EVALUATION OF ANTIEPILEPTIC ACTIVITY OF THE METHANOL EXTRACT OF TRACHYSPERMUM AMMI (L.)

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Abstract - This study aims to investigate the effect of a methanol extract of Trachyspermum ammi (L.) as an antiepileptic agent. Tests were conducted with a single- and multiple-dosing schedule of Trachyspermum ammi (L.), using a strychnine-induced seizure model for epilepsy. Twenty-one animals were divided into three groups; control (vehicle), standard (diazepam) and test (Trachyspermum ammi (L.) extract). Trachyspermum ammi (L.) demonstrated antiepileptic effects, since there was a highly significant delay in the onset of convulsions as compared to the control, whereas the percentage of animals that survived or ignored seizure was also greater compared to the control. However, the duration of convulsions was significantly increased with both Trachyspermum ammi (L.) and diazepam as compared to the control. The methanol extract of Trachyspermum ammi (L.) showed antiepileptic activity, which may be due to the presence of thymol.

Key words: Trachyspermum ammi (L.), strychnine, thymol, benzodiazepines, antiepileptic

INTRODUCTION

Herbs have, from prehistoric times until today, been used as a cure for many diseases. A number of antiepileptics are currently on the market but almost all are associated with some limitations. Hence, there is a considerable increase in the demand for medicinal plants (Perucca et al., 1993).

Trachyspermum ammi (L.) (Ajwain) belongs to the family Umbelliferae and is a popular spice in the Indo-Pak region. It is a small, egg shaped fruit, grayish in color, similar to that of the Bishop’s weed plant; because of their seed-like appearance, the fruit pods are sometimes called Ajwain seeds or mistakenly Bishop’s weed (Zachariah, 2008).

The phytochemical studies on Trachyspermum ammi (L.) seeds have demonstrated the presence of many constituents, including an aromatic volatile essential oil and a crystalline substance, stearoptene, also known as Ajowan-ka-phul or crude thymol (As-
The seeds contain essential oil that has about 40-50% thymol (Uma et al.1993; Nagalakshmi et al., 2000; Gersbach and Reddy, 2002).

MATERIALS AND METHODS

This experimental study was conducted in the Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi, after obtaining approval from the Board of Advanced Study and Research (BASR), University of Karachi.

Animals

Twenty-one locally bred male rats (180-220 g) were used. The rats were divided into three groups; control, standard and test, with 7 rats in each group. The rats were housed at the animal house of the Department of Pharmacology, University of Karachi, under controlled conditions of temperature (22±2°C) and humidity (50 to 60%) in an alternating 12 h light/dark cycle. The animals were kept in plastic cages and were given standard diet and water regularly. The use of animals in this experiment was in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Plant material and preparation of extract

The seeds of Trachyspermum ammi (L.) were purchased from a local herbal store in Karachi, and identified by the Center for Plant Conservation Herbarium and Botanic Garden. The voucher specimen was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi and the number TA-10-12 dated 03.27.12 was issued. The seeds were rendered free from all impurities manually and then 500 g seeds were soaked in 1500 ml of 99% methanol in a transparent glass bottle for 30 days until becoming dark green in color. Soaked material was then filtered through filter paper and the filtrate was collected separately. The filtrate was subjected to rotary evaporation and sent to the Hussain Ibrahim Jamal (HEJ) Research Institute of Chemistry, University of Karachi, for the procedure of freeze-drying. The extract of Trachyspermum ammi (L.) was given orally at a dose of 50 mg/kg. This is the minimum dose observed to produce pharmacologically significant effects (Hejazian et al., 2007).

Drugs

Diazepam tablets were purchased from a local medical store in Karachi. Gum tragacanth powder and strychnine was purchased from Merck. Five mg Diazepam tablets were crushed to a powder and suspended in 2% gum tragacanth, which was administered orally (1 mg/kg) with the help of an orogastric tube (Martinez et al., 2007).

Gum tragacanth powder was used as a suspending agent to prepare a suspension of the test substance and standard drug. It was also given to control animals at a dose of 10 ml/kg orally. Two % suspensions of the powder were prepared freshly at the time of administration (Madhu et al., 2009).

Strychnine was diluted in distilled water and was then administered intraperitoneally at a dose of 4 mg/kg for the induction of seizures (Duraisami et al., 2009).

Strychnine-induced seizures

Twenty-one male albino rats were divided into three groups, control, standard and test, and were given 2% gum tragacanth, diazepam and Trachyspermum ammi (L.) extract in respective doses for 14 days. On day 14, strychnine was administered (4 mg/kg; i.p.) to all animals 40 min after the administration of gum tragacanth, diazepam and the Trachyspermum ammi (L.) extract. The time of onset of convulsions, duration of convulsions and percentage of animals to survive or avoid seizures was noted. Strychnine produces seizures by directly antagonizing the inhibitory spinal cord and brain stem reflexes and by increasing spinal reflexes (Biggio et al., 2002). Rats that did not convulse 30 min after strychnine administration were considered protected (Duraisami et al., 2009).
Table 1. Comparison of Trachyspermum ammi (L.) and diazepam on strychnine-induced seizures in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses</th>
<th>Onset of convulsion (minutes)</th>
<th>Duration of convulsion (minutes)</th>
<th>Animals Survived or Avoided Seizures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gum tragacanth (Control)</td>
<td>10ml/kg</td>
<td>0.1457±0.010</td>
<td>0.880±0.14</td>
<td>0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1mg/kg</td>
<td>2.57±0.81*</td>
<td>10.19±2.4*</td>
<td>71.4</td>
</tr>
<tr>
<td>Trachyspermum ammi</td>
<td>50mg/kg</td>
<td>6.25±0.51**</td>
<td>5.47±1.2*</td>
<td>42.8</td>
</tr>
</tbody>
</table>

n=7; Mean ± S.E.M; *P<0.01 significant as compared to control; **P< 0.001 highly significant as compared to control.

**Statistical analysis**

All values were compared with the control by taking the mean and standard error to the mean using the t-test. Values of P<0.01 were considered as significant and P <0.001 as highly significant. All statistical methods were performed using SPSS software version 17.0.

**RESULTS**

Table 1 and Figs. 1, 2, 3 show the comparison of antiepileptic effect after 14 days continuous administration of Trachyspermum ammi (L.) extract (50mg/kg) and diazepam (1mg/kg). The time of onset of convulsions, duration of convulsions and percentage of animals surviving or protected from seizures...
was recorded. The onset of convulsions by *Trachyspermum ammi* (L.) extract was highly significantly delayed, i.e. by 6.25±0.51 min as compared to the control group (0.146±0.01 min), while the delay in onset of convulsions by diazepam was also significant, 2.57±0.81 min compared to the control but less than *Trachyspermum ammi* (L.). The survival rates of animals treated with diazepam and *Trachyspermum ammi* (L.) was 71.4% and 42.8%, respectively, compared to the control group (0%). However, the duration of convulsions was significantly increased with both *Trachyspermum ammi* (L.) and diazepam compared to the control.

**DISCUSSION**

Epilepsy is an important health-care issue, as about 30,000 people around the world develop epilepsy every year. The condition affects about one person in 20 sometime in their lives. There are about 20-70 new cases of epilepsy per 100,000 people per year (Shporvan, 1990). Approximately 1% of the world’s population is suffering from epilepsy, which is the second most common neurological disorder after stroke, and a large burden on health-care systems (Madhu et al., 2009). Evidence has suggested that the imbalance between inhibitory and excitatory neurotransmission in the brain is the main contributor to seizure development in both clinical and experimental conditions (Maria and Hector, 2008).

The present study was conducted to assess the potential of a methanol extract of *Trachyspermum ammi* (L.) as an antiepileptic agent through scientific evaluation.

*Trachyspermum ammi* (L.) exerts its anticonvulsant effect because of the presence of thymol, which excites GABA responses mainly by stimulating human GABAA receptors and increasing the chloride ion channel opening, a mechanism followed by many sedative/hypnotics, CNS depressants and anticonvulsants (Garcial et al., 2005). Hence, it may be concluded that *Trachyspermum ammi* (L.) mimics the antiepileptic effect of diazepam. However, further studies are required on different species and a large number of animals to investigate the exact mechanism of action.

**CONCLUSION**

The methanol extract of *Trachyspermum ammi* (L.) exhibits a potential anti-epileptic effect that could be due to the presence of thymol. It would thus act through a mechanism that is similar to that of benzodiazepines, making it potentially useful in epileptic states.

**REFERENCES**


