Original Article

Evaluation and Comparison of *Trachyspermum ammi* Seed Extract for Its Anti-inflammatory Effect

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Aims and Objectives: The present study was aimed to evaluate the anti-inflammatory effect of different seed extracts of *Trachyspermum ammi* at different doses. Materials and Methods: Three different seed extracts were prepared through Soxhlet extraction method by using n-hexane, chloroform and methanol solvents. Acute toxicity test performed at dose of 400 mg/kg, 800 mg/kg, 1600 mg/kg and 3200 mg/kg. Two different strengths of seed extracts (minimum therapeutic dose of 500 mg/kg and maximum therapeutic dose of 1000 mg/kg) were given to Wistar rats to measure anti-inflammatory activity through Carrageenan induced paw edema method. Results: The standard drug diclofenac sodium was (percentage of inhibition of paw edema 29.68%) more effective as compared to test drug. When efficacy of all extracts compared with each other, n-hexane extract showed more anti-inflammatory effect (percentage inhibition of paw edema 22.21%) at maximum effective dose 1000 mg/kg. Conclusion: Seed extracts of *T. ammi* showed anti-inflammatory activity by potentiating the neurotransmission of GABA and also by repression glutamate receptor.

KEYWORDS: Acute toxicity, anti-inflammatory, *Trachyspermum ammi*

INTRODUCTION

Inflammation occurs due to the defensive reaction of the body; it may be due to cell damage, tissue collapse, exposure to injurious synthetic materials, burns, or microbiological agents.[1] The indications of inflammation are redness, soreness, warmth, and ache.[2] Whenever an injury occurs, it increases the number of damage-associated molecular patterns system (DAMPS), which alarms the body’s immune system. These interpretations and transcriptions lead toward the inflammatory reaction and due to these reactions, the release of pro-inflammatory cytokines, different stimulatory particles, and chemokines take place and these proteins promote the accumulation of neutrophils and monocytes at the site of disruption and result in inflammation.[3]

Nowadays, a vast research being carried out for the development of natural pain-relieving and anti-inflammatory drugs, which have fewer side effects as compared to nonsteroidal anti-inflammatory drugs that cause stomach bruits and opiates that result in addiction and dependence.[4] *Trachyspermum ammi* is used in the traditional medicinal system for curing headache, neurological disorders, joint pain, and rheumatic arthritis, and it also diminishes inflammation[2] and seeds also have antispasmodic, antihypertensive, hepatoprotective, and bronco dilating activity.[5]

MATERIALS AND METHODS

Test drug and its identification

Seeds were procured from the local market, Lahore in July 2015 and authenticated by Dr. Iqbal Niazi

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Animal Ethics Committee of the University. The study was conducted in accordance with the Institutional diet and water during the entire period. The experimental daylight as well as 12 h in dark. Rats were fed on standard. They were kept in the animal house at 25°C, and 12 h stored in the refrigerator.

**Extraction of plant specimen**
The extraction of plant specimen was carried out through a continuous extraction method using a Soxhlet apparatus. 150 g of dried powdered seeds were taken and extracted with 1.5 L of $n$-hexane. The powdered seeds were filled in a thimble and placed in the Soxhlet apparatus for extraction in a round bottom flask, which filled with $n$-hexane. The extraction of plant specimen was started with $n$-hexane solvent for 72 h until the solvent in Soxhlet thimble become colorless. The residue obtains after $n$-hexane extraction treated with chloroform and then methanol through following the same procedure. The excess solvent was evaporated using a rotary evaporator and extract obtained after evaporation freeze dried and stored in the refrigerator.

**Ethical approval**
The ethical approval for this study was obtained from the Institutional Research Committee of the university.

**Test animals**
Wistar rats were obtained from the Department of Microbiology, the University of Lahore, Pakistan. The average weights of rats fall within the range of 200–250 g. They were kept in the animal house at 25°C, and 12 h daylight as well as 12 h in dark. Rats were fed on standard diet and water during the entire period. The experimental study was conducted in accordance with the Institutional Animal Ethics Committee of the University.

**Acute toxicity test**
The dose of crude extract was prepared in four different concentrations: 400, 800, 1600, and 3200 mg/kg body weight. The drug was administered to rat through the oral route with the help of a syringe equipped with the oral gauge. After administration of test drug, rats were observed for first 4 h to check any possible adverse effect. While, 24 h later rat groups were examined again to check the death rate.\[^8\]

**Carrageenan-induced paw edema method for anti-inflammatory activity**
In this study, male Wistar rats weighing 200–225 g were kept in standard conditions and the anti-inflammatory activity performed on them by measuring the paw size of normal hind using plethysmometer. The rats were divided into eight groups: Group 1, Group 2, Group 3, Group 4, Group 5, Group 6, Group 7, and Group 8. Each group contained four rats. Doses of methanolic, $n$-hexane, and chloroform extract were prepared in two different concentrations: 500 and 1000 mg/kg. All doses were calculated and prepared carefully. Group 1 was given normal saline dose of 10 mL/kg, Group 2 was given diclofenac sodium dose of 10 mg/kg, Groups 3, 4, and 5 were given chloroform, $n$-hexane, and methanol at the dose of 500 mg/kg, and Groups 6, 7, and 8 were given 1000 mg/kg dose of extract. Carrageenan (1%) was injected subcutaneously in the right paw of each rat after half an hour of the administration of the above doses. Immediately after the injection of carrageenan, the paw size was measured through a plethysmometer to obtain 0-h readings. Then after 1, 2, 3, and 4 h, paw size again measured with a plethysmometer and the difference in paw size among drug-treated rats was compared with the controlled groups. The percentage inhibition of edema was calculated through the following formula:\[^7\]

\[
\text{Percent inhibition} = \frac{A - B}{A} \times 100,
\]

where $A$ is the volume of edema of controlled paw and $B$ is the volume of edema of tested paw.

**Results**

**Acute toxicity test**
Acute toxicity test of *T. ammi* seeds was performed on Winster rats to check its adverse effect, as well as physiological behavior and safety. The crude seed extract was administered to rats up to 3200 mg/kg six deaths noted after 24 h and at the dose of 1600 mg/kg side effects such as skin allergy (red spot on skin) difficulty in breathing and convulsions were observed. Yet at dose 800 mg/kg, no such effects were noted and results showed that side effects of seed extract were dose-dependent and increased when dose increased.

**Anti-inflammatory activity**
The anti-inflammatory activity of *T. ammi* was examined on the rat at the dose of 500 and 1000 mg/kg through carrageenan-induced paw edema. Table 1 describes the percentage of inhibition of paw edema at dose of 500 mg/kg and 1000 mg/kg. Edema induced by the carrageenan was more significantly reduced by diclofenac sodium drug at the dose of 10 mg/kg, whereas the plant extract of $n$-hexane was most effective than other two extracts on both doses of 500 and 1000 mg/kg. Diclofenac sodium and plant extract have a peak effect on the first and second hours after administration of doses and then the response was gradually decreased up to 4 h. The chloroform extract was least effective than other extracts. Table 2 shows results of anti-inflammatory activity of *T. ammi* seeds extracts through measuring paw size analysis of variance (ANOVA) test revealed that Winster rat
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Discussion

Results obtained from this study proved that *T. ammi* has a prominent anti-inflammatory effect. The anti-inflammatory activity of seeds of *T. ammi* was investigated through the carrageenan-induced paw edema method. The carrageenan-induced edema is a two-phase response; the primary phase is mediated by the production of kinins, histamines, and serotonin, whereas the secondary phase is associated with the production of prostaglandin. This method most extensively used as a fundamental method for screening and to check the effectiveness of anti-inflammatory drugs.[8] The growth of edema relies on the leukocytes (polymorph nuclear), kinins and prostaglandin, histamines, and serotonin release.[9] The exact mechanism of action about anti-inflammatory activity of *T. ammi* seed extracts was unknown. But it was thought that anti-inflammatory activity of test drug was exerted by blocking the entrance of harmful stimuli into cells, that can reduce the generation of irritants and therefore also decrease the number of writhing in rats. Furthermore, inflammation is regulated centrally by involving different complex procedures[10] and it was thought that *T. ammi* seed extracts produce anti-inflammatory effect by different central mechanism (potentiating in neurotransmission of GABA) and as well as through peripheral mechanism, which inhibits endogenous compounds, leukotrienes and prostaglandins which are main components involve in inflammation.[2,11]

However, anti-inflammatory effect among test groups (rats groups treated with plant extract and standard drug) was noted through the reduction in paw volume and this effect may illustrate through inhibition or blockage of these mediators’ release. Inflammation is a protective phenomenon of the body which involves several complex actions along with mediators of inflammation that can provoke the number of disorders.[12] Therefore by treating with anti-inflammatory agents results in the reduction of those pathophysiological conditions associated with inflammatory reactions.[13]

Percentage inhibition of *T. ammi* at the dose of 500 mg/kg was noted and according to results percentage inhibition continuously increased with time up to 2 h and then started decreasing. Diclofenac sodium showed more consistent results at the dose of 10 mg/kg and the percentage of inhibition increased

| Table 1: Percentage inhibition of paw edema at dose of 500 and 1000 mg/kg |
|-----------------------------|--------------|--------|--------|--------|--------|--------|
| Treatment                  | Dose (mg/kg) | 0 h    | 1 h    | 2 h    | 3 h    | 4 h    |
| Diclofenac sodium          | 30           | 22.8   | 24.77  | 29.68  | 26.31  | 21     |
| Methanolic extracts        | 500          | 7.6    | 9.17   | 12.5   | 8.77   | 7.80   |
|                             | 1000         | 13.04  | 17.43  | 17.96  | 10.52  | 8.65   |
| *n*-hexane extract         | 500          | 6.17   | 6.88   | 13.28  | 14.03  | 10.57  |
|                             | 1000         | 17.39  | 20.01  | 22.21  | 18.42  | 18.26  |
| Chloroform extract         | 500          | 7.6    | 8.25   | 10.15  | 10.52  | 7.00   |
|                             | 1000         | 10.86  | 12.84  | 15.62  | 11.4   | 7.69   |

| Table 2: Results of anti-inflammatory activity of *Trachyspermum ammi* seeds extracts through measuring paw size |
|---------------------------------------------------------------|--------------|--------|--------|--------|--------|--------|
| Treatment                  | Dose (mg/kg) | Normal paw size | 0 h    | 1 h    | 2 h    | 3 h    | 4 h    |
| Normal saline              | 10           | 0.742±0.01      | 0.926±0.11 | 1.091±0.01 | 0.901±0.01 | 1.14±0.05 | 1.04±0.05 |
| Diclofenac sodium          | 30           | 0.595±0.13      | 0.826±0.14 | 0.821±0.11 | 0.901±0.03 | 0.842±0.02 | 0.831±0.01 |
| Methanol extract           | 500          | 0.756±0.06      | 0.890±0.07 | 0.99±0.09  | 1.12±0.09  | 1.04±0.08  | 0.97±0.04  |
|                             | 1000         | 0.701±0.01      | 0.800±0.02 | 0.901±0.07 | 1.05±0.08  | 1.02±0.07  | 0.95±0.04  |
| *n*-hexane extract         | 500          | 0.805±0.03      | 0.867±0.09 | 1.015±0.14 | 1.11±0.16  | 0.983±0.12 | 0.931±0.09 |
|                             | 1000         | 0.661±0.12      | 0.910±0.14 | 0.853±0.08 | 0.974±0.14 | 0.983±0.11 | 0.855±0.01 |
| Chloroform extract         | 500          | 0.775±0.10      | 0.901±0.09 | 1*±0.14   | 1.15±0.14  | 0.983±0.04 | 0.97±0.02  |
|                             | 1000         | 0.73±0.11       | 0.876±0.12 | 0.95±0.14  | 1.08±0.15  | 0.983±0.22 | 0.961±0.19 |

The result presented in table form explains the anti-inflammatory activity of standard drug and plant extract by measuring the size reduction in paw edema. All values were reported as mean ± SD and analyzed by one-way analysis of variance (ANOVA) test and results were significant range from *P < 0.05 and **P < 0.01. Values were closer to P < 0.01 after 2 h of dose administration.
from 22.8% to 29.68% after 2 h of carrageenan injection, but from plant extracts, \(n\)-hexane showed more anti-inflammatory activity than methanol and chloroform, respectively. Percentage inhibition of *T. ammi* at the dose of 1000 mg/kg given in Table 1 showed that reduction in inflammatory edema was dose-dependent as the dose of plant extract increased the anti-inflammatory action also increased. But when compared with standard drug plant extract was less effective and between plant extracts, \(n\)-hexane displayed more anti-inflammatory activity about 22.21% than the other two extracts.

**Conclusion**

In conclusion, extracts of *T. ammi* have anti-inflammatory potential, but standard drug (diclofenac sodium) was most effective and showed more percentage inhibition of paw edema. Furthermore, among plant extracts, \(n\)-hexane was most effective at the maximum dose (1000 mg/kg) as compared to other extracts. The exact mechanism of action of plant extract for its anti-inflammatory activity was unknown and further studies needed to explore the exact mechanism. In addition to this, some further tests are required to find out the active ingredients among plant extract, which are responsible for the anti-inflammatory activity.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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