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Original Article

EVALUATION OF ANXIOLYTIC ACTIVITY OF METHANOL EXTRACT OF TRACHYSPERMUM AMMI L

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ABSTRACT

Objective: The anxiolytic activity of the methanol extract of *Trachyspermum ammi.* L was investigated by hole board and passive avoidance response test.

Methods: The study was conducted using hole board and passive avoidance response tests. 21 male albino mice and rats for each hole board test and passive avoidance response test were used respectively. In both the experiments animals were equally divided into three groups; control, given 2% gum tragacanth (10 ml/kg), standard given diazepam (1 mg/kg) and test group given 50 mg/kg extract of *Trachyspermum ammi.* L. The data were subjected to analysis by taking mean and standard error to the mean using one sample t-test.

Results: The extract of *Trachyspermum ammi.* L revealed a decrease of 17.43 counts/3 minutes in number of head dips (hole board test) as compared to control, which was almost comparable to diazepam. The decrease in compartment change time (passive avoidance response test) of *Trachyspermum ammi L* treated animals was 107.2 seconds as compared to control. All of the changes were statistically highly significant.

Conclusion: The Methanol extract of *Trachyspermum Ammi. L* showed anxiolytic activity may be due to the presence of thymol in it, which has the mechanism similar to that of benzodiazepines but further studies are needed to reach a final conclusion.

Keywords: *Trachyspermum ammi. L*, Diazepam, Hole board test, Passive avoidance test.

INTRODUCTION

Anxiety is one of the leading psychiatric illnesses today as 1/8th of the population of the world suffers from anxiety disorder. Theoretically, anxiety is an adaptive emotion that permits physiological and behavioral changes, to appropriately react to a stressful situation in order to resolve it by fighting or escaping [1].

In 1980 American Psychiatric association's recognized anxiety disorder as one of the psychiatric illness. Lifetime prevalence of all anxiety disorders in men is 19.2% and in women its 30.5%. The prevalence of pathologic anxiety in the society is very high and is associated with lot of morbidity which can be prevented or cured by early diagnosis and effective treatment. Even in the advance world researchers are exploring their traditional remedies to find a suitable cure for mind affecting diseases [2].

Trachyspermum ammi L has been used traditionally in allergic conditions, colic and dysentery [3]. But recent studies have not only confirmed its traditional uses but also demonstrated its analgesic, anti-inflammatory, hepatoprotective, antispasmodic and antimicrobial effects [4, 5]. *Trachyspermum ammi* L is also known as *Carum copticum* or ajwain and belongs to the family *Umbelliferae*, originated from Middle East, possibly in Egypt.

Pakistan, India and Saudi Arabia are the leading importers [6]. The plant is a small erect, annual shrub, profusely branched, 60 to 90 cm in height with soft fine hair, bearing feathery leaves. It has many branches of leafy stem which has 4 to 6 rays of flowers head, each bearing 6-16 flowers [7].

The volatile oil of the *Trachyspermum ammi* L seeds contains 17 constituents, of which thymol and gamma-terpinene are highest i. e. 39.36% and 30.97%, respectively, while it also contains rho-cymene (19.47%), beta-pinene (5.45%) and alpha-pinene (1.48%) [8]. Thymol can be easily crystallized from the essential oil extract of *Trachyspermum ammi* L seeds [9].

A growing number of people in the world are using herbal products for preventive and therapeutic purposes, but further studies are needed to evaluate the pharmacological effects of herbal medicines, hence this study was conducted on *Trachyspermum ammi L* to investigate its anxiolytic potential.

MATERIALS AND METHODS

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

Animals

21 male albino mice (25-30 g) and locally bred male rats (180- 220g) for Hole board and Passive avoidance response tests were equally divided into three groups respectively. Each group consists of 7 animals, one group treated as a control while the other remaining group received standard (Diazepam) and test (*Trachyspermum ammi* L) drugs. Animals were housed at the animal house of the Department of Pharmacology, University of Karachi, under controlled condition 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 the standard diet and water regularly.

The research committee Department of Pharmacology permitted the use of animals in this experiment in accordance with the guidelines of NACLAR [10] and the National Institute of Health (NIH) for use of laboratory animals [11].

Plant material and preparation of extract

The seeds of *Trachyspermum ammi L* were purchased from a local herbal store in Karachi and identified, by Prof. Dr. Anjum Parveen Director Centre for Plant Conservation Herbarium and Botanic Garden University of Karachi, Karachi-75270. The voucher specimen (TA-10-12) was deposited in the department of Pharmacognosy, University of Karachi. The crude extract was prepared through a cold extraction process [12]. The seeds were rendered free from all impurities manually and then 500 g seeds were soaked in 1500 ml

80% methanol for 30 days with occasional shaking and stirring until it becomes dark green in color. The solvent was filtered through cotton and then through filter paper (Whatman No. 1). After filtration, the methanol extract was evaporated under reduced pressure in a rotary evaporator at 40°C - 45°C and then followed by freeze drying at -30°C. The extract so obtained was kept at -20°C until further used in the dose of 50 mg/kg orally [13]. The resultant yield of the extract was 75g of dry weight.

Drugs

Diazepam tablets were purchased from a local medical store in Karachi and gum tragacanth from Merck. Gum tragacanth suspension was prepared by adding 2 g gum tragacanth powder to 100 ml of warm distilled water which was used to prepare suspensions of extract and standard drug.

Suspensions were prepared freshly at the time of administration [14]. Diazepam 5mg tablets were crushed to powder and suspended in 2% gum tragacanth which was administered orally in a dose of 1mg/kg with the help of oro gastric tube [15], while animals of control group received 2% gum tragacanth in the dose of 10 ml/kg orally.

Hole board test

The Hole-board test measures head-dip activity in experimental animals, decrease in head dipping reflects anxiolytic behavior in rodents [16]. Mice were placed individually in the center of a perforated board and the number of head-dips was counted during a 3 minute trial. The board was made by using plastic floorboard, 40 cm×40 cm×25 cm in which 12 holes (3 cm in diameter each), were equally spaced in the walls. The roof was made of transparent plastic fixed in the center. The exploratory activity of the animal from the holes provides a measure of the number of head dips [15].

Drugs were administered daily through oral route for 14 days and then on fourteenth day test was performed forty minutes after treatment with *Trachyspermum ammi* L at a dose of 50 mg/kg and Diazepam 1mg/kg, while control group was given 2% gum tragacanth at a dose of 10 ml/kg. The extract was initially tested in the dose of 20 mg/kg for 14 days, but no significant response was observed.

Passive avoidance response test

A fear motivated phenomenon known as passive avoidance response is classically used to assess long term and short term memory in experimental animals such as rats and mice. Avoidance or delayed entrance of the animal in to the shock zone or increase compartment change time reflects anxiety like behavior in rodents whereas anxiolytic reduce compartment change time of the animal.

The Passive avoidance response test apparatus (Panlab, S. L, Barcelona- SPAIN) consists of a small black- painted dark compartment having a guillotine gate, which separates it from a large white painted illuminated compartment. This apparatus is controlled by a PC using the SHUTAVOID-01 software.

The test is performed in two phases

Conditioning phase

This phase starts with the exploratory period in which animal was placed in the illuminated white compartment and was allowed to explore it for few seconds leaving the guillotine gate closed. The guillotine gate was opened at the end of the exploratory time and the time elapsed before entering in to the black chamber was recorded (compartment change time or crossing latency).

If the animal could not enter in to the black compartment before a defined cut- off latency time of 90 seconds, the experiment was aborted and the animal was removed from the experiment.

In contrast, as soon as the animal enters the black compartment, the guillotine gate was lowered and a strong electrical shock with specific duration was given through the grid floor. The animal was removed from the black compartment and returned to its home cage immediately. Both compartments were thoroughly cleaned.

Test phase

The test phase was performed 24 hours after conditioning (for long term memory). The animal was placed in the white illuminated compartment for few minutes and the latency between door opening and entry in to the dark compartment was measured (compartment change time).

Better memory performance is indicated by longer latency to enter in the black compartment in the test phase than in the conditioning phase generally up to the maximum time of 180 seconds for mice and 280 seconds for rats [17].

The test was performed 40 minutes after oral administration of extract, standard drug and gum tragacanth in specified doses to the respective groups.

Statistical analysis

The data were subjected to analysis by taking mean and standard error to the mean using one sample t- test, $P< 0.01$ was considered as significant and $P < 0.001$ as highly significant. All statistical methods were performed using SPSS software version 17.

RESULTS

Table 1 reveals the comparison of anxiolytic effect after 14 days continuous administration of the extract (50mg/kg) and diazepam (1mg/kg) by head dip test. There was the highly significant decrease in number of head dips by both *Trachyspermum ammi* L and diazepam treated animal's i. e. 3.00 ± 0.44 and 3.43 ± 0.84 counts/3 minutes as compared to control i. e. 20.43 ± 0.61 counts/minute. Table 2 reveals the comparison of anxiolytic effect of the extract and diazepam measured through passive avoidance response after single dose administration in specified doses. The time interval between door opening and entry of animal into the black compartment was recorded. There was highly significant decrease in the compartment change time (latency test) of both *Trachyspermum ammi* L and diazepam treated animals i. e. 75.7 ± 14 and 74 ± 13 seconds as compared to control i. e. 182.9 ± 11 seconds.

Table 1: **Effect of fourteen days administration of *Trachyspermum ammi* L and diazepam on number of head dips in mice**

Groups	Doses	Head-Dips (counts/3 minutes)
Control	10 ml/kg	20.43±0.61
Diazepam	1mg/kg	3.43±0.84**
Trachyspermum ammi L	50mg/kg	3.00±0.44**

n=7, Mean ± S. E. M, **p< 0.001 highly significant as compared to control

Table 2: **Effect of single dose Administration of *Trachyspermum ammi* L and diazepam on passive avoidance response in rats**

Groups	Doses	Latency time (seconds)
Control	10 ml/kg	182.9±11
Diazepam	1mg/kg	74±13**
Trachyspermum ammi L	50mg/kg	75.7±14**

n=7, Mean ± S. E. M, **p< 0.001 highly significant as compared to control

DISCUSSION

Herbs have been used since prehistoric time as a cure for many diseases. Today active research has been underway to discover safe and pharmacologically active herbs. A number of anxiolytics are currently available in the market but almost all are associated with some limitations. Hence there is a considerable increase in demand for medicinal plants [18].

Present study was conducted to assess the potential of methanol extract of *Trachyspermum ammi* L as anxiolytic agent through scientific evaluation.

Anti-anxiety effect of *Trachyspermum ammi* L might be due to high contents of thymol in the extract which are thought to potentiate GABAA receptors and increase the opening of chloride ion channel, a mechanism followed by many sedatives/hypnotics. CNS depressants and anticonvulsants [19].

Studies on the essential oil of *Ducrosia anethifolia* revealed that it has anti anxiety and sedative effects mainly due to the presence of alpha-pinene, whereas studies on another specie of *Ducrosia Ismaelis also* showed highly significant dose dependent central nervous system depressant effects [20] having alpha-pinene as the major component. More recently study on the essential oil of *Alpinia zerumbet* has also demonstrated anxiolytic effect which contains alpha-pinene [21].

Since alpha-pinene is also present in the essential oil of *Trachyspermum Ammi. L*, hence it may be concluded that present results of *Trachyspermum ammi* L are due to presence of alpha-pinene in its essential oil, however further studies on large number of animals at different doses and species are needed to explore the exact mechanism of action and confirmation of the previous studies.

CONCLUSION

It may be concluded that methanol extract of *Trachyspermum ammi* L revealed anxiolytic activity due to the presence of high thymol contents in it which has been reported to have a mechanism similar to that of benzodiazepines, however further studies are required to reach at definite conclusion.

CONFLICT OF INTERESTS

Declared None.

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