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Original Article

AQUEOUS AND ETHANOLIC EXTRACTS OF TRACHYSPERMUM AMMI EXHIBIT COMPAREABLE ANTI-DEPRESSANT AND ANXIOLYTIC EFFECT TO IMIPRAMINE HYDROCHLORIDE AND DIAZEPAM

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ABSTRACT

The importance of herbal medicines in treating various ailments or diseases is phenomenal and cannot be denied due to their safe use and efficacy; *Trachyspermum ammi* commonly known as ajwain belongs to the family Umbelliferae and a commonly used spice in home cooking. It contains various phytoconstituents which have several pharmacological effects against number of diseases. The objective of the study is to screen the therapeutic potential such as anti-depressant and anxiolytic activities using aqueous and ethanolic extracts of the plant seeds on laboratory animals. Antidepressant activity was seen by both extracts ($p \leq 0.001$) in "forced swim test". Significant anxiolytic activity ($p \leq 0.001$) of both extracts of *Trachyspermum ammi* was observed by "open field test" and "Light and dark activity". Diazepam and Imipramine were used as standard drugs to evaluate anxiolytic and anti-depressant effects respectively. The comparable results of both extracts of *Trachyspermum ammi* with the respective standard drugs are suggestive of enhancing GABA receptors for anxiety suppression while inhibiting MAO to exhibiting antidepressant effects. We can conclude through this study that treatment through natural ways open doors for a new treatment protocol which can benefit us, with least possibility of facing any adverse effect.

Key words: *Trachyspermum ammi*, anxiolytic, anti-depressant, GABA receptors.

Author contributions: The authors have materially participated in the research and compiling the article. 1 Conception, study design, data collection, processing and critical review. 2 & 4 Conception, study design, data collection and processing. 3-4 Data analysis and interpretation. 3 & 4 Drafting of manuscript and critical review and incorporation of recommended corrections.

INTRODUCTION

Depression and anxiety are rampant neuro-pharmacological disorders which are not only limited to deteriorate mental health but also responsible for increasing socioeconomic burden (1). The use of conventional drugs such as benzodiazepines as anxiolytics and SSRIs, SNRIs, MAOIs and TCAs as antidepressants are well recognized. However their wide range of side effects cannot be ignored (2). Currently, a variety of plants are being tested for their potential use against various disorders which is undoubtedly becoming effective due to being cost effective and a fewer side effects (3). Depression is characterized by unhappy frame of mind or lack of interest while excessive fear apprehension and anxiousness characterize the anxiety disorders (4). Gamma-amino butyric acid (GABA) is one of the inhibitory neurotransmitters in the central nervous system which is responsible for anxiety regulation(5). According to the amine hypothesis of mood, the reduction in the levels of nor-epinephrine and serotonin lead to depression. The hypothesis is principally based on the mechanism of action produced by the antidepressants to assuage the symptoms by increasing the levels of nor-epinephrine and serotonin (6).

Depression and anxiety are sometimes undifferentiated disorders in terms of identical display of symptoms, neurotransmitters involved and stress being the predisposing factor. Selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MOAI) are effectively used to treat depression and certain anxiety disorders such as generalized anxiety disorder, panic disorder (PD) and social anxiety disorder (SAD), while some drugs are exclusively used for anxiety such as benzodiazepines. Despite the use of the same drugs, the effective dose is different in both neuroses such that the dose of SSRIs is higher when it is administered to treat depression than in some anxiety disorders (7). The side effects associated with the conventional medications for neuro-pharmacological disorders require the urge to begin the quest for medications with fewer to no side effects (8).

The seeds that are often referred to as fruit are part of *Trachyspermum ammi* plant with therapeutic efficacy. *Trachyspermum ammi* is a spice that belongs to the family Umbelliferae. It is primarily used in Indo Pakistan cuisine and is not only used in food but has also been used since primeval times for medicinal and aromatic purposes (9). Thymol is associated with many pharmacological activities (10). The value of herbal medicines in the treatment of various ailments or diseases is phenomenal and due to their safe use and effectiveness cannot be ignored. The research is therefore intended to assess *Trachyspermum ammi*'s pharmacological activity and therapeutic potential to explore its anxiolytic and anti-depressant activity testing.

The main objective of the study is to represent the importance of herbal treatment, which will provide us many opportunities in developing new treatment protocol with least likely, do good to us in a way, of facing minimum adverse events, providing economical treat protocol, least chances of developing any allergies. We have selected *Trachyspermum ammi*, a very common plant used in our cuisine on daily basis, this magical plant was chosen to identify therapeutic effect on depression and anxiety; a very common ailment we are currently facing globally.

MATERIALS AND METHODS

Plant Material

The seeds of *Trachyspermum ammi* were obtained from local Karachi market in Pakistan. These were then identified and confirmed by a skilled scientist and professor of pharmacognosy, Jinnah University for Women, North Nazimabad, Karachi, Pakistan (herbarium number: JUWH-1008).

Plant Extraction

The aqueous extract was arranged from Herbion (Pvt) Limited, Karachi, Pakistan using 2 kilograms of ground seeds extracted with 20 liters of distilled water and 2 grams of methyl paraben and 400 milligrams of propyl paraben as preservatives (batch number: Ext015/17). The percentage yield was 400 grams (20%).

For ethanolic extract, about 25 g of coarse powdered plant material was extracted by soxhlet apparatus (MS-E103; M Topo) using 300 ml ethanol. Rotary evaporator (Buchi, Switzerland) was used to dry the solvent in order to yield the extract (percentage yield 7.2%).

Animal Selection

24 Albino mice (20-25 grams) of either gender were chosen and held in a controlled environment (temperature of $25\pm 5^{\circ}\text{C}$ with $50\pm 15\%$ of *relative* humidity). Food and water ad libitum was allowed to animals.

2.4 Animal guidelines

For the proper treatment of animals, the guidelines of National Institute of Health guide for Care and use of Laboratory animals (NIH Publications No. 8023, revise 1978) is followed.

2.5 Institutional Approval

All experimental procedures and animal handling was ratified by Board of Advance Study and Research (BASR) of Jinnah University for Women (Resolution number: 5XVII).

2.6 Grouping and drug treatment

Mice were divided into four groups at random ($n = 6$). Group 1 as control group was administered distilled water, group 2 as standard group was given imipramine hydrochloride (10mg/kg) for evaluating anti-depressant activity and diazepam (1 mg/kg) for the determination of anxiolytic activities. While group 3 and 4 were test groups for administration of aqueous and ethanolic extracts (200mg/kg) respectively. The route of administration was oral once daily which continued for 30 consecutive days.

2.7 Behavioral tests

2.7.1 Forced swim test

Forced swim test apparatus is a transparent cylinder of plastic which is 65 cm by height and 25 cm by diameter. Water is filled in the cylinder up to 48 cm having $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ temperature. Each mouse placed in the cylinder, was observed for the struggling time for 5 minutes (11).

2.7.2 Open field test

Open field apparatus is a large square chamber of plastic walls and floor with 60cm x 60cm dimensions marked with 16 equally sized squares. It has central and peripheral areas (12). The OFT measures the locomotion, exploratory behavior and anxiety which is determined by total number of squares covered by animals on 4 paws for 5 minute in the arena (13).

2.7.3 Light and dark test

The apparatus used is divided into two equal compartments, one of which is quite well lit with translucent walls while the other is coated in black. Both compartments are partitioned in the lower center with a small

aperture. For a period of 5 minutes each mouse was put in the lit compartment and the time spent in the dark partition was observed using a stop watch. After the experiment, each mouse was led back to the home cage (14).

2.8 Statistical Analysis

Values are presented as mean $\pm$ S.D n=6. Data analysis was done by SPSS version: 20 using one-way ANOVA with post hoc Tuckey's test. Significant values were less than 0.05. Where 0.001 was denoted as highly significant, 0.01 moderately significant and 0.05 was being the least significant. The effects were marked at 200 mg/kg of test drugs.

Results

3.1 Effect of *Trachyspermum ammi* on forced swim test

Graph I recapitulates the results of forced swim test. The results of our study specify that all treatment groups i.e., group 2, 3 and 4 exhibited a highly significant increase ($p < 0.001$) in mobility time when compared to group 1 on day 30. No significant difference could be seen when group 3 and 4 drugs were compared with group 2 presenting the comparable effects.

3.2 Effect of *Trachyspermum ammi* on open field test

Graph II summarizes the results of open field test. The results of our study postulate that all treatment groups i.e., group 2, 3 and 4 exhibited a highly significant increase ($p < 0.001$) in exploring the peripheral boxes when compared to group 1 on day 15 and day 30 except for the group 4 which showed the activity only on day 30. No significant difference could be seen when group 3 and 4 were compared with group 2, thus, exhibiting the comparable effects. On the other hand, there was no significant change established in central box exploration by the test drugs compared to group 1 on any day of the treatment. However, group 2 displayed moderately significant increase ($p < 0.01$) in central arena crossing on day 15 when compared with group 1.

3.3 Effect of *Trachyspermum ammi* on light and dark test

Graph III sums up the results of light and dark test. The outcomes of our study stipulate all treatment groups i.e., group 2, 3 and 4 exhibited a highly significant increase ($p < 0.001$) in time spent in lit compartment when compared to group 1 on day 15 and day 30. No significant difference could be seen when the test drugs (group 3 and 4) were compared with group 2 showing the similar effects.

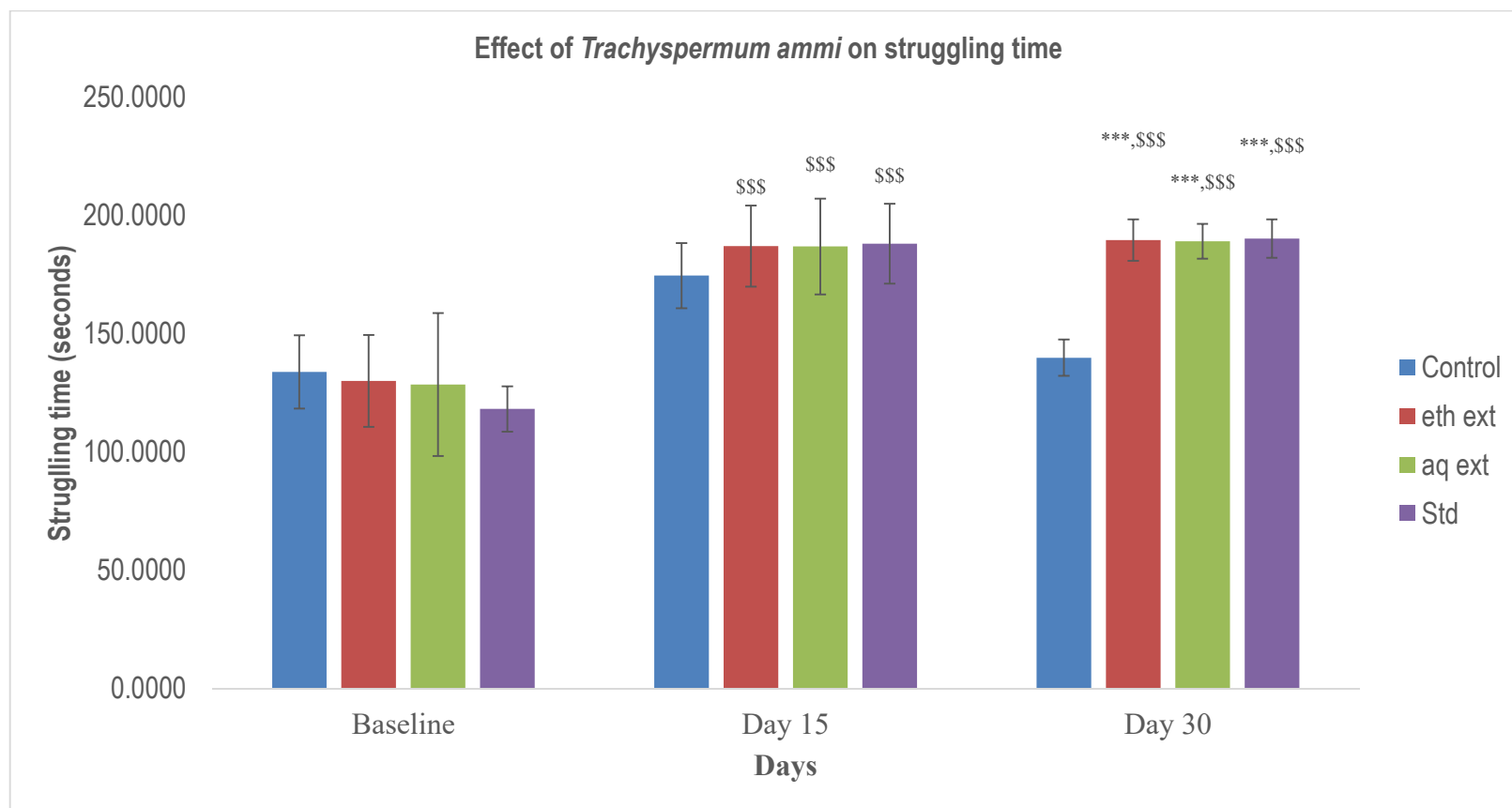


Figure 1: Effect of *Trachyspermum ammi* on forced swim test.

P*** ≤ 0.001 when compared with control.

4' P\$\$\$ ≤ 0.001 when compared to the baseline reading

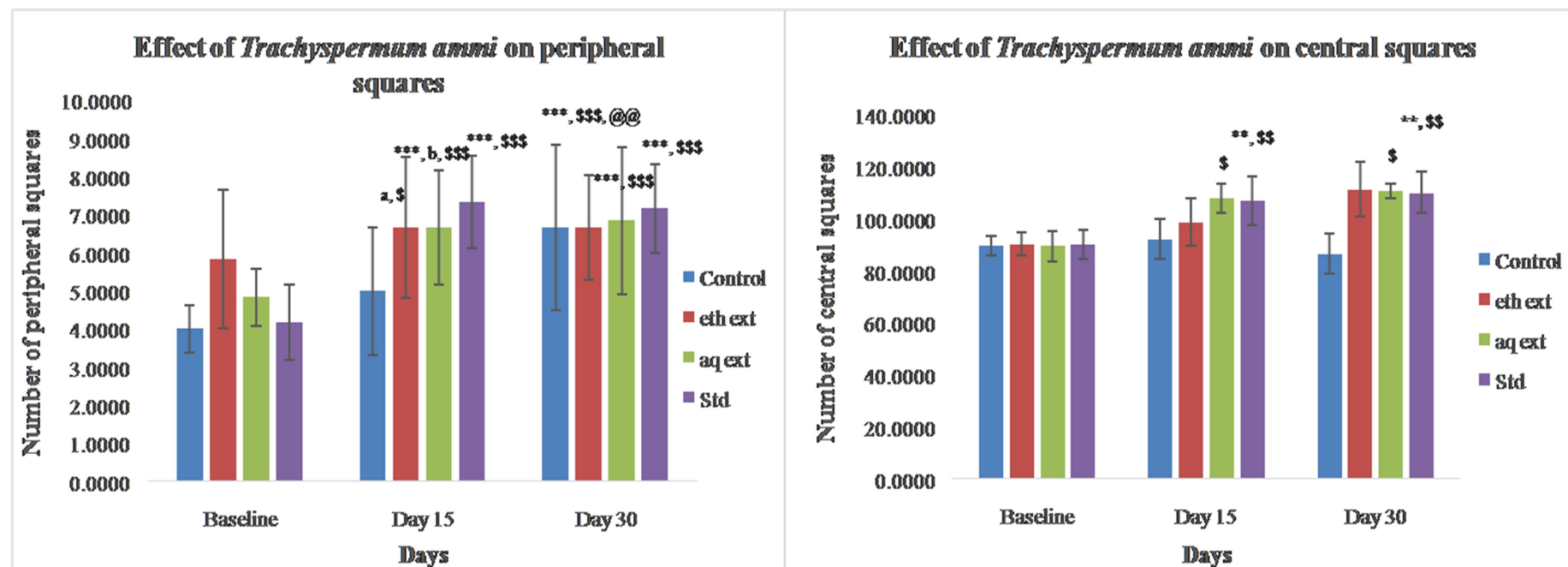


Figure 2: Effect of *Trachyspermum ammi* on open field test.

P**≤ 0.01, P***≤ 0.001 = when compared with control. Pa≤ 0.05 = when compared with standard.
Pb≤ 0.05 = when aqueous extract compared with ethanolic extract. P\$≤ 0.05, P\$\$≤ 0.01, P\$\$\$≤ 0.001 =
when compared to the baseline reading. P@@≤0.01 = when day 15 is compared with day 30

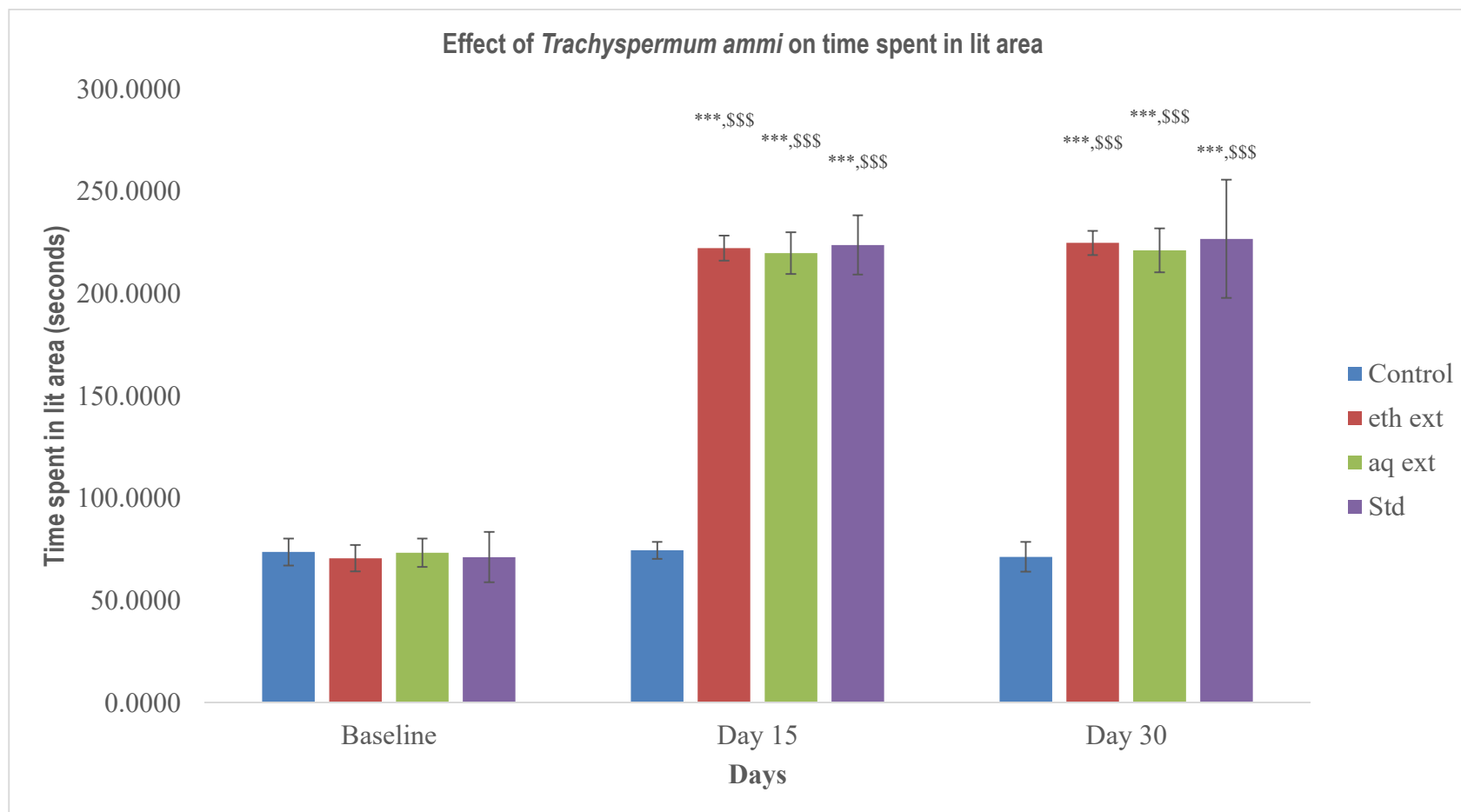


Figure 3: Effect of *Trachyspermum ammi* on light and dark test.

P*** ≤ 0.001 = when compared with control.

P\$\$\$ ≤ 0.001 = when compared with baseline.

DISCUSSION

In the current study, aqueous and ethanolic extracts of *Trachyspermum ammi* were used to appraise the anti-depressant and anxiolytic properties of the plants in laboratory animals using different behavioral tests such as forced swim test, open field test and light and dark exploratory test. Various studies have reported several hypothesis for the cause of depression, which is not as simple as reduced levels of neurotransmitters (15). Another possible concept is promotion of neurogenesis by anti-depressants which indirectly keeps the levels of neurotransmitters normal (16). *Trachyspermum ammi* showed comparable anti-depressant activity to imipramine hydrochloride. Imipramine is a tricyclic antidepressant which increases the levels of serotonin and norepinephrine by inhibiting the activity of monoamine oxidase enzyme. Hence the possible mechanism of action could be that of the standard drug belonging to monoamine oxidase inhibitors (MAOI) (17). The essential oil, thymol present in *Trachyspermum ammi* has been reported to have CNS effects and a study proved its ability to increase the levels of the neurotransmitters in hippocampus in various depression models (18).

The forced swim test (FST) is used to evaluate antidepressant-like effects which involves the observation of mobility (swimming and struggling) or immobility time of rodents finding no escape. If the immobility time is reduced or mobility or struggling time is increased, both indicate the antidepressant effect of a test drug (19). The results of our study showed that both extracts of the plant possess the antidepressant activity and have comparable results to the standard drug, imipramine hydrochloride.

For investigating anxiolytic activity of drugs, open field test can be used for evaluating emotional state of rodents. Animals express fear in terms of lack of movement and exploratory behavior when they are commenced to a new surrounding that they are not used to (20). Increased peripheral square crossing was seen by both test groups with comparable effects to standard drug diazepam. However, aqueous extract exhibited increased number of peripheral squares crossed compared to ethanolic extract.

Anxiolytic effects of drugs can also do be predicted by light and dark exploratory test. The greater time spent in the light compartment is a measure of anxiolytic-like activity which reveals reduced aversion toward light area. Normally animals prefer dark compartment as a display of anxiety (21). According to our study group 3 and 4 of *Trachyspermum ammi* spent more time in lit compartment showing anxiolytic effects of the test drug. Thus the effects were equivalent to the standard drug diazepam.

The anxiolytic effects of our test drugs tested on various behavioral paradigms were comparable with the standard drug diazepam, which is a conventional anxiolytic (benzodiazepine) that mainly targets GABA receptors, causing the conformational changes in GABA receptors and leading the chloride influx. Thus, causing hyperpolarization of neuron, which in turn reduces the excitability and produces the calming effect (22). The presence of flavonoids in *Trachyspermum ammi* (23) are indicative of the anxiolytic activity of the plant as the GABA receptor mediators. Flavonoids are compounds with low molecular weight found in higher plants as secondary metabolites, possessing diverse pharmacological activities (24). Also in another study, it is reported that thymol precursors (para-cymene and gamma-terpinene) are responsible to have

anxiolytic properties (25) by enhancing the GABA responses (26). It is reported in a study that *Ducrosia anthefolia* displayed as anti-depressant and anxiolytic effects due to the occurrence of alpha pinene the plant's essential oil which is also present in *Trachyspermum ammi*'s essential oil (27)((28).

CONCLUSION

It can be inferred that herbal drugs are considered to be even effective than modern medications with the lowest possible undesirable side effects, likely due to the presence of specific phyto-constituent compounds. Further studies are desirable to understand the exact mechanism involved in *Trachyspermum ammi*'s antidepressant and anxiolytic activities.

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