IJP (2017), Vol. 4, Issue 5 **(Research Article)**

Received on 27 February 2017; received in revised form, 20 April 2017; accepted, 25 April 2017; published 01 May 2017

ANXIOLYTIC ACTIVITY OF *TRASCHYSPERMUM AMMI* LEAVES

Safeena Nazeer [*1], Usman Ghani Khan [2] and Safila Naveed [3]

Department of Pharmacognosy [1], Clinical Pharmacy and Health Care [2], Department of Pharmaceutical Chemistry [3], Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan.

Keywords:

Anxiety, *Trachyspermum ammi*, Plant extracts, Light and dark model

Correspondence to Author:
Safeena Nazeer

Department of Pharmacognosy, Jinnah University for Women, Karachi, Pakistan.

E-mail: Safeeenanazeer@gmail.com

ABSTRACT: Anxiety is an obnoxious condition of inner disorder, often accompanied by thoughts, somatic complaints, and nervous behavior. Anxiety is initiated by external stimuli. It may be as a consequence of any underline disease condition such as Parkinson's disease, rheumatoid arthritis, or diabetes. There are different types of anxiety which are a social anxiety disorder, selective mutism, agoraphobia, specific phobia, panic attack, and separation anxiety disorder. In this study anxiolytic activity of methanolic and ethanolic extract of Leaves of *Trachyspermum ammi* has been determined. For this purpose methanolic and ethanolic extract of *Trachyspermum ammi* leaves were prepared and evaluate anxiolytic activity *in-vivo* on mice by using light and dark model of anxiety. Data analysis by using two way ANOVA gives highly significant results, *i.e.* (P<0.000) for both methanolic and ethanolic extract of *Trachyspermum ammi* leaves.

INTRODUCTION: Anxiety is an obnoxious condition of inner disorder, often accompanied by thoughts, somatic complaints and nervous behavior [1] when anxiety gets to be distinctly outrageous, it might be accepted as an anxiety disorder, and can fundamentally decrease the personal satisfaction actuating various psychosomatic ailments. Anxiety can be characterized as "a state of strong apprehension, hesitation, and dread that outcome from the desire of an alarming circumstance or scene, much of the time to the degree that hinders in the common physical and psychological functions" [2]. The American Association of Psychiatry declares that all types of anxiety disorder contribute to characteristics of fear plus anxiety.

QUICK RESPONSE CODE

DOI:

10.13040/IJPSR.0975-8232.IJP.4(5).179-84

The article can be accessed online on www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.4(5).179-84

"Fear is the emotional reaction to the perceived or genuine threat, though anxiety is an expectation of future risk" [3]. Symptoms of anxiety differ from individuals to individuals since it depends on the type of anxiety disorder except that common symptom of all anxiety disorders are categorized into 2 that is a physical and psychological sensation. In physical sensation patient go through insomnia, nausea, trembling as well as itchy feet and hands, palpitation, sweating or cold on hand or feet, dry mouth, churning in the stomach, shaking, feeling light headache, panic attacks, increase in blood pressure and tension in the muscle.

In psychological anxiety patient go through dread of worst, nervous, restlessness, focus, feeling that people will laugh at you, feeling of discomfort, irritability, feeling like the world is speeding up or slowing down, and on bad experience thinking many times on the situation [4]. The exact reason for anxiety is obscure. Anxiety is initiated by external stimuli. It may be as a consequence of any underline disease condition such as Parkinson's

disease [5], rheumatoid arthritis [6], or diabetes. Major depressive disorder [7] and a diminished measure of inhibitory neurotransmitter, *i.e.* GABA likewise the reason for anxiety disorder [8]. One more cause of anxiety is environmental conditions like stress, parenting factor, [9], socioeconomics [10], history of any trauma [11], cultural factor, *etc.* Chronic use or drug abuse or with drawl of many drugs that includes central nervous stimulant like caffeine, tobacco, sedatives, or alcohol may likewise be the reason for anxiety [10]. Stress identified with an individual relationship, worry at workplaces, worry because of fund and demise of a dearest individual or the oxygen insufficiency in conditions, these elements or combination of above causes may add to anxiety. Types of anxiety are a social anxiety disorder, selective mutism, agoraphobia, specific phobia, panic attack, and separation anxiety disorder [3]. As per the severity of anxiety treatment of anxiety can be changed. Normally treatment of anxiety starts with adjustment in the nourishment intake and also an amendment in the way of life. Psychotherapy is likewise utilized for anxiety treatment. But change over to medications when these remedies are not efficient [12].

Classification of Anxiolytics:

TABLE 1: CLASSIFICATION OF ANXIOLYTICS

Pharmacological class	Drugs	Adverse Effects
Selective Serotonin Reuptake Inhibitor	Paroxetine, Fluoxetine, Fluvoxamine, Sertraline, Escitalopram	Nausea, insomnia, headache, diarrhea, sexual dysfunction, somnolence
Serotonin-Norepinephrine Reuptake Inhibitor	Duloxetine, Venlafaxine	Nausea, insomnia, headache, diarrhea, sexual dysfunction, somnolence, hypertension
Benzodiazepines	Diazepam, Chlordiazepoxide, Lorazepam Alprazolam, Clonazepam, Oxazepam.	Appetite change, cognitive problems, somnolence, fatigue, (Effects of class)
Tricyclic Antidepressant	Doxepine, Clomipramine, Imipramine	Dry mouth, urinary retention, weight gain, constipation, dizziness, orthostasis, somnolence, sexual dysfunction, (Effects of class)
MAO inhibitor	Phenelzine	Dry mouth, orthostasis, sexual dysfunction, constipation, weight gain, dizziness, headache, somnolence
Antihistamine	Hydroxyzine	Dry mouth, headache, sedation, dizziness
Other	Buspirone	Nausea, headache, dizziness

Presently available treatments are effective for about two third of the anxiety patients. Furthermore, anxiety disorder also produces various systemic adverse effects and show tolerance and dependence to the long term treatment which now turns into a major concern about the currently using medications [13] so modern sciences is in search of a drug which has greater efficacy, minor unwanted effects having least or no dependence as well as tolerance.

Herbs which are extensively established resource of medicine, that take part a significant role in the programme of health care worldwide [14]. Therefore various conventionally used plant show pharmacological action with prospective therapeutic uses in the cure of CNS disorders, like anxiety [15, 16]. As a consequence of the increasing demand of people for herbal medicines in this study we try to evaluate the anxiolytic property of *Trachyspermum ammi* leaves. *Trachyspermum ammi (T. ammi)* is an inhabitant of Egypt and cultivated in many countries like Pakistan, Iraq, Afghanistan, and Iran. In several cities of India, it is also grown like Maharashtra, Madhya Pradesh, Gujarat, Uttar Pradesh, Rajasthan, Bihar and West Bengal [17].

T. ammi is extensively cultivated in scorched and semi-scorched areas [17] where soil has elevated level of salts [18]. *Trachyspermum ammi* is generally used as a spice in curries because of its characteristic aroma & pungent taste. *T. ammi* seeds are utilized in a minute amount to give flavor to the number of foods, also utilize in medicine as a preservative as well as in perfumery for the production of its essential oil [19].

Medicinally it is used in India, and used as a home remedy for treating disorders of the stomach, for

relieving colic pains (fruits are crushed and make a paste then externally applied), and for treatment of asthma hot and dry fomentation of *T. ammi* fruit apply on chest [20]. *Trachyspermum ammi* has been revealed to have anthelmintic; antihyperlipidemic, anti-aggregatory effects [21-23], insecticidal [24], kidney stone inhibitory [25], antimalarial [26], molluscicidal [27-29]; for the treatment of amenorrhoea *T. ammi* seeds are sopped in juice of lemon along with *Prunus amygdalus* (badam) [30] and also used as antipyretic, febrifugal as well as in the treatment of typhoid fever [31, 32].

FIG. 1: LEAVES OF *TRACHYSPERMUM AMMI*

MATERIALS AND METHODS: To study the anxiolytic activity of *Trachyspermum ammi* we experimented on mice. At first, we prepared an ethanolic extract of *Trachyspermum ammi* leaves and then methanolic extract of *Trachyspermum ammi.* For the preparation of ethanolic extract, we purchase leaves of *Trachyspermum ammi* from the local nursery of Karachi which were identified by Prof. Dr. Iqbal Azhar, Dean, Faculty of Pharmacy, University of Karachi.

For the preparation of ethanolic extract, first leaves were separated from their stems then washed with distilled water then dry it. Dried leaves were then ground by the help of mortar and pestle and then macerated with 250 ml of ethanol for 15 days at room temperature after that filter it by the help of Watmann filter paper. The filtrate is then allowed to dry at room temperature resultant paste is of dark green and, *i.e.* an ethanolic extract. For the preparation of methanolic extract, residue obtains from the filtrate of ethanolic extract was then macerate with 250 ml of methanol for 15 days after that filter it by the help of Watmann filter paper. Filterate is then allowed to dry at room temperature resultant paste is of dark green and, *i.e.* a methanolic extract.

Animals: To experiment with Swiss albino mice with average weights of 20 g were selected. Mice were taken from the animal house of Jinnah University for Women, Karachi. Mice are kept under standard conditions with 12 h day and night cycle in the animal house. Mice were familiarized to laboratory conditions minimum of 1 h before they initiate the experiment. Noise, light, and temperature should remain the same for all mice. Fecal matter and Urine are removed after each experiment to clean the apparatus 70% ethanol is used.

Grouping: We take 18 mice and divided into 3 groups which are Group 1 that is the controlled group that receives normal saline, Group 2 that receives a methanolic extract of *Trachyspermum ammi* leaves, Group 3 that receives an ethanolic extract of *Trachyspermum ammi* leaves.

Treatment Schedule: Group 1 was treated with normal saline. Group 2 was treated with ethanolic extract of *T. ammi* leaves, and Group 3 was treated with a methanolic extract of *T. ammi* leaves. The doses of extracts were calculated to administer 2 mg/ml of the extract solution. The dose was given once daily. It is a 45 days study. Anxiolytic activity was examined by using the light/dark box.

Light and Dark Box: Light and dark test is another commonly used anxiety model. This test focuses on the intrinsic hatred of animals towards illuminated regions that are bright as well as on the exploratory behavior of animals in the reaction of stressor that is mild that is novel light & experiment. The light and dark model allows an animal to freely explore 2 compartments which are interconnected; compartments differ in its size (2:1), differ in color (white: black) and also differ in illumination (bright: dim).

Therefore, the mice of the controlled group when placed into the bright light compartment it speedily goes in the darker area. After treated with anxiolytic drug apparent anxiety of remaining in or moving towards the light area is apparent apprehension of remaining in or moving to the light area is eradicated. Since, then the L/D test has been widely adopted as an anxiolytic screening test in

mice, extended for use with rats and has been subject to several modifications [33].

FIG. 2: LIGHT AND DARK BOX

Statistical Analysis: Analysis of experimental data was done in SPSS by making use of two-way analysis of variance (ANOVA) with Scheffe test of Post Hoc.

RESULTS AND DISCUSSION: Data analysis by using two way ANOVA with scheffe test of Post Hoc analysis gives highly significant results, *i.e*. (P<0.000) for both MET and EET that shows a decrease in duration in dark box and increase duration in lightbox throughout the experimental period, *i.e*. day 7-day 45.

TABLE 2: EFFECT OF MET AND EET VERSUS CONTROLLED GROUP IN LIGHT AND DARK MODEL (IN LIGHT COMPARTMENT

Duration	Groups	Mean ± Standard deviation
Day 1	Controlled	25.5±0.54
	MET	79.66±0.81**
	EET	78.5±0.54**
Day 7	Controlled	24.5±0.83
	MET	84.5±0.54**
	EET	82.83±1.16**
Day 14	Controlled	25.83±1.16
	MET	95.66±0.51**
	EET	88.66±1.36**
Day 21	Controlled	25±0.63
	MET	101.66±0.81**
	EET	95.16±0.75**
Day 28	Controlled	25.16±0.98
	MET	108±0.63**
	EET	101.33±0.81**
Day 35	Controlled	25±1.26
	MET	113±2.00**
	EET	104.66±1.21**
Day 45	Controlled	25±1.26
	MET	118.16±0.75**
	EET	110.33±0.81

** p<0.000 is highly significant.

Effect of MET and EET in comparison with the controlled group on mice by using Light and dark model (in the light compartment) has been shown in **Table 2**, the highly significant result has been obtained, *i.e.* (P<0.000).

Effect of MET and EET in comparison with the controlled group on mice by using Light and dark model (in dark compartment) has been shown in **Table 3**, the highly significant result has been obtained, *i.e*. (P<0.000). Comparison of the mean of MET and EET with the controlled group by using the Light and dark model (in the light compartment) has been shown in **Fig. 3**. Comparison of mean of MET and EET with the controlled group by using Light and dark model (in the light compartment) has been shown in **Fig. 4.**

TABLE 3: EFFECT OF MET AND EET VERSUS CONTROLLED GROUP IN LIGHT AND DARK MODEL (DARK COMPARTMENT)

Duration	Groups	Mean ± Standard deviation
Day 1	Controlled	274.5±0.54
	MET	220.33±0.81**
	EET	221.5±0.54**
Day 7	Controlled	275.5±0.83
	MET	215.5±0.54**
	EET	217.16±1.16**
Day 14	Controlled	274.16±1.16
	MET	204.33±0.51**
	EET	211.33±1.36**
Day 21	Controlled	275±0.63
	MET	198.33±0.81**
	EET	204.83±0.75**
Day 28	Controlled	274.83±0.98
	MET	192±0.63**
	EET	198.66±0.8**
Day 35	Controlled	275±1.26
	MET	187±2.00**
	EET	195.33±1.21**
Day 45	Controlled	275±1.26
	MET	181.83±0.75**
	EET	189.66±0.81**

** p<0.000 is highly significant.

Trachyspermum ammi seeds possess anxiolytic effect may be due to extract containing more content of thymol which is considered to potentiate GABA receptors and increase chloride ion channel opening, a mechanism followed by various hypnotics / sedatives, CNS depressants, and anticonvulsants [34]. Studies on the essential oil of *Ducrosia anethifolia* revealed that it has anti-anxiety and sedative effects mainly due to the presence of α-pinene, whereas studies on another

species of *Ducrosia ismaelis* also showed highly significant dose-dependent central nervous system depressant effects [35] having α-pinene as the major component. The more recent study on the essential oil of *Alpinia zerumbethas* also demonstrated anxiolytic effect which contains α-pinene [36]. Since, α-pinene is also present in the essential oil of *Trachyspermum ammi* L., hence it can say that present results of *Trachyspermum ammi* are due to the presence of α-pinene in its essential oil, however further studies on a large number of animals in different extracts at different doses and species are needed to explore the exact mechanism of action and confirmation of the current study.

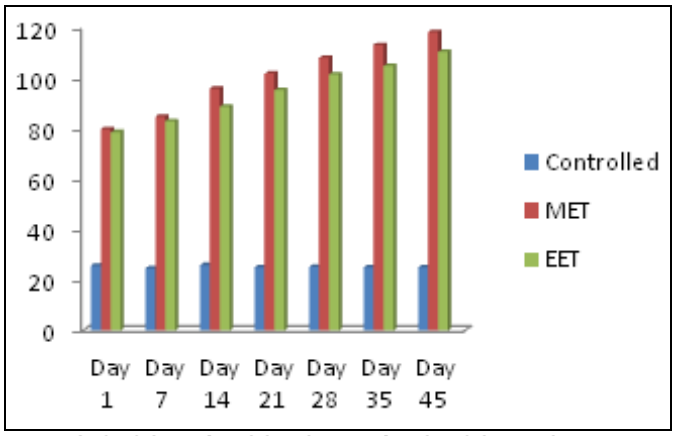

FIG. 3: COMPARISON OF MEAN OF CONTROLLED GROUP *vs.* MET GROUP AND EET GROUP IN LIGHT AND DARK MODEL (LIGHT COMPARTMENT)

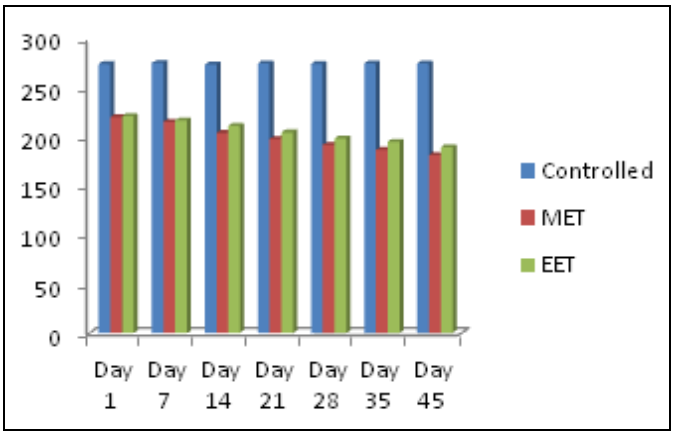

FIG. 4: COMPARISON OF MEAN OF CONTROLLED GROUP *vs.* MET GROUP AND EET GROUP IN LIGHT AND DARK MODEL (DARK COMPARTMENT)

MET: Methanolic extract of *Trachyspermum ammi*, EET: Ethanolic extract of *Trachyspermum ammi*

CONCLUSION: From our study, we may conclude that both methanolic extract and ethanolic extract of *Trachyspermumm ammi* leaves extract on light and dark model possess anxiolytic activity.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Doukkali Z, Taghzouti K, Bouidida EH, Nadjmouddine M, Cherrah Y and Alaoui K: Evaluation of anxiolytic activity of methanolic extract of *Urtica urens* in a mice model. Behavioral and Brain Functions 2015; 11(1): 1.
2. Dictionary A: The American Heritage Medical Dictionary. Houghton Mifflin Company, 2007.
3. Association AP: Diagnostic and statistical manual of mental disorders (DSM). American Psychiatric Association, Washington DC, 1994: 143-7.
4. Bell-Dolan DJ, Last CG and Strauss CC: Symptoms of anxiety disorders in normal children. Journal of the American Academy of Child & Adolescent Psychiatry. 1990; 29(5): 759-65.
5. Schoenhuber R and Gentilini M: Anxiety and depression after mild head injury: a case-control study. Journal of Neurology, Neurosurgery and Psychiatry 1988; 51(5): 722-4.
6. El-Miedany YM and El Rasheed AH: Is anxiety a more common disorder than depression in rheumatoid arthritis? Joint Bone Spine 2002; 69(3): 300-6.
7. Healy D: Psychiatric drugs explained: Elsevier Health Sciences 2008.
8. Etkin A, Prater KE, Schatzberg AF, Menon V and Greicius MD: Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. Archives of General Psychiatry 2009; 66(12): 1361-72.
9. McLeod BD, Wood JJ and Weisz JR: Examining the association between parenting and childhood anxiety: A meta-analysis. Clinical Psychology Review 2007; 27(2): 155-72.
10. Quitkin FM, Rifkin A, Kaplan J and Klein DF: Phobic anxiety syndrome complicated by drug dependence and addiction: a treatable form of drug abuse. Archives of General Psychiatry 1972; 27(2): 159-62.
11. Moore EL, Terryberry-Spohr L and Hope DA: Mild traumatic brain injury and anxiety sequelae: a review of the literature. Brain Injury. 2006; 20(2): 117-32.
12. Dalrymple KL: Combined treatments (medications plus psychotherapy). The Encyclopedia of Clinical Psychology. 2015.
13. Chatterjee M, Verma R, Lakshmi V, Sengupta S, Verma AK and Mahdi AA: Anxiolytic effects of *Plumeria rubra* var. acutifolia (Poiret) L. flower extracts in the elevated plus-maze model of anxiety in mice. Asian Journal of Psychiatry 2013; 6(2): 113-8.
14. Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N and Shimotohno K: Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. Phytotherapy Research 2000; 14(7): 510-6.
15. Gilani A, Jabeen Q, Ghayur M, Janbaz K and Akhtar M: Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the *C. copticum* seed extract. Journal of Ethnopharmacology. 2005; 98(1): 127-35.
16. Ahsan S, Shah A, Tanira M, Ahmad M, Tariq M and Ageel A: Studies on some herbal drugs used against kidney stones in Saudi folk medicine. Fitoterapia 1990; 61(5): 435-8.

17. Ayurvedic Pharmacopoeia of India. The government of India.
18. Ashraf M: Salt tolerance of cotton: some new advances. Critical Reviews in Plant Sciences 2002; 21(1): 1-30.
19. Kaur GJ and Arora DS: The bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family Umbelliferae-Current status. Journal of Medicinal Plants Research 2010; 4(2): 087-94.
20. Mukherjee PK, Kumar V, Kumar NS and Heinrich M: The Ayurvedic medicine *Clitoria ternatea*-from traditional use to scientific assessment. Journal of ethnopharmacology. 2008; 120(3): 291-301.
21. Srivastava K: Extract of a spice-Omum (*Trachyspermum ammi*)- shows antiaggregatory effects and alters arachidonic acid metabolism in human platelets. Prostaglandins, Leukotrienes and Essential Fatty Acids. 1988; 33(1): 1-6.
22. Javed I, Iqbal Z, Rahman Z, Khan F, Muhammad F and Aslam B: Comparative antihyperlipidaemic efficacy of *Trachyspermum ammi* extracts in albino rabbits. Pakistan Veterinary Journal 2006; 26(1): 23.
23. Lateef M, Iqbal Z, Akhtar M, Jabbar A, Khan M and Gilani A. Preliminary screening of Trachyspermum ammi (L.) seed for anthelmintic activity in sheep. Tropical animal health and production. 2006;38(6):491-6.
24. Chaubey MK: Fumigant toxicity of essential oils from some common spices against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Journal of Oleo Science 2008; 57(3): 171-9.
25. Kaur T, Bijarnia RK, Singla SK and Tandon C: Purification and characterization of an anti-calcifying protein from the seeds of *Trachyspermum ammi* (L.). Protein and Peptide Letters 2009; 16(2): 173-81.
26. Mathew N, Misra-Bhattacharya S, Perumal V and Muthuswamy K: Antifilarial lead molecules isolated from *Trachyspermum ammi*. Molecules 2008; 13(9): 2156-68.
27. Singh S, Singh V and Singh D: Molluscicidal activity of some common spice plants. Biological Agriculture & Horticulture 1997; 14(3): 237-49.
28. Singh V, Singh S, Singh S and Singh D: Effect of an active molluscicidal component of spices on different enzyme activities and biogenic amine levels in the nervous tissue of *Lymnaea acuminata*. Phytotherapy Research 1999; 13(8): 649-54.
29. Singh K and Singh D: Effect of different combinations of MGK-264 or piperonyl butoxide with plant-derived molluscicides on snail reproduction. Archives of environmental contamination and toxicology 2000; 38(2): 182-90.
30. Shome U, Rawat A and Mehrotra S: Time tested household herbal remedies. Ethnobiol Human Welf Deep Publications, New Delhi, 1996.
31. Umadevi I and Daniel M: Phenolics of some fruit spices of the Apiaceae. National Academy of Science Letters 1990; 13(12): 439-41.
32. Vedavathy S and Rao D: Herbal folk medicine of Tirumala and Tirupati region of Chittoor district, Andhra Pradesh. Fitoterapia 1995; 66(2): 167-71.
33. Bourin M: Animal models for screening anxiolytic-like drugs: a perspective. Dialogues in Clinical Neuroscience 2015; 17(3): 295.
34. García DA, Bujons J, Vale C and Suñol C: Allosteric positive interaction of thymol with the GABA A receptor in primary cultures of mouse cortical neurons. Neuropharmacology 2006; 50(1): 25-35.
35. Hajhashemi V, Rabbani M, Ghanadi A and Davari E: Evaluation of antianxiety and sedative effects of essential oil of *Ducrosia anethifolia* in mice. Clinics 2010; 65(10): 1037-42.
36. Satou T, Murakami S, Matsuura M, Hayashi S and Koike K: Anxiolytic effect and tissue distribution of inhaled *Alpinia zerumbet* essential oil in mice. Natural Product Communications 2010; 5(1): 143-6.

How to cite this article:
Nazeer S, Khan UG and Naveed S: Anxiolytic activity of *Traschyspermum ammi* leaves. Int J Pharmacognosy 2017; 4(5): 179-84. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.4(5).179-84.