesic Properties of *Tragia involucrate* Anti-inflammatory, Analgesic Activity of *Tragia involucrata*

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ABSTRACT

The diverse bioactive properties of *Tragia involucrate* are highlighted by numerous studies that reveal its anti-inflammatory, antioxidant, analgesic, and potential anti-diabetic effects. Leaves of *T. involucrata* were gathered, extracted with various solvents, and analyzed for phytochemical components through biochemical methods and high-performance thin-layer chromatography (HP-TLC). The extracts underwent evaluation for cytotoxicity, antioxidant capacity, and in vitro anti-inflammatory effects. An acute oral toxicity assessment was conducted to determine in vivo toxicity in rats, while carrageenan-induced paw edema was utilized to assess in vivo anti-inflammatory activity. The phytochemical constituents include tannins, flavonoids, saponins, terpenoids, and steroids. Notably, the aqueous extract exhibited significant membrane-stabilizing effects on human red blood cell membranes and demonstrated potent activity against stable free radicals. Results from acute toxicity studies and paw edema tests indicated that the extract was well tolerated at a dose of 2000 mg/kg body weight and effectively reduced edema. Additionally, the aqueous extract produced analgesic effects at an oral dosage of 300 mg/kg body weight. These results imply that AETI possesses promising analgesic, antioxidant, and anti-inflammatory properties, suggesting that *T. involucrata* may be a therapeutic option for managing pain and inflammation.

Keywords: Tragia involucrata, anti-inflammatory, analgesic, antioxidant activity, Ayurveda.

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INTRODUCTION

Exploring medicinal plants has unveiled a treasure trove of bioactive compounds with therapeutic potential in natural product research. Among these, *Tragia involucrata*, a member of the Euphorbiaceae family, has emerged as a subject of keen scientific inquiry owing to its diverse pharmacological properties. The multifaceted nature of *T. involucrate's* bioactivity is underscored by many studies elucidating its anti-inflammatory, antioxidant, analgesic, and anti-diabetic attributes.

Recent investigations into the phytochemical composition of *T. involucrata* leaf extracts have shed light on its therapeutic potential. Studies conducted by Vigneswaran *et al.* (2023) have explored the anti-inflammatory effects of specific fractions, unveiling mechanisms involving the inhibition of NF-κB signaling pathways. This molecular insight is pivotal for understanding the intricate interplay between phytoconstituents and inflammatory cascades. Furthermore, the analgesic properties of *T. involucrata* have also been investigated (Alimuzzaman *et al.*, 2005). These findings validate its traditional use as a pain-relieving agent and underscore its potential as a natural alternative to conventional analgesics.

In addition to its anti-inflammatory and analgesic effects, *T. involucrata* exhibits notable antioxidant activity (Romero-Benavides *et al.*, 2023). Flavonoids, known for their potent antioxidant properties, constitute a significant portion of *T. involucrata*'s phytochemical profile, contributing to its free radical-scavenging capacity. Moreover, the ethno-pharmacological significance of *Tragia* species, including *T. involucrata*, has spurred research endeavors to unravel their biological activities and phytochemical composition. Comprehensive reviews provide invaluable insights into the traditional uses, chemical constituents, and pharmacological potentials of *Tragia* species, laying the groundwork for further exploration (Reddy *et al.*, 2017).

MATERIALS AND METHODS

Plant Collection and Preparation

T. involucrate leaves were harvested in the months of May and June 2011 from a plant-grown wild in the Haffkine Institute for Training, Research, and Testing (Parel) garden. The plant was authenticated at Navsari Agriculture University, Navsari, Gujarat. The leaves were chopped and dried in the shade. A mechanical grinder was used to form a coarse powder. The resulting powder was kept in airtight bottles, out of direct sunlight, in a cold and dry location to avoid any moisture.

Extraction with Solvents

Using Soxhlet's extraction method, phytoconstituents from *T. involucrata* were extracted using a series of organic solvents, including hexane, dichloromethane (DCM), methanol, and distilled water. Rota-vaporization was also used to create methanol cold extract. The extraction was carried out in succession using hexane-dichloromethane-methanol (non-polar to polar). The solvent was heated at different temperatures depending on the solvent to be extracted on the heating mantle to reflux (Hexane: 68°C; Dichloromethane: 40°C; Methanol: 62°C; Water: 100°C).

The number of cycles was recorded and the extraction was continued until the solvent in the extraction tube's siphon turned colorless. Following the extraction, distillation was used to recover the solvent. The temperature changed based on the boiling point of the solvent in use. The extracts of *T. involucrata* were diluted in water and used for pharmacological tests and additional analysis.

To prepare Cold Methanol Extract, 100 mL of Methanol and 30g of powdered leaves of *T. involucrate* were added. The solvent was kept for incubation at room temperature for 24 hrs. Then, the solvent was filtered and stored in the freezer during the experimental period. The remaining powder was again dissolved in 100 mL of methanol and repeated 3-4 times. Once the extracts were obtained by using the solvent, the same was recovered by an artificially prepared rotary evaporator at 30-35°C.

Assessment of phytochemical activity

For phenolic compounds, 2 mL of 3% extract and 2 mL of 1% $FeCl_3$ (Ferric chloride) were added in 0.5 N HCl (Hydrochloric acid). The deep blue coloration of the solution formed indicated the presence of phenolic compounds in the extract. For terpenoids, 2 mL of chloroform was added to 0.5 g each of the extract, followed by 3 mL of concentrated H_2SO_4 carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids. For flavonoids, two tests were performed; sodium hydroxide test included dissolving 2 mL of the extract in 10% aqueous NaOH solution and filtered to form yellow coloration which if turns colorless on the addition of diluted HCl, indicates presence; in the Sulphuric test, the sample was dissolved in strong sulphuric acid, and the disappearance of the yellow color indicated the presence of flavonoids. For tannins, 15% ferric chloride was added to the water extract, and the color that emerged was noted. A blue tint represents condensed tannins, while a green tint shows hydrolysable tannins. For saponins, to 0.5 g of extract, 5 mL of distilled water was added in a test tube, and the solution was shaken vigorously and observed for a stable persistent froth. The emulsion formation was

checked after vigorously shaking three drops of olive oil to the froth. Finally, for alkaloids, 0.5 g of the extract and 5 mL of 1% aqueous HCl were swirled over a water bath, and 3 mL of the filtrate was filtered. After adding a few drops of freshly made Dragendorff's reagent, the mixture was monitored to see if an orange-to-brownish precipitate formed.

High-Performance Thin Layer Chromatography (HPTLC) "CAMAG Linomat 5" HPTLC and 0.2 mm thick silica gel plates were used for the experiment. The Anchrom Laboratory protocol was followed for the experiment. The silica gel was used in a stationary, and mobile phase for different chemical constituents. For flavonoids, Ethyl acetate: Formic acid: Glacial acetic acid: Water (10: 0.5: 0.5: 1.3) and alkaloids Toluene: Ethyl acetate: Diethyl amine (7: 2: 1).

The extracts were spotted on silica gel plates. 15 µL of each dried extract was applied and was individually developed in solvent systems 1 and 2. Sulfuric acid was used to detect flavonoids, Dragendorff's reagent was used to identify alkaloids, and derivatization was used to attach a chromophore to molecules that are not visible in ultraviolet light. The image was obtained by CAMAG Visualizer.

IN-VITRO STUDIES

MTT assay for cell viability

Porcine-stable kidney cells were seeded in a 96-well plate (5×10^3 cell/ 100 µL) with Dulbecco's Minimal Essential Medium (DMEM) complete media and were incubated overnight at 37°C in a humidified incubator kept at 5% CO2. Test chemicals, such as Negative control (DMEM), Positive control (DMSO) and Vehicle control, were added to the plate at a final amount of 100 µL per well. The plate was incubated overnight at 37°C. The media was discarded and 100 µL of MTT dissolved media was added in each well. The plate was incubated at 37°C for 3 hrs, after which 100 µL of DMSO was added to each well. The absorbance was measured at 550 nm using a plate reader, and results were calculated as percentage viability.

$$\text{Viability (\%)} = \text{O.DT} / \text{O.DC} \times 100$$

Trypan blue dye exclusion test

About 4 mL of RPMI media was injected intraperitoneally into Wistar rats for macrophage cell extraction. The lavage was removed from the rat, keeping the needle inside the peritoneal cavity. About 0.4% trypan blue was mixed with the cell suspension at a 1:1 ratio and incubated for 3 mins at room temperature. A drop of the mixture was added to a hemocytometer, and the unstained (viable) and stained (non-viable) cells were counted individually using a microscope. The formula to calculate the percentage of viable cells is as follows-

Viable cells (%) = Total number of viable cells per mL of aliquot / Total number of cells per mL of aliquot × 100

HRBC membrane stabilization assay

The blood of healthy volunteers was drawn with sterilized Alsever solution (anti-coagulant). Upon centrifugation at 3000 rpm, the packed cells were washed with isosaline (0.9%, pH 7.2) and mixed to prepare a 10% (v/v) solution. The test drug, 1 mL of phosphate buffer (0.15 M, pH 7.4), 2 mL of hyposaline (0.36%), and 0.5 mL of HRBC solution were all included in the assay mixture. The standard drug was 50 mg of diclofenac sodium. The negative controls were 1 mL isotonic saline-free red blood cells (Control I) and 1 mL extract solution-free red blood cells (Control II). Furthermore, 2 mL of distilled water instead of hyposaline was used as the positive control. After 30 mins. of incubation at 37°C, the supernatant was separated, and readings were obtained at 560 nm using a spectrophotometer.

By assuming that 100% of the hemolysis would occur in the presence of distilled water, the percentage of hemolysis and protection was calculated using the below formula-

Percentage of hemolysis = O. D. of test sample /O. D. of control × 100

Percentage Protection = 100 – O. D. of test sample / O. D. of control× 100

Assessment of Antioxidant activity by DPPH radical scavenging assay

The stock solution of the methanol plant extract (5 mg/mL) was prepared in methanol from which a working concentration of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg/mL was prepared with a total volume of 1 mL using methanol. DPPH (0.0002% w/v) was prepared in methanol and was added to the diluted plant extract, and the reaction was incubated in the dark for 30 mins. After 30 mins, the absorbance was taken at 517 nm using methanol as blank and DPPH as control. Butylated Hydroxyl Toluene (BHT) was used as a standard against which the graph of the sample was plotted. The percentage inhibition of DPPH activity was calculated using the formula-

$$\text{Percentage Inhibition} = \frac{\text{O. D.}_{\text{Test}} - \text{O. D.}_{\text{Control}} \times 100}{\text{O. D.}_{\text{Control}}}$$

IN VIVO STUDIES

Experimental Animals

Wistar Rats (100-200 g) of either sex was procured from a CCSEA-authorized vendor as per approval from the Institutional Animal Ethics Committee (IAEC) [HITRT/IAEC/13/2011]. The animals were housed in polypropylene cages (38 × 23× 10 cm), with no more than six ani-

mals per cage, in a well-ventilated room with a 12:12 light/dark cycle. Drinking water and standard pellet feed were given to the animals ad libitum. The animals were acclimatized to the laboratory environment one week before the experiment.

Acute oral toxicity studies

An acute toxicity study was carried out for the aqueous extract of the *T. involucrata* plant using the Fixed Dose Method (OECD Guideline No. 423) using three female Wistar rats per step. The rats were given a single dosage of the plant extract at 2000 mg/kg body weight through the oral route of administration. Following the dosing, the animals were observed for clinical signs of toxicity and mortality constantly for 4 hrs and then every day for 14 days. The animals' body weights were recorded on the 1st, 3rd, 5th, 10th, and 14th days.

Carrageenan-induced rat paw edema

Wistar rats (100-200 g) were used for the study with either sex divided into five groups of six animals each. Before study initiation, the individual body weight of the animals was determined to establish the appropriate treatment dosage.

Group I (negative control) received vehicle only (WFI water). Aqueous extract of T. involucrate (AETI) was administered orally to the animals of groups II, III, and IV at doses of 100, 200, and 300 mg/kg body weight, respectively. Group V (positive control) received diclofenac sodium at 50 mg/kg body weight. Thirty minutes after oral drug administration, edema was induced. Subcutaneous injection of 100 µL of 1% carrageenan solution (w/v solution in WFI water) was used to cause paw edemas in the right hind paw's sub-plantar aponeurosis. Measurements were taken by Vernier caliper immediately prior to the carrageenan infusion and after 30 mins, followed by different time intervals (60, 90, 120, and 180 mins). The mean increase of the hind paw volumes (or thickness) of rats given the plant extract were calculated and compared with that of the control rats. Edema volumes were calculated in groups treated with test substances (Vt) and control (Vc). The percentage inhibition was calculated by using the formula-

$$\text{Percentage Inhibition} = \frac{Vc - Vt \times 100}{Vc_1}$$

Where Vc = Edema volume of Control rats and Vt = Edema volume of Test rats.

Analgesic activity: Acetic acid-induced writhing

Wistar rats were used to test the analgesic activity of the samples by inducing writhing in them using acetic acid. Five groups, each consisting of six individuals of both sexes. Group I (negative control) received vehicle only (WFI water). Aqueous extract of T. involucrate (AETI) was administered orally to the animals of groups II, III, and IV at doses of 100, 200, and 300 mg/kg body weight, respectively. Group V (positive control) received diclofenac sodium at 50 mg/kg body weight. After 30 mins of dosing, each animal received an intraperitoneal injection of 0.7% acetic acid at the dose of 10 mL/kg b.w.. Up to 30 mins after the acetic acid injection, the number of writhes (abdominal constrictions) was counted. An anti-nociceptive reaction was defined as a marked decrease in writhing in the test animals when compared to the control group. Using the following formula, the percentage inhibition of abdominal constrictions was determined. 01

$$\text{Percentage Inhibition} = \frac{\text{Mean no. of writhes (Control)} - \text{Mean no. of writhes (Test)} \times 100}{\text{Mean no. of writhes (Control)}}$$

Statistical Analysis

The data was expressed as Mean ± Standard Deviation (S.D.). One Way Analysis of Variance (ANOVA) was used to determine the significant differences between the groups. Dunnett's post-hoc test was carried out to compare the treatment groups with the control using computerized Graph Pad Instat version 3.05 (Graph Pad Software, U.S.A.). P values less than 0.05 ($P < 0.05$) were considered significant. Regarding analgesic activity, the outcomes were compared with the vehicle control group. Statistical significance is expressed as (i) $P < 0.05$, (ii) $P < 0.01$ and (iii) $P < 0.001$

RESULTS

Soxhlet Extraction

The percentage extractive value of the plant extract in each solvent is represented in Table 1.

Table 1: Extractability percentage of sample (*T. involucrata*)

Solvent used	Boiling Point (°C)	Weight of raw material (g)	Weight of beaker (g)	Weight of beaker + extract (g)	Weight of extract (g)	Percent extractive value (%)
n-Hexane	68	60	48.337	50.78	2.443	4.01
Dichloromethane	40	60	27.62	29.260	1.64	2.70
Methanol	62	60	27.743	29.65	1.907	3.17
Distilled water	100	60	26.251	29.221	2.97	4.95
Cold Methanol	65	30	48.337	50.78	2.443	4.01

Table 2: Phytochemical screening of plant extracts

Test for compound	DCM	METI	METI (cold)	AETI
Phenolic compounds	+	+	+	-
Terpenoids	+	-	-	+
Flavonoids	+	+	+	+
Alkaloids	-	-	-	-
Tannins	-	+	+	+
Saponins	+	+	+	+

Preliminary Phytochemical screening

Dichloromethane extract (DCM), Methanol extract Soxhlet (METI), Methanol extract Cold (METI cold), and Aqueous extract (AETI) underwent preliminary phytochemical screening. The results of preliminary phytochemical tests show the presence of phytoconstituents in different extracts in Table 2. Alkaloids were absent from all of the extracts, although flavonoids and saponins were detected in each. Phenolic compounds were absent in AETI, terpenoids were absent in METI, and tannins were present in all extracts except in DCM.

HPTLC analysis

The presence of flavonoids and alkaloids in the DCM, AETI and METI leaf extracts was determined by HPTLC analysis. The instrument used was CAMAG Linomat 5". It was executed by Anchrom Test Lab Pvt. Ltd., Mumbai. According to the HPTLC analysis, no alkaloids were detected in all the extracts evaluated on the TLC plate. In contrast, flavonoids were present in DCM, AETI, and METI extracts **(Fig. 1A)**.

MTT assay for cell viability

The test showed purple formation production in lower concentrations, but the color formation was too complex to be observed at higher concentrations. This was caused by the plant sample's significant color interference. Hence, the cell cytotoxicity at higher concentrations was difficult to calculate. Another test, the Trypan blue dye exclusion test, was performed to verify the viability of the cells at different doses **(Fig.1B)**.

Trypan blue dye Exclusion Test

In the protocol presented here, a viable cell will have a clear cytoplasm **(Fig. 1B)**. The test carried out showed a positive

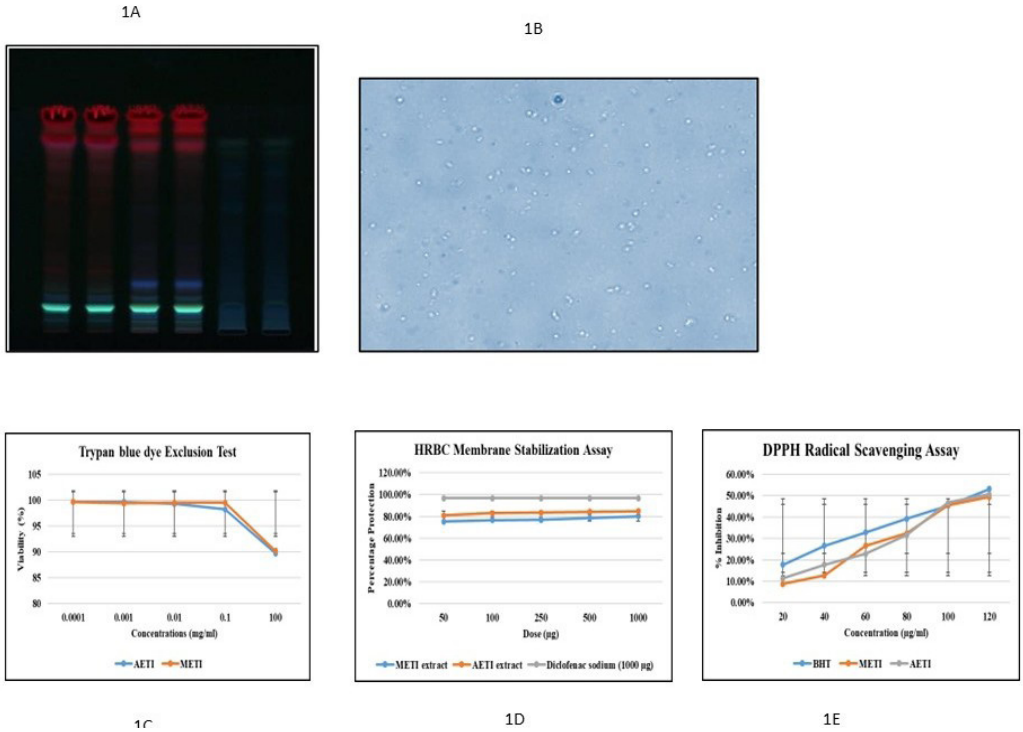

Fig. 1A: Presence of flavonoids in DCM, AETI, and METI extracts; Fig. 1B: Cell viability by Trypan blue dye exclusion (Cells marked are viable); Fig. 1C: Percentage viability by Trypan blue dye exclusion test; Fig. 1D: HRBC membrane stabilization activity of AETI and METI; Fig. 1E: Percentage inhibition of radicals by extract

result. In this case, as seen in Graph 1, AETI and METI showed significant viability at varying concentrations. AETI showed 89.77% viability at 100 mg/mL, whereas METI showed 90.25% viability. Hence, the extracts were found to be non-cytotoxic **(Fig. 1C)**.

HRBC Membrane Stabilization Assay

Compared to the reference drug Diclofenac sodium, the two plant extracts, AETI and METI, had notable activity in *in vitro* anti-inflammatory activity screening, as seen in Graph 2. Among the two plants' extracts, AETI possessed better activity than METI with a percentage protection of 84.81% at 1000 µg/mL for *T. involucrata.* At 1000 µg/mL, the standard drug Diclofenac sodium produced 96.80% protection **(Fig. 1D)**.

DPPH Radical Scavenging Assay

Up to a concentration of 120 µg/mL, the *T. involucrata* aqueous (AETI) and methanol (METI) extracts demonstrated a promising concentration-dependent free radical scavenging efficacy of DPPH. Scavenging activity was higher in AETI than in METI. The reference standard, butylated hydroxytoluene (BHT), also showed significant radical scavenging potential up to a concentration of 120 µg/mL **(Fig. 1E)**.

The DPPH activity was interpreted using IC_{50} values. The concentration of the sample needed to scavenge 50% of DPPH free radicals is indicated by the IC_{50} values. The half maximal inhibitory concentration (IC_{50}) of Aqueous extract, Methanol extract, and BHT was found to be 146.1 µg/mL, 149.9 µg/mL, and 110.9 µg/mL, respectively.

IN-VIVO STUDIES

Effect of AETI on acute toxicity studies

Following a 2000 mg/kg body weight dose, the animals were monitored closely for 6 hrs. During this time, none of the animals displayed typical toxicity-related signs, such as convulsions, ataxia, diarrhea, or increased diuresis. Also, after 24 hrs of dosing, no toxic signs and symptoms were observed.

Since no mortality was observed, it can be concluded that the plant extract's LD50 was higher than the dosage given to the animals. It is also possible to conclude that a dose below 2000 mg/kg body weight would be safe for the study. Hence, the doses selected for the main study were less than 2000 mg/kg body weight.

Carrageenan-induced rat paw edema

Table 5 illustrates the effect of *T. involucrate* aqueous extract (AETI) on carrageenan-induced rat paw edema. During the study, the control animals progressively exhibited increasing paw volume in response to carrageenan injection. The anti-inflammatory activity became noticeable after 60 mins at a dose of 200 mg/kg body weight. In contrast, the same activity became evident at 90 mins at the concentration of 300 mg/kg body weight. The oral administration of 100 mg dose produced a low significant effect by 60 mins. However, doses of 200 and 300 mg of the preparation produced a significant ($p< 0.05$,$p < 0.01$, and $p<0.001$) inhibition of the rat paw edema when compared to control. Compared to the control group, the maximum paw edema was seen at dosages of 200 and 300 mg/kg body weight; nevertheless, this was less than the dose reported with Diclofenac sodium.

Table 3: Anti-inflammatory activity of AETI and Diclofenac drug on rats using carrageenan-induced paw edema

Treatment	Pre-treatment	Post-treatment					
	0 mins	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
G5 Control (Distilled water)	0.5 ± 0.05	0.7 ± 0.06	0.8 ± 0.05	0.9 ± 0.08	0.9 ± 0.00	0.9 ± 0.05	0.9 ± 0.06
G4 Standard (Diclofenac sodium)	0.5 ± 0.05	0.6 ± 0.04* (14.28%)	0.7 ± 0.05*** (14.28%)	0.7 ± 0.09*** (22.22%)	0.6 ± 0.04* (33.33%)	0.6 ± 0.05* (33.33%)	0.5 ± 0.05 (44.44%)
G1 100 g/kg b. w.	0.5 ± 0.9	0.6 ± 0.08 (14.28%)	0.7 ± 0.08*** (14.28%)	0.6 ± 0.04 (33.33%)	0.6 ± 0.04 (33.33%)	0.6 ± 0.05 (33.33%)	0.6 ± 0.05 (33.33%)
G2 200 g/kg b. w.	0.5 ± 0.05	0.6 ± 0.05* (14.28%)	0.7 ± 0.05*** (14.28%)	0.7 ± 0.08*** (22.22%)	0.6 ± 0.04* (33.33%)	0.6 ± 0.05* (33.33%)	0.5 ± 0.04 (44.44%)
G3 300 g/kg b. w.	0.6 ± 0.05	0.7 ± 0.04*	0.8 ± 0.04***	0.7 ± 0.04* (22.22%)	0.6 ± 0.08 (33.33%)	0.6 ± 0.06 (33.33%)	0.6 ± 0.05 (33.33%)

*Results are expressed in Mean ± SD (n=6) significance levels * P<0.05, ** P < 0.01, and ***P<0.001 as compared with the respective control.*

Figure 2: Rat paw edema before and after treatment

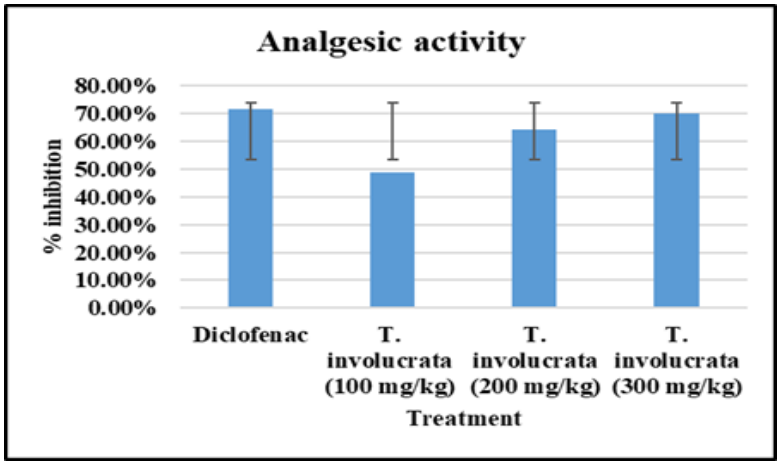

Figure 3: Analgesic effect on acetic acid-induced nociception

Analgesic activity: Acetic acid-induced writhing

Graph 4 represents the results from the rats' acetic acid-induced writhing test. Acetic acid administered intraperitoneally induced writhing reactions in rats; at doses of 200 and 300 mg/kg body weight, the AETI inhibited such responses. The mean number of writhes at these doses was significantly lower than that of the control group. At a dosage of 300 mg/kg body weight, the nociceptive response was maximally inhibited by 70.23%. Diclofenac sodium produced a significant protective effect at a 50 mg/kg body weight with a 71.40% protection.

DISCUSSION

Certain herbs are said to relieve pain and inflammation in the Indian medical system. The therapeutic repute that has been asserted needs to be scientifically confirmed. *T. involucrata* is one such plant and its leaves were used in the current investigation. Using specific in vitro and in vivo experiments, the anti-inflammatory, analgesic, and antioxidant properties of *T. involucrata* leaf extracts were evaluated. Both endogenous and exogenous reactive oxygen species (ROS) have been linked to the pathophysiology of a number of diseases, including diabetes, cancer, atherosclerosis, arthritis, and the aging process. Inflammation is a multifaceted process, and reactive oxygen species (ROS) play an essential role in the development of inflammatory disorders. Antioxidants that are able to scavenge ROS are, therefore, predicted to help alleviate such diseases (Ilavarasan *et al.*, 2004).

Preliminary phytochemical analysis of the different extracts showed the presence of tannins, phenolic compounds, saponins, and flavonoids. Numerous researchers have reported the analgesic and anti-inflammatory properties of flavonoids and tannins; therefore, the extract's analgesic and anti-inflammatory actions can be attributed to the flavonoids and tannins separately or in combination. (Musa *et al.*, 2009).

The in vitro HRBC membrane stabilization method has been used to test these extracts' anti-inflammatory properties. Since the components of the HRBC membrane resemble those of the lysosomal membrane, the ability of a medication to inhibit hypotonicity-induced HRBC membrane lysis is used to gauge its anti-inflammatory effect (Mounnissamy *et al*., 2008). The chemical components found in these extracts, including flavonoids and tannins, which are widely known for their anti-inflammatory properties, may operate via a similar process. METI and AETI extract possess anti-inflammatory activity, showing maximum percentage inhibition of hemolysis of HRBC membrane at dose 1000 µg, i.e., 80.25% by METI extract and 84.81% by AETI as compared to Diclofenac sodium showing 96.80% percentage protection.

The ability of the extracts to scavenge the synthetic DPPH was used to assess their free radical scavenging activities. This assay's odd number of electrons gave useful data on the chemicals' reactivity with stable free radicals. The half maximal inhibitory concentration (IC50) of Aqueous extract, Methanol extract, and BHT were found to be 146.1 µg/mL, 149.9 µg/mL, and 110.9 µg/mL, respectively. When compared to methanol extract, the aqueous extract exhibits significantly more reducing activity against stable free radicals. This biological activity of the experimental plant extracts may be due to the presence of various phytochemical compounds in plants, and these compounds may jointly or independently show antioxidant activity.

The AETI and METI extracts were tested by MTT assay for its cytotoxicity. These extracts were showing false positive results; hence, an alternative method was used, i.e., the Trypan blue dye exclusion test. The dye exclusion test can determine the amount of viable cells in a cell solution. The principle behind this is that live cells do, whereas dead cells lack cell membranes that block certain dyes like propidium, eosin, or trypan blue. It was found that the extracts had no cytotoxic properties.

Rats given an aqueous extract of *T. involucrata* at a dose of 2000 mg/kg body weight did not exhibit any significant toxic effects. Even after giving the extract to the Wistar rats for 14 days, no mortality was observed with a dose of 2000 mg/kg body weight.

Carrageenan-induced paw edema was taken as a prototype of the exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals, and necrotized cells activate the different mediators through a common trigger mechanism (Das *et al*., 2010). When compared to normal diclofenac sodium, the highest inhibitory impact on carrageenan-induced inflammation during a 2-hour period was reported at a concentration of 200 mg/kg of AETI extract ($P<0.05$, $P<0.01$, $P<0.001$). Extracts significantly inhibited the edema formation in the rat paw edema model generated by carrageenan from *T. involucrata*. This effect began to manifest within the first hour. It persisted throughout all phases of inflammation, indicating that the prostaglandin production pathway and other inflammatory mediators may be the primary mechanisms of action of the examined extracts. The extract thus effectively suppressed the inflammation. The aqueous extract was found to possess tannins, saponins, and flavonoids. Therefore, the existence of these chemical ingredients may be responsible for the plant's anti-inflammatory activity. Flavonoids have been found to have anti-inflammatory properties and also inhibit the prostaglandin manufacturing enzyme, namely the endo-peroxides (Das *et al*., 2010). *T. involucrata* extracts may have analgesic properties because they were highly effective in treating rat paw edema models generated by carrageenan.

Because the acetic acid-induced abdominal constriction approach is so sensitive and can identify anti-nociceptive effects of compounds at dose levels that may look inactive in other methods, it is frequently employed to assess peripheral anti-nociceptive activity. Local peritoneal receptors may partially mediate the abdominal constriction reaction (Mbiantcha *et al*., 2011). The analgesic efficacy of the aqueous extract was evaluated using the abdominal constriction method generated by acetic acid. As for the visceral pain model, which involves writhing in the abdomen in response to acetic acid, the results indicate that a dose of 300 mg/kg body weight had a considerable analgesic effect. When the extract concentration increased from 100 mg/kg to 200 mg/kg to 300 mg/kg, the extract's percentage of inhibition increased. The extract concentration of 300 mg/kg body weight shows a significant inhibition of 70.25%. Whereas the inhibitory effect produced by diclofenac sodium at 50 mg/kg body weight doses was 71.42%. This may be partially explained by its anti-inflammatory properties since, in the visceral pain model, the processor releases arachidonic acid through prostaglandin production and cyclooxygenase, which contributes to the nociceptive pathway (Adedapo *et al*., 2009). As a result, the outcomes of the writhing test and the carrageenan-based oedematogenic test are quite comparable.

CONCLUSION

The extracts were found to be non-cytotoxic in nature as their percentage viability was found to be above 50%. The findings of the study indicate that, at a dose level of 1000 µg/mL, the aqueous extract of *T. involucrate* protects the HRBC membrane from hyposaline-induced lyses by around 85%, as opposed to 100% lysis induced in control. Aqueous extract (AETI), out of all the extracts, demonstrated significant anti-inflammatory activity in a concen-

tration-dependent manner. When compared to methanol extract, the aqueous extract exhibits significantly more reducing activity against stable free radicals. Studies on acute toxicity demonstrated that AETI is not toxic. Until the end of the study period, none of the doses that were chosen showed signs of fatality or adverse reactions. The extract showed analgesic activity, as seen in both Paw edema and writhing studies.

ETHICS APPROVAL

The animal procurement and use was approves by the Institutional Animal Ethics Committee (IAEC) and the approval number is HITRT/IAEC/13/2011.

AUTHORS CONTRIBUTION

The authors' contribution as follows-

Dr. Mrunal Ghag Sawant: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing- Original Draft, Writing- Review and Editing, Visualization, Supervision, Project Administration.

Mr. Anurag Killedar: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing- Original Draft, Writing- Review and Editing, Visualization, Project Administration.

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