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Comparative Assessment on the Analgesic, Anti-Inflammatory, And Acute Oral Toxicity Profile of *Trachyspermum Ammi* Seeds Extracted with Different Polarity Solvents

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KEYWORDS

Trachyspermum ammi, anti-inflammatory, analgesic, paw edema, granuloma, indomethacin, dexamethasone

ABSTRACT:

Trachyspermum ammi, commonly known as 'ajwain' or 'caraway' (family: Apiaceae), is utilized for various purposes in both dietary practices and traditional medicine. The current study was formulated to investigate the anti-inflammatory, analgesic, and toxicity profiles of T. ammi extracts, using different polarity solvents, including petroleum ether (TAPE), chloroform (TACE), and hydroalcoholic extract (TAHE), using experimental animals. The anti-inflammatory property of the test compounds was screened using carrageenan-induced paw edema (CIPE) and cotton pellet-induced granuloma methods. Central and peripheral pain-relieving activity was analyzed utilizing hot plate and acetic acid-induced writhing test, respectively. Toxicological profiling was conducted using the Up-and-Down Procedure method outlined in Test No. 425: Acute Oral Toxicity. Study findings revealed that TAPE, TACE, and TAHE at 2000 mg/kg were determined to be safe. The CIPE and cotton pellet-induced granuloma models revealed a notable ($p<0.05$) anti-inflammatory activity. It also demonstrated a positive impact on serum and liver tissue biochemical parameters, including SGOT, SGPT, total proteins, and acid phosphatase. Furthermore, the plant extracts displayed both central and peripheral analgesic effects, as evidenced by a significant ($p<0.05$) rise in latency time in the hot plate test and inhibition in the number of writhes, respectively. In conclusion, various polarity solvent extracts of T. ammi, namely TAPE, TACE, and TAHE, exhibited significant dose-dependent anti-inflammatory and anti-nociceptive effect. Notably, TAHA displayed a more pronounced effect in reducing inflammation and nociceptive pain compared to TACE. Although TAPE also possesses anti-inflammatory and pain-relieving properties, the effects were observed to be less pronounced compared to TACE.

INTRODUCTION

Trachyspermum ammi, (Family: Apiaceae) commonly known as ajwain or carom seeds, is an aromatic spice native to the Indian subcontinent. These small, oval-shaped seeds are used in culinary applications, particularly in Indian and Middle Eastern cuisines, for their distinctive flavor. Ajwain has a pungent and slightly bitter taste, often described as a mix of thyme and cumin [1]. In addition to its culinary uses, *T. ammi* is also known for its potential medicinal properties. It has been traditionally used in Ayurvedic and other traditional medicine systems for its fungicidal, antimicrobial, anti-aggregatory effects digestive, anti-inflammatory, antispasmodic anthelmintic, carminative, laxative, and stomachic and carminative properties. Ajwain is often employed in various forms, including as whole seeds, ground powder, or as an essential oil [2].

Inflammation is a defensive reaction of the body, triggered by factors such as cell damage, tissue collapse, exposure to injurious synthetic materials, burns, or microbiological agents. The indicators of inflammation

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include redness, soreness, warmth, and ache [3]. Under certain circumstances, inflammation, which is typically a defensive reaction of the body, can become harmful, potentially giving rise to serious pathological conditions. Chronic inflammation is recognized as a key component in the development of a range of diseases, including diabetes, heart conditions, cancer, digestive issues, autoimmune disorders, and neurodegenerative conditions [4].

The pain syndrome demonstrates an inflammatory profile marked by the presence of distinct inflammatory mediators linked to that specific pain syndrome. In cases of inflammation resulting from mild tissue damage or infection, like postoperative pain, toothache, or cystitis, afferent fibers are triggered by lower intensity stimuli, resulting in pain that varies in quality and may persist for a longer duration [5, 6]

Indeed, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and opiates have traditionally been utilized in the classical treatment of pain and inflammation. However, long term use of these anti-inflammatory drugs leads to sever side effects such as renal dysfunction, constipation, possible dependence, peptic ulcer, gastrointestinal disturbances, respiratory depression, and bleedings [7]. Opioid analgesics are linked to numerous undesirable side effects and toxicities, which may include hearing loss, physical dependence, addiction, drowsiness, pruritus, tolerance, nausea, vomiting constipation, disruptions in hormonal homeostasis, and respiratory problems [8].

In recent years, there has been a growing inclination towards the discovery of new anti-inflammatory and analgesic drugs, with a particular emphasis on natural sources and medicinal plants. The utilization of plant extracts and isolated phytoconstituents has significantly contributed to the discovery of new drugs especially in pain and inflammation. Numerous studies have documented the significant anti-inflammatory and analgesic activities of secondary metabolites from medicinal plants, such as alkaloids polyphenols, terpenoids, and flavonoids [9].

Globally, there is an increasing demand for traditional system of medicine for the treatment of various diseases. In India, approximately 80% of the rural population relies on medicinal herbs or indigenous systems of medicine. The Indian herbal industry utilizes nearly 960 plant species, contributing to a turnover exceeding rupee 80 billion [10, 11]. While medicines of plant origin are generally expected to have low toxicity, certain medicinal plants used in traditional medicines have been reported to exhibit toxic effects [12].

Several scientific reports on *T. ammi* have explored its potential for anti-inflammatory and analgesic studies. However, as of now, there is a lack of research specifically comparing extracts from different polarity solvents to assess anti-inflammatory, analgesic, and toxicological profiles. The extraction of pharmacologically active phytonutrients can vary with solvents of different polarity, influencing overall biological activity. In the present study, petroleum, chloroform, and hydroalcoholic extracts were comparatively evaluated for their anti-inflammatory, analgesic, and acute oral toxicity profiles using experimental animals. Anti-inflammatory activity was examined using the Carrageenan-Induced Paw Edema (CIPE) model and the cotton pellets induced granuloma pouch model. Central and peripheral analgesic activity was evaluated through the hot plate and acetic acid induced writhing tests, respectively. The toxicological profile was assessed using the Up-and-Down Procedure (Test No. 425) for acute oral toxicity.

MATERIALS AND METHODS

Chemicals and reagents

λ-Carrageenan was obtained from Sigma Aldrich, St. Louis, MO, USA. Various other chemicals and reagents, including normal saline (sodium chloride injection IP 0.9% w/v), indomethacin, dexamethasone tablets, and Tramadol injection, were sourced from the local market. For biochemical analysis, chemicals and reagents of the highest commercial quality were employed.

Collection and authentication of plant material

The seeds of the *T. ammi* plant were obtained from the local market. The sample underwent identification at the Agarkar Research Institute, an autonomous body under the Department of Science and Technology, Government of India, located in Pune. A voucher specimen (AUTH 22-158) was subsequently deposited in the Herbarium of the Agarkar Research Institute for future reference.

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Preparation of plant extracts

The dried powdered seeds of the *T. ammi* plant underwent extraction using petroleum ether (60–80°C) and successive extraction with chloroform and 70% ethanol (ethanol: water; 7:3 ratio) in a soxhlet extractor. The obtained extracts were concentrated through solvent recovery and dried to completeness at 50°C in a hot air oven. Extractive values were determined for all extracts, accurately labeled, and then stored in sealed containers.

Experimental animals

Male Sprague Dawley Rats and Swiss albino mice were procured from Global Bioresearch Solution Pvt Ltd., Bhor, Pune. These animals were accommodated in polypropylene cages under controlled conditions, including a room temperature of 22±1°C, a relative humidity of 60%-70%, and a 12:12-hour light and dark cycle. The housing was provided in an animal facility (615/PO/Re/S/2002/CPCSEA; dated on 11th June 2002). All procedures for the study adhered strictly to the protocols set by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. The experimental protocols for animal studies (RDCOP/Pcol-11/IAEC/2022-23/11) were approved by the Institutional Animal Ethical Committee (IAEC) at Rajgad Dnyanpeeth's College of Pharmacy, Bhor, Dist. Pune – 412206, India, before the commencement of the experiment.

Phytochemical analysis

The examination of various phytoconstituents in the seeds of *T. ammi* was conducted using petroleum ether, chloroform, and hydroalcoholic extracts, following the procedure outlined by Khandelwal [13].

Acute Oral Toxicity

An investigation into acute oral toxicity was carried out using the Up-and-Down procedure as described in Test No. 425 [14]. Healthy female albino mice weighing 30-35 g were selected and acclimatized for one week under standard conditions. The limit test was conducted at 2000 mg/kg *p.o.* as a single dose, with mice fasting overnight before dosing but having access to water *ad libitum*. A single mouse from each group received the dose of the vehicle or the respective test compound. Observations were closely monitored during the initial 0.5 hour, followed by continuous monitoring for 4 hours. Feed was provided 2 hours post-dosing. Once the treated mouse survived, the same dose was administered to all other animals. A similar procedure was followed for a vehicle-treated control group (0.25% Na-CMC). The various animal groups were carefully observed for potential toxic effects within the initial 6 hours and subsequently at regular intervals throughout a 14-day period. Surviving mice were continuously observed for any toxic reactions, and their body weights were consistently monitored. After 14-days, blood was collected through the retro-orbital plexus method for hematological analysis. Blood serum was separated for biochemical evaluations. Vital organs were excised post-euthanasia via cervical dislocation, washed with normal saline, and weighed.

Assessment of Anti-inflammatory activity:
Carrageenan induced rat paw edema model

The evaluation of anti-inflammatory activity employed the carrageenan-induced rat paw edema assay according to the modified method of Balkrishna A *et al*. [15] and Salem S *et al*. [16]. Edema was induced by injecting 0.1 mL of a 1% freshly prepared aqueous carrageenan solution into the right hind paws of the animal. Prior to carrageenan injection, animals were treated with a single dose of vehicle or indomethacin (10 mg/kg), TAPE, TACE, or TAHE administered 60 minutes in advance. All the test compounds viz. TAPE, TACE, or TAHE were treated at 100, 200 and 400 mg/kg dose levels. Paw volume was measured at 0 (before carrageenan injection) and 1, 2, 3, 4, and 5 hours post-injection. Paw edema was calculated as the difference between the paw volume at "0 hour" and the respective hours.

Cotton pellet induced granuloma pouch model:

The modified methods of Nair V *et al*. [17] and Wilches I *et al.* [18] were followed to execute cotton pellet-induced granuloma pouch model in rats. Wistar rats were divided into distinct groups, each comprising six rats. Pellets, constructed from absorbent cotton wool and weighing 20±1 mg, were sterilized at 120ºC for 2 hours in a hot air oven. The abdominal area was carefully shaved and cleaned with 70% ethanol. In anesthetized rats, two cotton pellets were subcutaneously implanted in the abdominal region. Vehicle, dexamethasone, or test compounds were administered orally throughout the 7-day experimental period. On the 8th day post-implantation, animals were anesthetized, and the pellets were dissected, dried at 50ºC for 22 hours, and weighed. The weight of cotton pellets (wet and dry) for all groups of animals was

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recorded. Subsequently, the exudate weight, granuloma weight, and biochemical parameters were calculated.

At the end of the experiment, blood samples were collected from the animals via the retro-orbital plexus, centrifuged, and serum was obtained. The levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and total protein in the serum were then estimated. Following humanely sacrifice of animals, liver tissue was excised, cleaned with normal saline, homogenized using phosphate buffer saline, and the levels of GOT, GPT, total protein, and alkaline phosphatase in the liver tissue were analyzed.

Assessment of Analgesic activity:

Hot plate test

The hot plate test, modified from the procedure outlined by Acharya et al. [15] and Franzotti EM *et al.* [19] was employed to assess thermal hyperalgesia with slight adjustments. Mice were positioned within the perspex cylinder of the hot plate apparatus (Ugo Basile, Italy) maintained at 55.0 ± 0.5°C. The time taken for a discomfort behavior (such as paw licking or jumping) was recorded as the response latency at 0 (before drug treatment), 30, 60, 90, and 120 minutes. This test was conducted one hour after the administration of vehicle, tramadol (40 mg/kg; i.p.), TAPE (100, 200 & 400 mg/kg), TACE (100, 200 & 400 mg/kg), or TAHE (100, 200 & 400 mg/kg). A maximum cut-off time of 18 seconds was established to prevent potential accidental paw damage.

Acetic acid (AA) writhing test

To evaluate the peripheral analgesic properties of various extracts derived from *T. ammi* seeds, the AA-induced writhing test was utilized following the protocol outlined by Ayanaw MA *et al.* [20] and Olukunle JO *et al*. [21] with slight modifications. Normal control animals were treated with vehicle, 0.25% Na- CMC. Indomethacin was used as a standard drug (10 mg/kg). Test extracts viz. TAPE, TACE and TAHE were administered at the doses of 100, 200 & 400 mg/kg. Treatment was given via oral gavage at the dose volume of 10 ml/kg. Pain induction was achieved by intraperitoneally administering 1% AA (0.1 mL/10 g body weight), 40 minutes after drug administration. Following the AA injection, the cumulative count of abdominal writhing responses was observed for a duration of 10 minutes, starting five minutes post-injection. The percentage inhibition of writhing, indicative of analgesic activity, was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{(No. of writhes in control animals – No. of writhes in drug treated animals)}}{\text{(No. of writhes in control animals)}} \times 100$$

Statistical analysis

The data are expressed as mean ± standard deviation (SD; n=6, except n=5 for acute oral toxicity) for each group. Statistical analysis was carried out using GraphPad Prism software 5.0. One-way or Two-way analysis of variance (ANOVA) was performed, followed by Dunnett's multiple comparison t-test or Bonferroni's multiple comparison t-test, respectively, to determine statistical differences. Significance levels were indicated as *p<0.05, **p<0.01, ***p<0.001 compared to the disease control group, and #p<0.05 compared to the normal control.

RESULTS

Phytochemical analysis

The yield of the TAPE, TACE and TAHE was found to be 9.63, 12.36 and 13.47% respectively. The phytoconstituents of *T. ammi* seeds were examined in petroleum ether, chloroform, and hydroalcoholic extracts. TAHE revealed the presence of carbohydrates, saponins, glycosides, alkaloids, phytosterols, phenolic compounds, tannins, proteins, amino acids, and flavonoids, all observed at levels ranging from moderate to strong. In the case of TACE, carbohydrates, saponins, glycosides, alkaloids, phytosterols, fixed oils and fats, phenolic compounds, and tannins were identified at mild levels, with moderate levels of flavonoids. Conversely, TAPE demonstrated moderate to strong levels of fixed oils and fats and mild presence of saponins and phytosterols.

Acute oral toxicity

The impact of Petroleum ether, chloroform, and hydroalcoholic extracts of *T. ammi* seeds at a dosage of 2000 mg/kg on behavioral patterns and body weight is presented in Tables 2 and 3. No instances of convulsions, tremors, or coma were observed in animals treated with TAPE, TACE, and TAHE at the specified dose. All parameters including eyes, fur and skin condition, mucous membrane, salivation, sleep, and

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urination (color) were found to be normal. Somatomotor activity and behavior patterns were slightly elevated during the initial 30 minutes in animals treated with TAHE. Similarly, respiration rate was slightly elevated and lowered in animals treated with TACE and TAHE respectively. No animal mortality was recorded in any group up to day 14. Throughout the experimental period, animals treated with TAPE, TACE, and TAHE exhibited no itching behavior. While TAPE, TACE and TAHE did not show a significant effect on absolute body weight. However, % body weight change was significantly increased in animals treated with TAPE, TACE and TAHE at day-7 and day-14.

The absolute weight of heart and kidney were significantly ($p>0.05$) unaltered by the treatment of TAPE, TACE and TAHE. Plant extracts showed no significant changes in the absolute liver weight. However, absolute liver weight was considerably ($p<0.01$) elevated in TAPE treated groups. Similarly, animals treated with TACE and TAHE displayed no significant ($p>0.05$) alterations in relative heart, kidney and liver weights. However, relative liver weight ($p<0.01$) was prominently increased by the treatment of TAPE (Table 4).

The impact of TAPE, TACE, and TAHE on renal and liver function tests is presented in Table 5. Serum creatinine and urea levels did not show significant ($p>0.05$) alterations in the treatment groups compared to normal mice. Additionally, in liver function tests, SGOT, SGPT, total proteins, total bilirubin, alkaline phosphatase, albumin, and globulin levels were observed to be normal ($p>0.05$) in animals treated with TAPE, TACE, and TAHE (Table 5). The levels of total cholesterol, triglycerides, HDL-c, and LDL-c showed no significant ($p>0.05$) alterations in the mice treated with TAPE, TACE, and TAHE compared to normal animals, as indicated in Table 6.

Table 6 presents the effect of TAPE, TACE, and TAHE on hematological parameters tested at 2000 mg/kg. Blood parameters including hemoglobin, RBCs, WBCs, platelets, and differential WBCs such as monocytes, neutrophils, lymphocytes, eosinophils, and basophils showed no significant ($p>0.05$) variations with the treatment of test compounds (Table 7).

Carrageenan induced rat paw edema model

Table 8 illustrates the variations in paw volume at different time points (1 h, 2 h, 3 h, 4 h, 5 h) across various treatment groups with different doses. While the control group experienced an increase in paw volume change over time, the treatment groups, specifically TAPE at 200 and 400 mg/kg, showcased noteworthy reductions in paw volume at 3 hr ($p<0.01$), 4 hr ($p<0.001$), and 5 hr ($p<0.001$), as well as from 2 ($p<0.05$) to 5 ($p<0.01$) hr, respectively. The observed dose-dependent responses in multiple treatment groups emphasize the significance of dosage in influencing the outcomes. Furthermore, TACE at 200 and 400 mg/kg demonstrated considerable reductions in paw edema at 3 hr ($p<0.001$), 4 hr ($p<0.01$), and 2 hr ($p<0.05$) to 5 hr ($p<0.01$), respectively. Similarly, TACE at 200 and 400 mg/kg exhibited significant reductions in paw edema at 3-5 hr ($p<0.01$), and 2 to 5 hr ($p<0.001$), respectively. The anti-inflammatory effect demonstrated by the test compounds, TAPE, TACE, and TAHE, was found to be dose dependent. Additionally, the standard drug, indomethacin (10 mg/kg), displayed a significant ($p<0.001$) reduction in paw edema at all time points.

Cotton pellet induced granuloma pouch model

The outcomes presented in Table 9 indicate significant variations in inflammatory parameters among different treatment groups assessed using the cotton pellet-induced granuloma pouch model. Animals treated with high dose TAPE (400 mg/kg) demonstrated a substantial reduction in wet weight ($p<0.001$), dry weight ($p<0.05$), and granuloma weight ($p<0.05$; Inhibition: 28.15±10.46%). Conversely, low doses of TAPE (100 and 200 mg/kg) exhibited a marginal effect ($p>0.05$) on lowering wet weight, dry weight, and granuloma weight. Additionally, TACE at doses of 200 ($p<0.05$) and 400 mg/kg ($p<0.01$) significantly reduced wet weight, dry weight, and granuloma weight, with TACE 400 mg/kg displaying a prominent effect ($p<0.001$) in wet weight reduction compared to normal rats. Treatment with high-dose TAHE (400 mg/kg) demonstrated a significant effect on reducing wet weight ($p<0.001$), dry weight ($p<0.01$), exudate weight ($p<0.05$), and granuloma weight ($p<0.01$). Notably, TAHE at the high dose (400 mg/kg) exhibited a prominent reduction ($p<0.01$) in exudate weight (50.63±10.76% inhibition) and an inhibition of granuloma weight by 40.86±18.82%. The standard drug, dexamethasone, showed significant inhibition ($p<0.001$) in wet weight, dry weight, granuloma, exudate, and overall granuloma weight at a dose of 5 mg/kg.

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In the biochemical analysis, disease control animals exhibited a significant ($p<0.001$) elevation in serum GOT, GPT, and total protein levels compared to normal. However, animals treated with TAPE at 400 mg/kg demonstrated a noteworthy reduction in SGOT ($p<0.001$) and SGPT levels ($p<0.01$). At 200 mg/kg, TAPE showed a significant ($p<0.05$) decrease only in SGOT levels, with no significant changes observed in total protein levels. Similarly, the treatment of TACE at 200 and 400 mg/kg was effective in a considerable decline in elevated levels of SGOT ($p<0.01$) and SGPT ($p<0.001$). Total protein levels were significantly lowered at the 400 mg/kg dose of TACE. Furthermore, TAHE at 400 mg/kg considerably ($p<0.001$) declined serum GOT, GPT, and total protein levels. However, the 200 mg/kg dose of TEHA displayed a substantial ($p<0.001$) reduction in SGOT and SGPT levels (Figure 1 A-C). Similarly, elevated levels of liver tissue GOT were significantly lowered by TACE ($p<0.05$) and TAHE ($p<0.01$) at 400 mg/kg. GPT levels were prominently ($p<0.01$) decreased by TAPE and TACE at 400 mg/kg. Additionally, the 200 mg/kg dose of TAHE displayed a considerable ($p<0.01$) reduction in liver tissue GPT levels. Total protein levels were significantly declined by TAPE ($p<0.05$), TACE ($p<0.001$), and TAHE at 400 mg/kg ($p<0.001$). However, TAHE displayed an additional effect on total protein reduction at 200 mg/kg ($p<0.05$). The elevated level of ALP was considerably lowered by TACE ($p<0.05$) and TAHE ($p<0.05$) at the 400 mg/kg dose (Figure 2 A-D). Dexamethasone, the standard drug, was found effective ($p<0.001$) in lowering elevated levels of all the serum and liver tissue biochemical parameters.

Hot plate test

Figure 3 depicts the analgesic effect of TAPE, TACE, and TAHE using the hot plate test. Mice treated with TAPE at 400 mg/kg displayed a significant ($p<0.05$) rise in latency time (LT) at 60 min compared to normal animals. Furthermore, the 200 mg/kg dose of TACE exhibited a substantial rise in LT at 60 and 90 min after drug administration ($p<0.05$). However, TACE at 400 mg/kg showed a significant ($p<0.001$) increase in LT at 30, 60, 90, and 120 min, confirming TACE's promising analgesic effect. Similarly, mice treated with TAHE at doses of 200 mg/kg and 400 mg/kg experienced a considerable ($p<0.001$) rise in LT at 30, 60, 90, and 120 minutes, supporting its analgesic effects. Notably, the Tramadol-treated group demonstrated a significant ($p<0.001$) increase in LT compared to the normal control, suggesting a prolonged analgesic effect.

Acetic acid induced writhing test

The results depicted in Figure 4 illustrate the writhing responses and corresponding percentage of inhibition. Indomethacin demonstrated a highly significant reduction in writhes ($p<0.001$), with a substantial 55.11% inhibition, affirming its potent analgesic effect. In contrast, mice treated with TAPE exhibited only marginal reductions in writhes at all dosage levels, with no statistically significant differences ($p>0.05$). Mice administered TACE displayed a significant reduction in writhes ($p<0.01$), specifically at a high dose of 400 mg/kg. Additionally, animals treated with TAHE exhibited a substantial decline in writhing count at the 400 mg/kg dosage, accompanied by a % inhibitory activity of 22.57% ($p<0.001$). These observations imply a dose-dependent relationship for the analgesic effects of the investigated compounds.

DISCUSSION

In the present investigation, we studied the comparative evaluation of petroleum, chloroform, and hydroalcoholic extracts of *T. ammi* for their anti-inflammatory, analgesic, and acute oral toxicity profiles using experimental animals. Undoubtedly, the importance of conducting a preliminary toxicological assessment cannot be overstated in ensuring the safety of herbal medicines. Despite their natural origin, herbal medicines may harbor bioactive compounds with the potential for unintended adverse effects on human health. Clinical signs and symptoms stand out as the primary indicators among various toxicity markers, shedding light on the adverse effects of drugs on crucial organs within the body [22, 23]. In this study, the acute oral toxicity of TAPE, TACE, and TAHE was investigated in Swiss albino mice following the OECD guideline-425. The findings revealed that various behavioral observations, including eyes, fur and skin condition, mucous membrane, salivation, sleep, and urination (color), all exhibited normal patterns. Somatomotor activity and behavior patterns were generally normal, with slight alterations observed in TACE and TAHE. Importantly, no instances of animal mortality were recorded in any group within the 14-day observation period. Additionally, renal, liver function tests, and hematological parameters displayed no

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significant alterations. Noteworthy changes in heart and liver weight were recorded in animals treated with TAPE, TACE, and TAHE compared to normal animals, indicating potential physiological responses that necessitate further investigation. This study offers valuable insights into the acute oral toxicity of TAPE, TACE, and TAHE, suggesting an overall favorable safety profile due to the absence of animal mortality and the observation of normal behavioral and physiological parameters. However, the observed changes in liver weight emphasize the need for additional investigations to better understand the underlying physiological responses. Interpreting these findings is crucial within the context of the intended use and dosage of herbal medicines, and ongoing monitoring is essential for a comprehensive understanding of their safety profile.

The induction of paw edema using carrageenan has been established as an experimental model for investigating acute inflammatory diseases. This model has found widespread use in the study of novel analgesic and anti-inflammatory agents. The study explored the anti-inflammatory capabilities of *T. ammi* seed extracts, specifically, employing a rat paw edema model induced by carrageenan. Treatment of TAPE, TACE and TAHE at high doses, 400 mg/kg displayed significant reduction in paw edema at 2 hr. A dose-dependent decline in paw edema, particularly evident with TAPE, TACE, and TAHE at 400 mg/kg, was observed during the early phase of inflammation (0–2 hours post-carrageenan injection). This phase involves inflammatory mediators like histamine, 5-hydroxytryptamine (5-HT), and bradykinin, suggesting that the extracts may modulate these early-phase mediators. Furthermore, a significant reduction in paw edema at 3-5 hours was observed for TAPE, TACE, and TAHE, corresponding to the late accelerating phase (2–6 hours after carrageenan injection). This phase is characterized by increased production of polyphenols (PGs), oxygen-derived free radicals, and cyclooxygenase-2 [24, 25]. The findings suggest that *T. ammi* seed extracts have the potential to influence both early and late phases of inflammation, indicating their promise as anti-inflammatory agents. To further support the efficacy of the extracts, the standard drug indomethacin was included as a reference. In conclusion, these results suggest that *T. ammi* seed extracts could effectively modulate the inflammatory response at various stages.

The cotton pellet granuloma method is a widely utilized technique for assessing various aspects of subacute inflammation, including transudative, exudative, and proliferative features [26]. In this method, subcutaneous implantation of a cotton pellet in rodents leads to the development of a granuloma at the implantation site. The initial stages involve the accumulation of fluid and proteinaceous material, accompanied by the infiltration of neutrophils, fibroblasts, and macrophages, along with the proliferation of small blood vessels. These processes collectively contribute to the formation of granulation tissue, presenting as a highly vascularized reddish mass. The wet weight of the granuloma is primarily influenced by the fluid absorbed by the pellet, while the dry weight serves as a reliable measure of the formed granulomatous tissue [27]. The present study findings suggested that, TAPE, TACE and TAHE displayed substantial reduction in wet weight of granuloma, confirm their effect on vascular permeability. To assess the impact of the test compounds on the proliferative phase of inflammation, the dry weight of the granuloma was measured. TAPE, TACE and TAHE demonstrated a positive influence on the proliferative phase of inflammation. It's noteworthy that various studies have reported that NSAIDs such as diclofenac exhibit only a marginal inhibitory effect on granuloma formation. In contrast, steroidal drugs show significant inhibition of granuloma formation [27]. For instance, dexamethasone at 5 mg/kg dose notably reduced granuloma weight in this context.

In the biochemical examination, TAPE, TACE and TAHE exhibited significant reductions in the levels of GPT, GOT, and total proteins in both blood serum and liver tissue. Notably, TAPE showed an exception by not affecting serum total proteins and liver tissue GOT. Furthermore, a dose-dependent inhibition of acid phosphatase activity in liver tissue was observed with the administration of TAPE, TACE, and TAHE. The observed inhibitory effects on GOT and GPT activities by these plant extracts may influence the formation of polypeptides such as bradykinin and other kinin-like substances released during the inflammatory process. Additionally, the dose-dependent inhibition of acid phosphatase suggests a potential interference with the synthesis of lysosomal enzymes by these anti-inflammatory drugs. This action could contribute to stabilizing the lysosomal membrane and influencing the

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inflammatory process, as indicated by findings from previous studies [28].

The analgesic properties of the test compounds were evaluated using the hot plate and AA-induced writhing tests. The hot plate test is designed to investigate central analgesic activity in animal models by utilizing thermal stimuli as pain inducers, emphasizing modifications above the spinal cord level for a meaningful representation of centrally mediated anti-nociceptive responses [29, 30]. In the present study, TACE, and TAHE displayed a significant rise in LT. However, TAPE displayed significant elevation in latency time only at 400 mg/kg at 60 min. The pain-relieving activity of these test compounds suggests a central anti-nociceptive effect. In the hot plate test, pain is induced through a supra-spinal reflex that engages μ1, κ3, δ1, and σ2 opioid receptors. The study outcomes suggest that the analgesic effect of the plant extracts may be attributed to alterations in pain induction via supra-spinal reflex pathways.

Further, in vivo antinociceptive effects of test compounds, TAPE, TACE and TAHE were evaluated using the AA-induced writhing test. The AA-induced writhing test is a widely employed model for assessing peripheral antinociceptive effects. Here, pain is induced by various inflammatory mediators such as Prostaglandin E2 and Prostaglandin E2 alpha, bradykinin, histamine, serotonin, and cytokines (Tumour Necrosis Factor-α, Interleukin-1β, Interleukin-6, and Interleukin-8). These inflammatory mediators not only generate pain but also contribute to increased vascular capillary permeability, decreased pain threshold, and heightened sensitivity of nerve terminals of nociceptive fibers (31, 25). The study outcomes suggest the significant inhibition of latency time by TACE and TAHE, confirms its anti-nociceptive property by inhibiting this pain inducing inflammatory mediators.

CONCLUSION

In conclusion, the administration of a single oral dose of 2000 mg/kg of TAPE, TACE and TAHE was deemed safe. Furthermore, the extracts demonstrated noteworthy dose-dependent anti-inflammatory effects, likely attributed to the presence of pharmacologically active compounds inhibiting various inflammatory mediators, including serotonin, histamine, prostaglandins, substance P, and bradykinin. The plant extracts also exhibited both central and peripheral analgesic effects, potentially through the inhibition of supra-spinal reflex pathways and inflammatory mediators, respectively. Remarkably, TAHA displayed a more pronounced effect in reducing inflammation and nociceptive pain compared to TACE. While TAPE also possesses anti-inflammatory and pain-relieving properties, the effects were found to be less pronounced compared to TACE. However, additional studies are recommended to unravel the precise mechanism of action.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

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REFERENCES

1. Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare Department of Ayush. Part 1. 1: (1999-2011). pp 170–171.
2. Krishnamoorthy V., Madalageri M.B., 1999. Bishop weeds (Trachyspermum ammi): An essential crop for north Karnatka. J Med Aromat Plant Sci. 21, 996–998.
3. Thangam C., Dhananjayan R., 2003. Antiinflammatory potential of the seeds of Carum copticum Linn. Indian J Pharmacol. 35, 388–391.
4. Braz-Silva P.H., Bergamini M.L., Mardegan A.P., De Rosa C.S., Hasseus B., Jonasson P., 2019. Inflammatory profile of chronic apical periodontitis: a literature review. Acta Odontol. Scand. 77, 173-180.
5. Omoigui S., 2007. The Biochemical Origin of Pain: The origin of all Pain is Inflammation and the Inflammatory Response. PART 2 of 3 – Inflammatory Profile of Pain Syndromes. Med Hypotheses. 69(6), 1169–1178.

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www.jchr.org

JCHR (2024) 14(1), 2702-2722 | ISSN:2251-6727

6. Dray A., 1995. Inflammatory mediators of pain. Br. J. Anaesth. 75 (2), 125–131.
7. Tamrat Y., Nedi T., Assefa S., Teklehaymanot T., Shibeshi W., 2017. Anti-inflammatory and analgesic activities of solvent fractions of the leaves of Moringa stenopetala Bak. (Moringaceae) in mice models. BMC Complement Altern Med. 17, 473.
8. Yimer T., Birru EM., Adugna M., Geta M., Emiru Y.K., 2020. Evaluation of Analgesic and Anti-Inflammatory Activities of 80% Methanol Root Extract of Echinops kebericho M. (Asteraceae). J Inflamm Res. 13, 647-658.
9. Bouyahya A., Guaouguaou F-E., Omari N.E., Menyiy N.E., Balahbib A., El-Shazly M., Bakri Y., 2022. Anti-inflammatory and analgesic properties of Moroccan medicinal plants: Phytochemistry, in vitro and in vivo investigat ions, mechanism insights, clinical evidences and perspectives. J Pharm Anal. 12(1), 35-57.
10. Sahoo N. and Manchikanti P. 2013. Herbal Drug Regulation and Commercialization: An Indian Industry Perspective. J Altern Complement Med. 19(12), 957–963.
11. Mukherjee P.K., Wahile A. 2006. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharmacol. 103, 25–35.
12. Hazarika I., Geetha K.M., Sivakami S.P., Divya M. 2019. Acute oral toxicity evaluation of extracts of Hydrocotyle sibthorpioides in Wister albino rats as per OECD 425 TG. Toxicol. Rep. 6, 321-328.
13. Khandelwal K. Practical Pharmacognosy. Pragati Books Pvt. Ltd.; Sep 7. 2008.
14. OECD. Acute oral toxicity: Up and down procedure. Guideline for the Testing of Chemicals, 425, OECD. 2008. pp. 1-2
15. Balkrishna A., Sakat S.S., Joshi K., Paudel S., Joshi D., Joshi K., Ranjan R., Gupta A., Bhattacharya K., Varshney A., 2019. Anti-Inflammatory and Anti-Arthritic Efficacies of an Indian Traditional Herbo-Mineral Medicine "Divya Amvatari Ras" in Collagen Antibody-Induced Arthritis (CAIA) Mouse Model Through Modulation of IL-6/IL-1β/TNF-α/NFκB Signaling. Front Pharmacol. 10, 659.
16. Salem S., Leghouchi E., Soulimani R., Bouayed J. 2021. Reduction of paw edema and liver oxidative stress in carrageenan-induced acute inflammation by Lobaria pulmonaria and Parmelia caperata, lichen species, in mice. Int J Vitam Nutr Res. 91(1-2),143-151.
17. Nair V., Kumar R., Singh S., Gupta Y.K. 2012. Anti-granuloma activity of Terminalia chebula retz. In wistar rats. Eur J Inflamm. 10: 185–191.
18. Wilches I., Tobar V., Peñaherrera E., Cuzco N., Jerves L. Heyden Y.V., León-Tamariz F., Vila E., 2015. Evaluation of anti-inflammatory activity of the methanolic extract from Jungia rugosa leaves in rodents. J. Ethnopharmacol. 173, 166–171.
19. Franzotti E.M., Santos C.V., Rodrigues H.M., Mourão R.H., Andrade M.R., Antoniolli A.R., 2000. Anti-inflammatory, analgesic and acute toxicity of Sida cordiforlia L. J Ethnopharmacol. 72, 273–277.
20. Ayanaw M.A., Yesuf J.S., Birru E.M., 2023. Evaluation of Analgesic and Anti-inflammatory Activities of Methanolic Leaf and Root Extracts of Gomphocarpus purpurascens A. Rich (Asclepiadaceae) in Mice. J Exp Pharmacol. 15, 1–11.
21. Olukunle J.O., Adenubi O.T., Oladele G.M., 2011. Studies on the anti-inflammatory and analgesic properties of Jatropha curcas leaf extract. Acta Vet. Brno. 80, 259–262.
22. Saleem U., Amin S, Ahmad B., Azeem H., Anwar F., Mary S., 2017. Acute oral toxicity evaluation of aqueous ethanolic extract of Saccharum munja Roxb. roots in albino mice as per OECD 425 TG. Toxicol Rep. 4, 580-585.
23. Subramanion L.J., Zakaria Z., Chen Y., Lau Y.L., Latha L.Y., Sasidharan S. 2011. Acute oral toxicity of methanolic seed extract of cassia fistula in mice. Molecules. 16(6), 5268-5282.
24. Vinegar R, Truax J.F., Selph J.L., Johnston P.R., Venable A.L., McKenzie K.K., 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed Proc. 46(1), 118–26.
25. Karim N., Khan I., Khan W., Khan I., Khan A., Halim S.A., Khan H., Hussain J., Al-Harrasi

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 2702-2722 | ISSN:2251-6727

A., 2019. Anti-nociceptive and Anti-inflammatory Activities of Asparacosin A Involve Selective Cyclooxygenase 2 and Inflammatory Cytokines Inhibition: An in-vitro, in-vivo, and in-silico Approach. Front. Immunol. 10, 581.
26. Purnima A., Koti B.C., Thippeswamy A.H.M., Tikare V.P., Dabadi P., Viswanathaswamy A.H.M., (2010). Evaluation of anti-inflammatory activity of Centratherum anthelminticum (L) kunze seed. Indian J Pharm Sci. 72(6), 697–703.
27. Dzoyem J.P., McGaw L.J., Kuete V., Bakowsky U., 2017. Anti-inflammatory and Anti-nociceptive Activities of African Medicinal Spices and Vegetables. Medicinal Spices and Vegetables from Africa Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases. Academic Press. pp 239-270.
28. Dorfman R.I., Dorfman A.S., 1965. Antiinflammatory activity of nonsteroidal agents in a rat granuloma assay. Proc Soc Exp Biol Med. 119, 859-862.
29. Wigdor S., Wilcox G.L., 1987. Central and systemic morphine-induced antinociception in mice: contribution of descending serotonergic and noradrenergic pathways. J Pharmacol Exp Ther. 242(1), 90-95.
30. Chapman C.R., Casey K.L., Dubner R., Foley K.M., Gracely R.H., Reading A.E., 1985. Pain measurement: an overview. Pain. 22(1), 1-31.
31. Moallem S.A., Imenshahidi M. Shahini N., Javan AR, Karimi M., Alibolandi M., Ghandadi M., Etemad L., Motamedshariaty V., Hosseini T., Hadizadeh F., 2013. Synthesis, Anti-inflammatory and anti- nociceptive activities and cytotoxic effect of novel thiazolidin-4-ones derivatives as selective cyclooxygenase (COX-2) inhibitors. Iran J Basic Med Sci.,16(12), 1238–1244.

TABLES AND FIGURES

Table 1. Phytochemical analysis of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi*

Phytochemical tests/reagent(s) used	Pet. ether extract	Chloroform extract	Hydroalcoholic extract
Alkaloids			
Dragendroff's test	-	+	+++
Hager's test	-	++	+++
Wagner's test	-	+	++
Carbohydrates			
Molisch's test	-	++	+++
Barfoed's test	-	++	++
Benedicts test	-	++	+++
Glycosides			
Molisch's test after hydrolysis	-	++	+++
Phytosterols			

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Liebermann's burchard's test	++	++	+++
Fixed oils and fats			
Spot test	++	++	-
Saponification test	+++	+	-
Saponins			
Foam test	+	++	+++
Haemolysis test	+	++	+++
Phenolic compounds and tannins			
Ferric chloride test	+	++	+++
Lead acetate test	+	++	+++
Proteins and amino acids			
Biuret test	-	++	++
Ninhydrin test	-	++	++
Flavonoids			
Shinoda test	-	+++	+++

Mild: +; Moderate: ++; Strong: +++; - Absent

Table 2. Effect of petroleum ether, chloroform and hydroalcoholic extract of T. ammi on behavioural observations (acute oral toxicity study)

Parameters	**Observations**																							
	30 Min				**4 h**				**24 h**				**48 h**				**Day-7**				**Day-14**			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	**A**	**B**	**C**	**D**
Convulsions & tremors	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b
Coma	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b	A b
Faeces consistency	N r	N r	N r	N r	N r	N r	N r	N r	Nr	N r	N r	N r	Nr	N r	N r	N r	Nr	N r	N r	N r	Nr	N r	N r	N r
Eye	N r	N r	N r	N r	N r	N r	N r	N r	Nr	N r	N r	N r	Nr	N r	N r	N r	Nr	N r	N r	N r	Nr	N r	N r	N r
Fur & Skin	N r	N r	N r	N r	N r	N r	N r	N r	Nr	N r	N r	N r	Nr	N r	N r	N r	Nr	N r	N r	N r	Nr	N r	N r	N r

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Itching	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Mucous membrane	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Mortality	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Respiration	Nr	Nr	E	L	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Salivation	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Somatomotor activity & behaviour pattern	Nr	Nr	Nr	E	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Sleep	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Urination (colour)	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr	Nr

A: Normal control; B: TAPE; C: TACE; D: TAHE; Nr: Normal; Ab: Absent; NF: Not Found; E: Elevated; L: Lowered

Table 3. Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on animal body weight (acute oral toxicity study)

Groups; Dose (mg/kg); p.o.	**Absolute body weight (g)**				**Body weight change (%)**		
	Day-0	**Day-1**	**Day-7**	**Day-14**	**Day-1**	**Day-7**	**Day-14**
NC	33.28±2.3	33.87±2.4	33.59±2.3	34.19±2.0	1.77±0.9	0.94±1.0	2.80±2.1
TAPE-2000	31.67±1.5	32.28±1.1	33.29±0.8	34.29±0.9	1.98±1.7	5.24±3.1*	8.38±3.3***
TACE-2000	32.10±1.0	32.77±0.9	33.65±1.0	34.74±1.0	2.08±0.9	4.83±2.5*	8.23±1.6**
TAHE-2000	32.50±1.7	33.10±1.4	34.44±1.0	35.58±0.8	1.88±1.2	6.06±2.4**	9.63±3.8***

Values in the results are expressed as mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to control at respective time points. (A Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison t-test)

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Table 4. Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on organ weight (acute oral toxicity study)

Groups; Dose (mg/kg); p.o.	Absolute Organ Weight (g)			Relative Organ Weight (per 10g)		
	Heart	Kidney	Liver	Heart	Kidney	Liver
NC	0.247±0.01	0.478±0.01	2.200±0.08	0.072±0.00	0.140±0.01	0.644±0.01
TAPE-2000	0.252±0.01	0.509±0.15	2.358±0.06**	0.074±0.00	0.149±0.04	0.688±0.02**
TACE-2000	0.257±0.01	0.586±0.03	2.284±0.06	0.074±0.00	0.169±0.01	0.658±0.02
TAHE-2000	0.255±0.01	0.563±0.02	2.278±0.06	0.072±0.00	0.158±0.01	0.641±0.03

Values in the results are expressed as mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to control at respective time points. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Table 5. Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on renal and liver function tests (acute oral toxicity study)

Groups; Dose (mg/kg); p.o.	Renal Function Test		Liver Function Test						
	Creatinine (mg/dL)	Urea (mg/dL)	SGOT (U/L)	SGPT (U/L)	Alkaline Phosphatase (U/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
NC	0.69±0.1	57.47±6.4	90.22±11.1	55.40±7.6	158.61±9.0	0.26±0.0	7.28±1.5	3.55±0.4	3.33±0.2
TAPE-2000	0.78±0.1	55.45±9.3	95.42±6.3	59.06±7.4	165.54±5.9	0.27±0.1	6.39±1.1	3.62±0.4	3.45±0.4
TACE-2000	0.64±0.8	62.54±10.9	87.69±6.9	55.09±8.3	153.21±10.2	0.29±0.0	8.06±1.1	3.35±0.4	3.22±0.2
TAHE-2000	0.68±0.8	58.10±8.2	92.02±6.9	53.43±5.3	166.11±14.8	0.26±0.1	7.08±1.6	3.57±0.4	3.55±0.3

Values in the results are expressed as mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to control at respective time points. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Table 6. Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on lipid profile (acute oral toxicity study)

Groups; Dose (mg/kg); p.o.	Lipid Profile			
	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)
NC	90.56±7.24	98.20±8.79	36.44±5.60	34.22±4.36

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TAPE-2000	91.71±6.00	95.06±5.11	36.53±3.91	32.61±6.38
TACE-2000	93.70±5.86	91.20±9.50	35.54±5.36	37.57±4.48
TAHE-2000	93.63±4.31	91.34±8.26	29.73±1.36	32.61±4.72

Values in the results are expressed as mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to control at respective time points. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Table 7. Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on haematology parameters (acute oral toxicity study)

Groups; Dose (mg/kg); p.o.	Haematology Parameters								
	Hb (g/dL)	RBCs (10^6/ µL)	WBCs (10^3/ µL)	Platelets (10^3/µ L)	Monocytes (%)	Neutrophiles (%)	Lymphocytes (%)	Eosinophiles (%)	Basophiles (%)
NC	15.55±0.6	5.90±0.1	5.59±0.4	763.80±39.3	1.80±0.5	32.40±5.6	60.40±1.9	2.00±1.0	0.40±0.6
TAPE-2000	15.22±0.8	5.89±0.6	6.02±0.6	786.80±44.9	1.20±0.5	30.40±4.3	62.00±7.8	1.60±0.6	0.20±0.5
TACE-2000	15.30±0.2	5.76±0.3	5.79±0.4	794.00±38.7	1.20±0.5	29.60±4.0	63.60±6.7	2.00±0.8	0.20±0.5
TAHE-2000	15.52±0.4	6.21±0.3	5.33±0.5	770.40±64.6	1.00±0.0*	28.80±94.0	58.80±8.1	2.40±1.3	0.40±0.6

Values in the results are expressed as mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to control at respective time points. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

Table 8. Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on rat paw edema

Groups; Dose (mg/kg); p.o.	Paw Edema (mL) at Different Time Points (% Anti-inflammatory activity)				
	1 h	2 h	3 h	4 h	5 h
NC	0.22±0.08 (0.00±0.00)	0.88±0.17 (0.00±0.00)	1.09±0.09 (0.00±0.00)	1.03±0.06 (0.00±0.00)	0.90±0.13 (0.00±0.00)
INDO-10	0.11±0.06* (51.91±26.67)	0.20±0.06*** (77.17±7.35)	0.35±0.06*** (67.90±5.36)	0.50±0.16*** (50.97±15.62)	0.43±0.08*** (51.96±8.77)
TAPE-100	0.17±0.02 (22.14±7.10)	0.84±0.03 (4.72±3.75)	0.99±0.02 (8.45±2.23)	0.94±0.04 (8.60±3.87)	0.80±0.05 (10.24±5.08)
TAPE-200	0.17±0.02 (22.90±10.61)	0.84±0.04 (5.09±4.26)	0.93±0.09** (14.59±8.62)	0.86±0.05*** (16.23±5.19)	0.79±0.06** (11.55±7.19)
TAPE-400	0.16±0.04 (26.72±20.28)	0.76±0.07* (14.53±8.22)	0.86±0.06*** (21.04±5.49)	0.81±0.07*** (21.43±6.63)	0.76±0.06** (15.27±6.97)
TACE-100	0.16±0.01 (25.95±6.74)	0.85±0.02 (03.58±1.95)	1.02±0.07 (5.99±6.33)	0.97±0.02 (5.36±2.26)	0.83±0.04 (7.45±4.26)
TACE-200	0.16±0.04 (29.01±19.38)	0.79±0.04 (10.38±4.38)	0.84±0.06*** (22.27±5.73)	0.88±0.06** (14.45±5.79)	0.80±0.04 (10.24±4.98)

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TACE-400	0.13±0.01 (40.46±5.79)	0.76±0.05* (13.58±5.25)	0.82±0.06*** (24.42±5.28)	0.78±0.05*** (23.70±4.38)	0.74±0.06** (16.95±7.02)
TAHE-100	0.16±0.03 (25.95±15.44)	0.87±0.07 (4.21±4.12)	0.94±0.05** (12.06±6.95)	0.93±0.03 (9.90±3.36)	0.78±0.05 (13.22±5.18)
TAHE-200	0.13±0.04 (38.93±18.92)	0.81±0.04 (8.87±4.79)	0.84±0.07*** (22.73±6.66)	0.80±0.06*** (21.92±5.89)	0.75±0.06** (15.83±6.80)
TAHE-400	0.10±0.05 (54.96±1.56)	0.68±0.08*** (23.21±9.10)	0.67±0.07*** (38.56±6.43)	0.65±0.11*** (36.36±10.28)	0.67±0.05*** (25.70±5.77)

Values in the results are expressed as mean ± SD (n=6). *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to control at respective time points. (A Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison t-test)

Table 9: Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on cotton pellet induced granuloma pouch model

Groups; Dose (mg/kg); p.o.	**Wet weight (mg)**	**Dry weight (mg)**	**Exudate weight (mg)**	**Exudative Inhibition (%)**	**Granuloma weight (mg)**	**Granuloma Inhibition (%)**
NC	211.27±10.7	86.83±8.2	124.44±16.9	0.00±0.00	66.83±8.2	0.00±.00
DEXA-5	130.89±11.9***	44.59±12.8***	86.30±20.3**	55.10±16.34	24.59±12.8***	63.20±19.17
TAPE-100	198.86±8.3	81.67±13.0	117.19±11.2	30.27±8.98	61.67±13.0	10.70±17.09
TAPE-200	196.82±8.2	76.06±10.9	120.76±15.4	27.40±12.39	56.06±10.9	16.12±16.31
TAPE-400	182.92±9.8***	68.02±7.0*	114.90±9.4	32.11±7.55	48.02±7.0*	28.15±10.46
TACE-100	199.49±8.05	85.83±10.15	113.66±9.18	33.11±7.38	65.83±10.15	6.92±9.83
TACE-200	193.08±11.49*	70.64±7.97*	122.44±18.11	26.05±14.55	50.64±7.97*	24.23±11.93
TACE-400	173.75±15.18***	66.00±7.33**	103.78±17.76	37.85±14.45	46.00±7.33**	28.26±16.35
TAHE-100	201.60±5.0	80.33±15.2	121.27±17.9	26.99±14.41	60.33±15.2	13.74±18.21
TAHE-200	177.74±13.2***	69.74±17.0	108.00±25.8	37.66±20.77	49.74±17.0	26.72±23.74
TAHE-400	151.38±10.0***	59.52±12.6**	91.86±13.4*	50.63±10.76	39.52±12.6**	40.86±18.82

Values in the results are expressed as mean ± SD (n=6); *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to control at respective time points. (A One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test)

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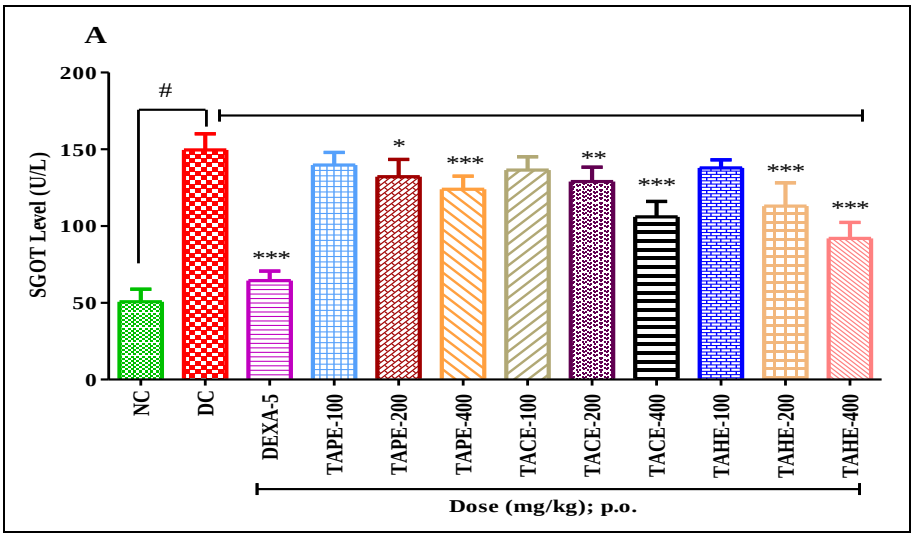

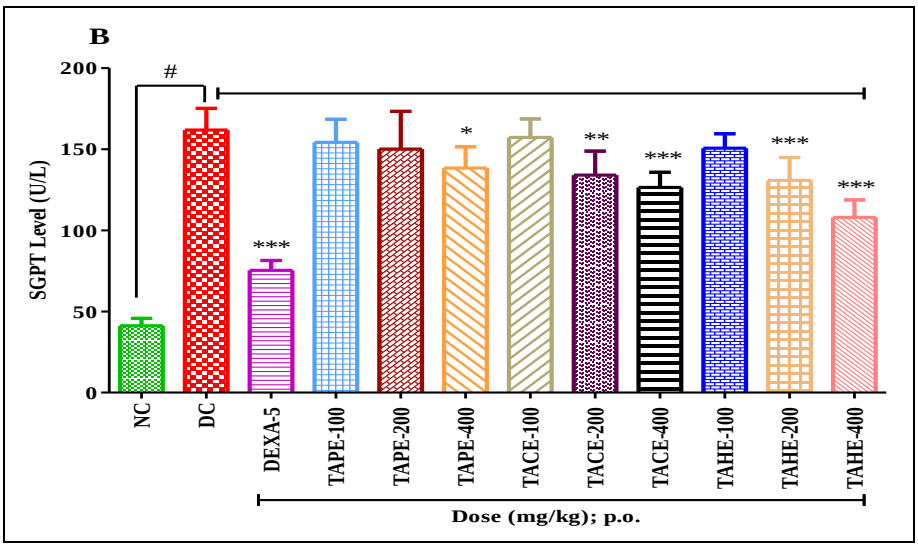

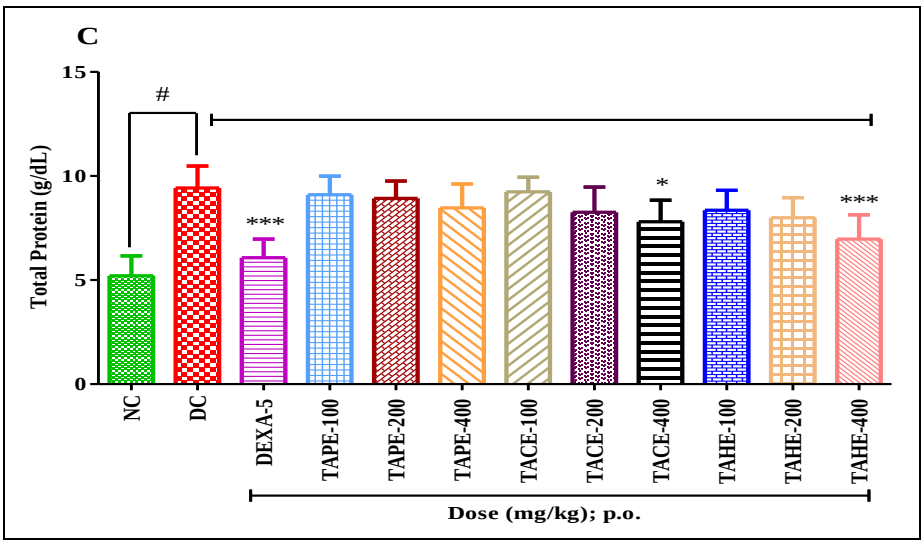

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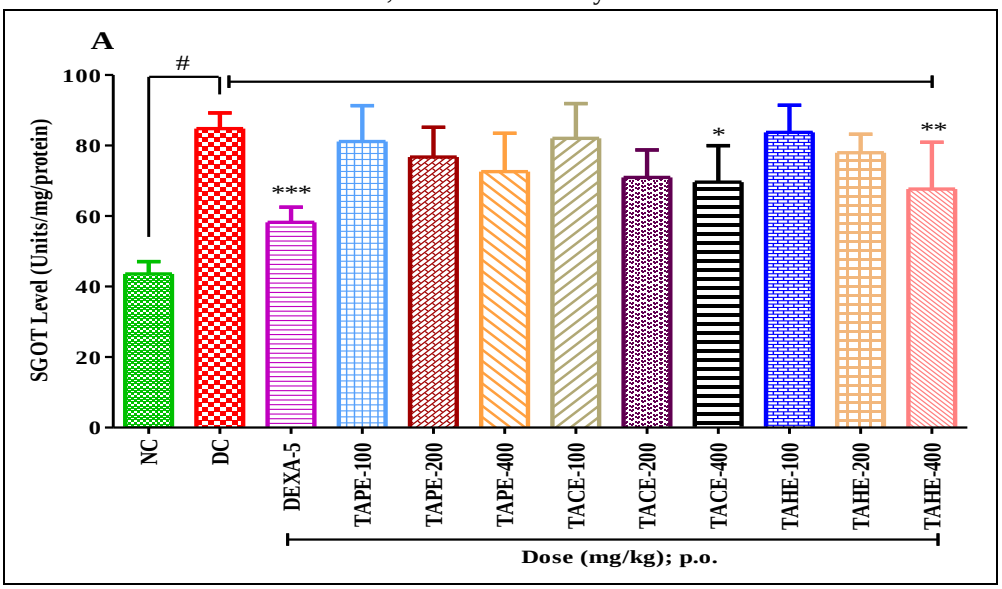
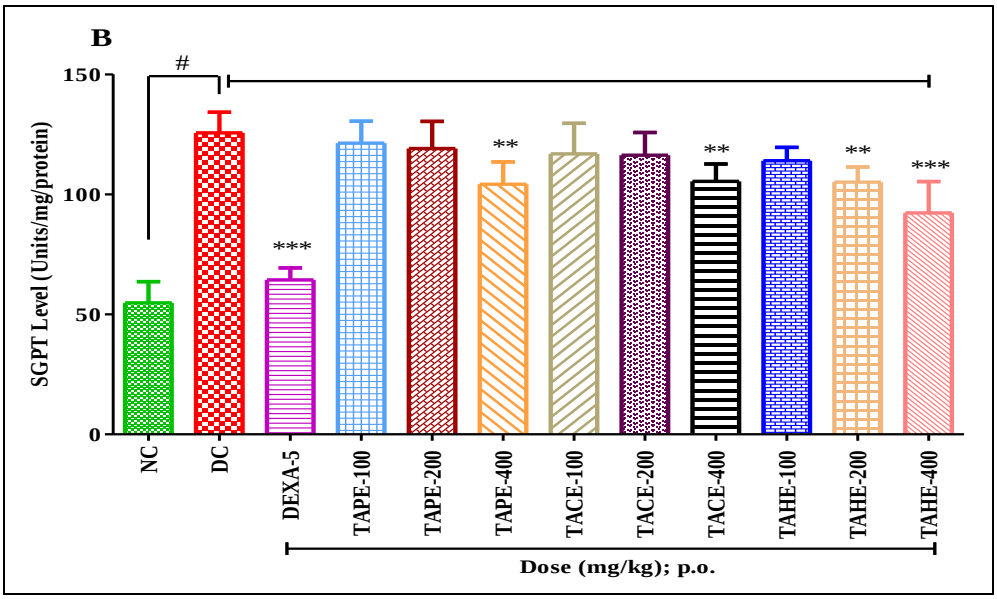
Figure 1: Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on serum enzymes in rats with cotton pellet-induced granuloma A) SGOT level B) SGPT level C) Total Protein Values in the results are expressed as mean± SD, (n=6); Data is analysed by One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test; *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to vehicle control). Data displays in the percentages are % inhibition. **Abbreviations:** NC: Normal control; TAPE: *T. ammi* Pet. Ether extract; TACE: *T. ammi* Chloroform extract; TAHE: *T. ammi* hydroalcoholic extract





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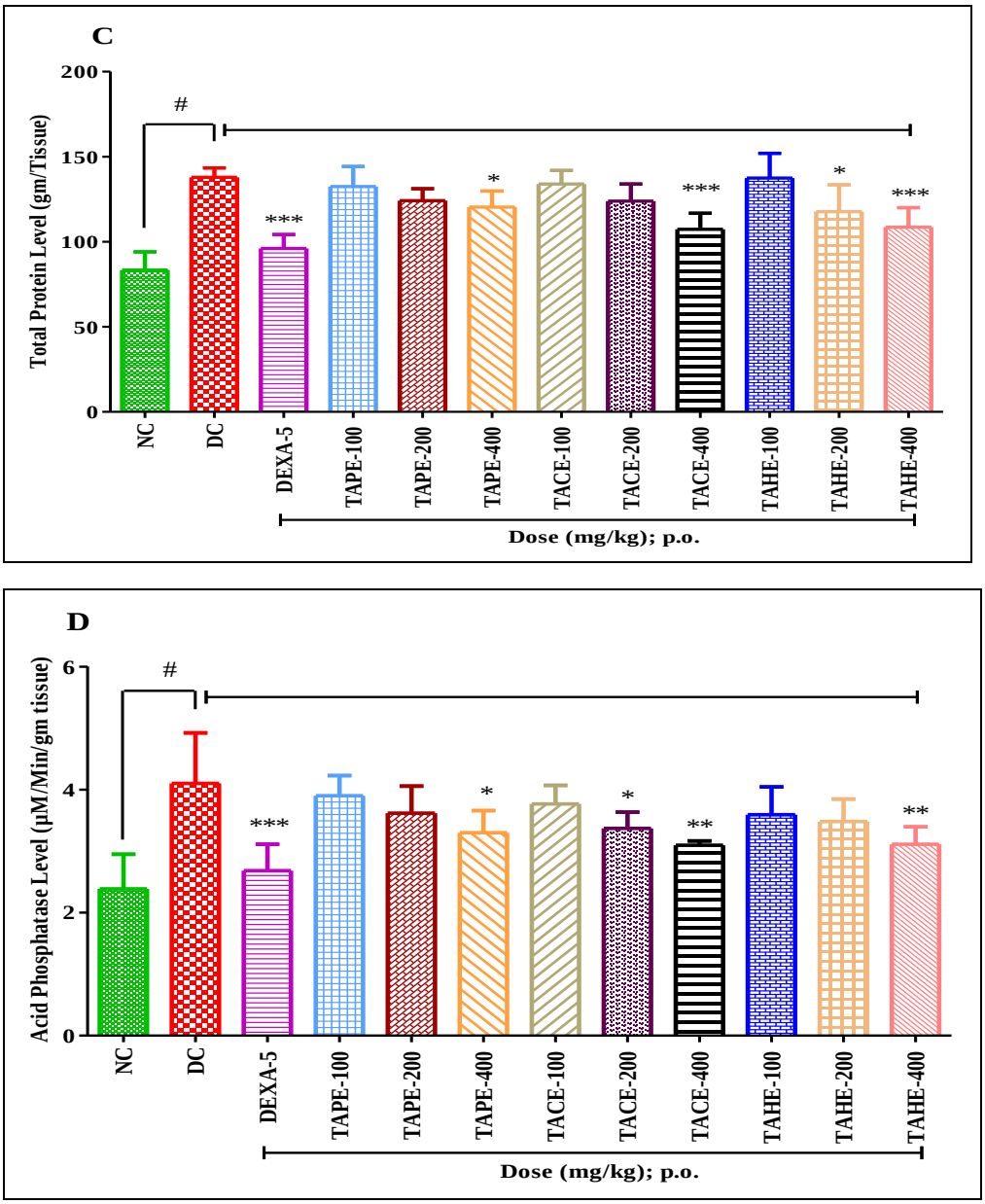

Figure 2: Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on liver enzymes in rats with cotton pellet-induced granuloma A) SGOT level B) SGPT level C) Total Protein D) Acid Phosphatase level**.** Values in the results are expressed as mean± SD, (n=6); Data is analysed by One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test; *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to vehicle control). Data displays in the percentages are % inhibition. **Abbreviations:** NC: Normal control; DC: Disease control; TAPE: *T. ammi* Pet. Ether extract; TACE: *T. ammi* Chloroform extract; TAHE: *T. ammi* hydroalcoholic extract

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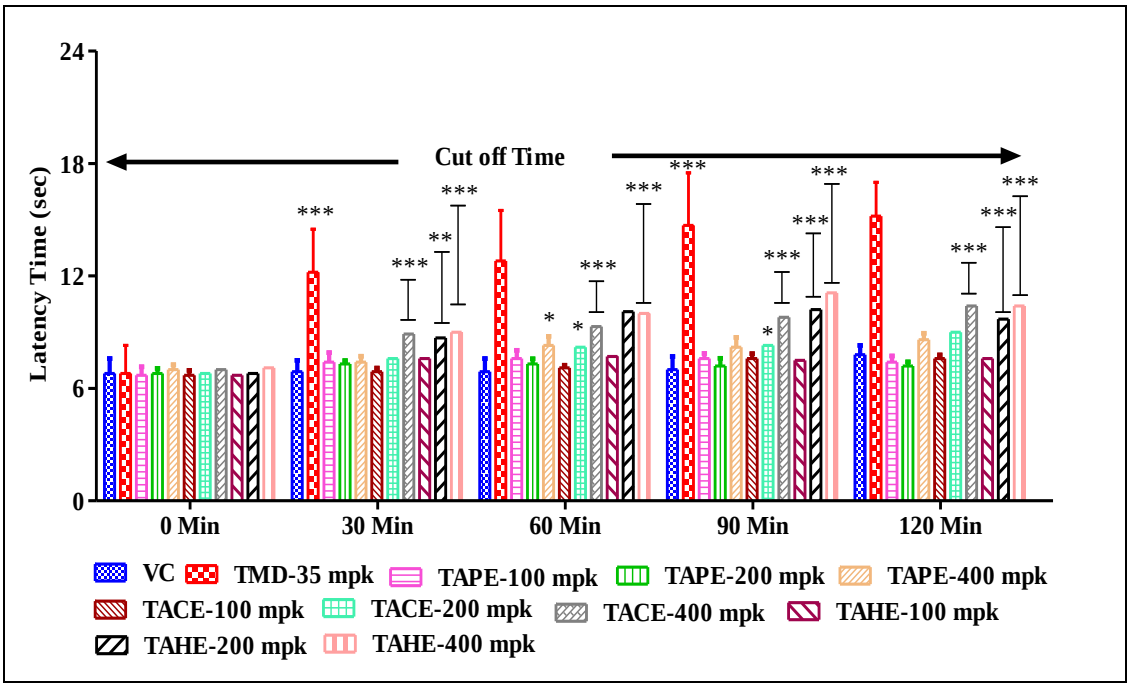

Figure 3: Effect of petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on latency time (Hot plate test). Values in the results are expressed as mean± SD, (n=6); Data is analysed by One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test; *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to vehicle control). Data displays in the percentages are % inhibition. **Abbreviations:** NC: Normal control; TAPE: *T. ammi* Pet. Ether extract; TACE: *T. ammi* Chloroform extract; TAHE: *T. ammi* hydroalcoholic extract

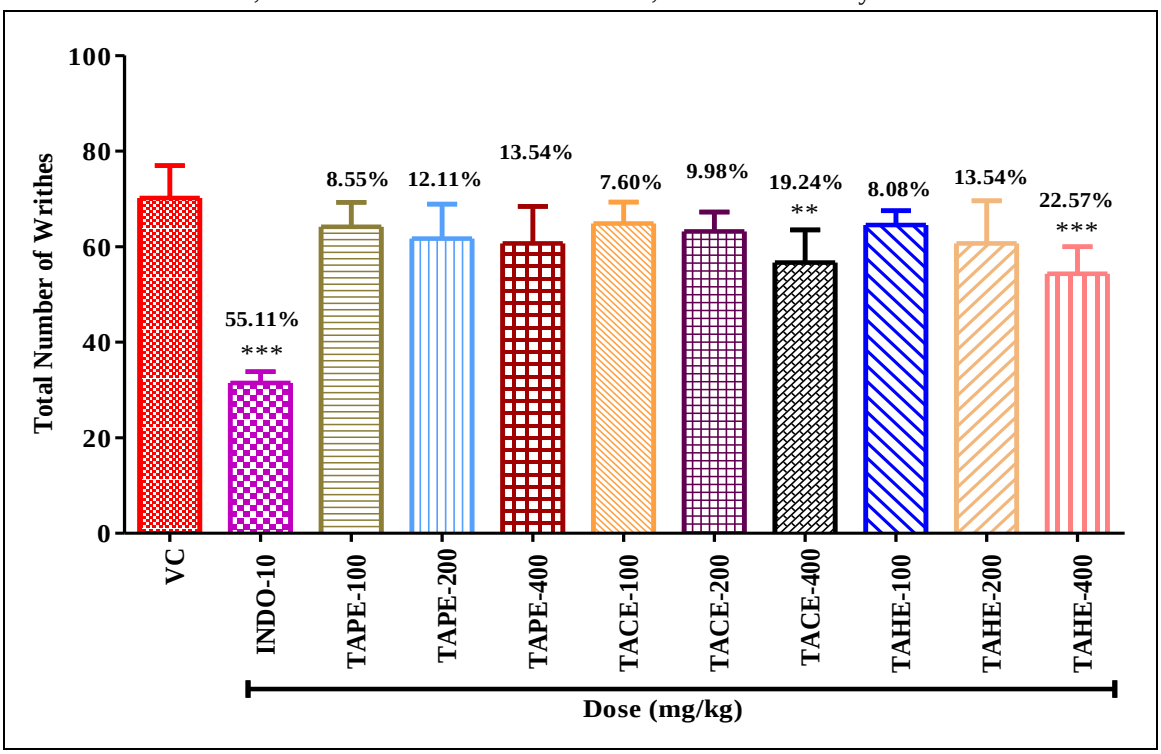

Figure 4: Effect of Petroleum ether, chloroform and hydroalcoholic extract of *T. ammi* on acetic acid writing test. Values in the results are expressed as mean± SD, (n=6); Data is analysed by One-way analysis of variance (ANOVA) followed

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Figure Legends

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