REPEATED ADMINISTRATION OF NIGELLA SATIVA DECREASES 5-HT TURNOVER AND PRODUCES ANXIOLYTIC EFFECTS IN RATS

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ABSTRACT

The black cumin or *Nigella sativa* L. seeds have many acclaimed medicinal properties. Pharmacological studies have been conducted on the aqueous and methanol extracts of *N. sativa* L. seeds to evaluate their effects on the central nervous system. In the present study, *N. sativa* oil was used to study its effect on anxiety in rats. Open field and elevated plus maze models were selected for the evaluation of anxiolytic effect of drug. After four weeks of daily administration of drug, the rats exhibited an increase in open field activity. The drug also produced anti-anxiety effect in rats when tested in elevated plus maze. Concentrations of 5-HT, 5-HIAA in brain and concentrations of plasma and brain tryptophan determined by HPLC-EC detector**.** Result shows that oral administration of *N. sativa* oil increased brain levels of 5-HT but the levels of brain 5-HIAA decreased significantly. Brain and plasma levels of tryptophan also increased significantly following oral repeated administration of N. sitiva oil. Based on this, it may be suggested that *N. sativa* oil is a useful choice for the treatment of anxiety.

Keywords: *N. sativa*, anxiety, 5-HT.

INTRODUCTION

The field of behavioral pharmacology uses concepts and techniques derived from pharmacology and psychology for the study of interaction between drugs and behavior. The discovery of new compounds which act on CNS processes will stimulate not only their clinical use but will also contribute useful information for the validation of animal models (Al-Naggar *et al*., 2003). Among the promising medicinal plants, Nigella Sativa, a dicotyldon of the Ranunclaceae family is an amazing herb with a rich historical and religious background (Huffman and Hirata, 2003).

Seeds of Nigella sativa are frequently used in folk medicine in the Middle East and some Asian countries for the promotion of good health and treatment of many ailments (El-Kadi and Kandil, 1987) including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems (Al-Rowais, 2002). Several studies conducted since 1959 confirmed the pharmacological effectiveness of *N. sativa* seed constituents (Salem, 2005). Several pharmacological properties of *N. sativa*, including hypotensive, anti-nociceptive, uricosuric, choleretic, anti-fertility, anti-diabetic, and anti-histaminic have been reported (Maxwell, 1995). It has been shown that *N. sativa* L. seeds contain> 30 % of a fixed oil and 0.40-0.45 % w/w of a volatile oil. The volatile oil has been shown to contain18.4-24 % thymoquinone and a total of 46 % of many monoterpenes such as p-cymene and pinene (El-Tahir *et al*., 1993).

Previous study shows that *N. sativa* seed constituent (aqueous extract, fixed oil, volatile oil, and major constituents of the volatile oil) produced anti-anxiety effect in different tests used as models for exploration-induced anxiety (Raza *et al*., 2006). Stress and anxiety can lead to mental and physical health (e.g., depression, nervous breakdown, and heart diseases). Role of 5-HT in anxiety related behavior is well documented (Handley and McBalney, 1993) It is reported that anxiolytic drugs increases 5-HT and decreases 5-HIAA levels (Collinge *et al*., 1983). During the last decade the use of herbal medicines has been tried to alleviate the symptoms of depressive disorders using animal research (Sharma, 1993). *N. sativa* is one of such herbal medicine that is used. Previous work in our laboratory show long term administration of *N. sativa* increases 5-HT levels in rat brain and improves learning and memory in rats (Perveen *et al*., 2008).

In view of these studies, present work is designed to investigate the anti-anxiety effect of repeated oral administration of *N. sativa* oil in male rats.

MATERIALS AND METHODS

Locally breed white albino wistar rats weighing about 150-250g (purchased from HEJ Research Institute of Chemistry, University of Karachi) were caged individually in specifically designed cages in a quite room with free access to water and cubes of standard rat food for at least 1 week before starting the experiment. Animals were divided into control and test. To test

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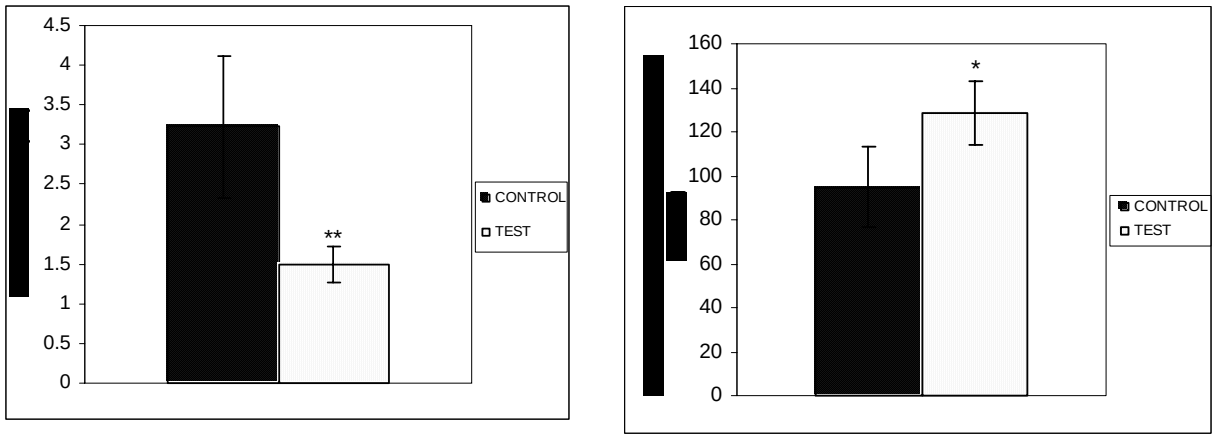

Fig. 1: Effect of repeated administration of *N. sativa* oil (0.1ml/day) for four weeks on open field activity in rats. Values are means + SD (n=6). Significant differences by Student's t-test. **p<0.01 and *p<0.5 from respective controls.

animals, the *N. sativa* oil at a dose of 0.1ml/day was given orally, daily for four weeks. *N. sativa* oil extract of Amir Laboratory was purchased from local market. The control animals were only given tap water.

After four weeks of repeated administration of drug, the activities of animals were monitored in different behavioral models. Open field and Plus maze activity were monitored for anti-anxiety effect of *Nigella sativa*.

Open field is the square area of 76x76 cm with opaque walls of 42 cm height. The floor was divided by lines into 25 equal squares. The test was performed in a quiet room under white light to avoid any noise effect as described by Kennet *et al*. (1985) and Haleem *et al*. (1988). Animals were placed in the center square of the open field (one at a time). Activity in open field was determined by monitoring latency period and counting number of squares crossed for five minutes as described earlier (Haleem *et al*., 2002). Activities of control rats and drug treated rats were monitored in a balance design to avoid order effect.

The anxiolytic activity of drug in elevated plus maze model was measured according to method as previously reported (Haider *et al.,* 2006). Plus maze apparatus consist of four equal size arms. The two opposite arms are open while two were closed. The length of each arm was 50 cm and width is 10 cm. Arms were joined by the central area of 5 cm^2. The length of the wall of the closed arm was 40 cm. The maze was elevated from ground at a height of 60 cm. To determine activity a rat was placed in the center of the plus maze and the time spent in the open arm was monitored for 5 minutes.

After four weeks of repeated administration of *Nigella sativa* oil and monitoring activities of animals in behavioral models, animals were decapitated. Plasma and brain samples were collected and stored at -70°C for the estimation of Plasma Tryptophan, brain Tryptophan, 5HT and 5HIAA by HPLC using EC dectector. HPLC-EC determination was carried out as described before (Haleem *et al*., 2002). A 5II Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by a mobile phase containing methanol (14%), Octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1M phosphate buffer at pH 2.9 at an opening pressure of 2000-3000psi on Schimadzun LEC 6A detector at an operating potential of 0.8V for biogenic amines and 1.0V for tryptophan.

Data were analyzed by student's t-test. *P* values <0.05 were considered as significant.

RESULTS

The effect of drug *N. sativa* on open field activity showed significant decrease (p<0.01) of latency period in drug treated animals whereas number of square crossings in test animals were significantly greater (p<0.05) than control animals (fig. 1). The drug significantly increased (p<0.05) the time spent by animals in open arms of plus maze (fig. 2). The 5HT levels were significantly greater (p<0.01) in test animals as compared to controls. But the concentration of 5HIAA was significantly lower (p<0.05) in drug treated animals than their control counterparts. The tryptophan levels were significantly greater (p<0.01) both in plasma and brain in animals treated with *N. sativa*.

DISCUSSION

Anxiety disorders are among the most common and prevalent forms of psychopathology. Although anxiety is a normal emotional response to a fearful situation, individuals who suffer from anxiety disorders show a greater sensitivity in both their physiological and behavioral reactions in a variety of situations. The prevalence of anxiety mental condition has risen in recent years (Andrews *et al*., 2000). Therefore the search for new compounds as therapeutic alternatives for such disorders has progressed constantly (Irie *et al*., 2004 and Klodzinska *et al*., 2004). An increasing number of studies have demonstrated that plant derived essential oils exhibit a variety of biological properties. Several of these described effects are frequently attributed to monoterpenes, which are the major chemical components of those essential oils. Several herbal medicines are commonly used in developed and developing countries for psychiatric illness.

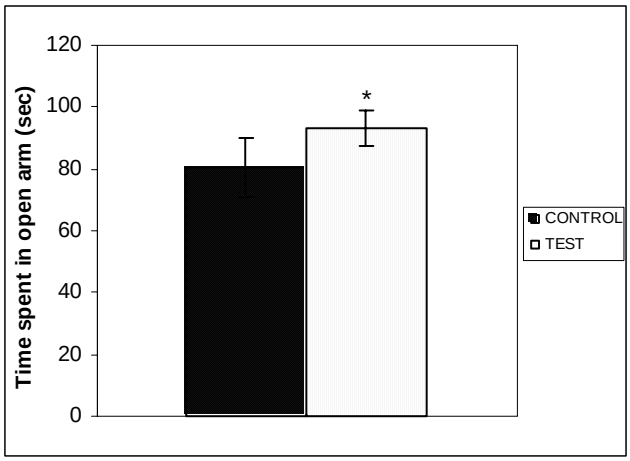

Fig. 2: Effect of repeated administration of *N. sativa* oil (0.1ml/day) for four weeks on plus maze activity in rats. Values are means + SD (n=6). Significant differences by Student's t-test. *p<0.05 from respective controls.

In the present work, the effects of *N. sativa* oil were studied in several animal behavioral models, such as open-field and elevated plus maze to investigate its possible anxiolytic activity. These tests are classical models for screening central nervous system actions providing information about psychomotor performance, anxiety and depression (Maria *et al*., 2007).

Behavior in the open field is used as a measure of exploration, anxiety, and locomotor behavior (Frye *et al*., 2008). Animals removed from their acclimatized cage and placed in a novel environment express anxiety and fear, by showing alteration in all or some parameters, such as decreases in ambulation and exploration, and immobilization or freezing due to augmented autonomic activity (Novas *et al*., 1988 and Bhattacharya *et al*., 1991). These paradigms are attenuated by classical anxiolytics, and potentiated by anxiogenic agents (Cicero, 1987). The animals treated with *N. sativa* oil showed increased motor activity in open field. The numbers of squares crossed by the drug treated animals are significantly greater than their control counterpart. The test animal also had significantly lower latency period (fig. 1). This all leads to suggest the anxiolytic behavioral drugs. Behavior in the elevated plus maze is also utilized to assess exploration, anxiety, and motor behavior (File, 1993 and Saida *et al*., 2006). Anxiolytic compounds reduce the animal's aversion to the open arms and promote the exploration thereof ((Maria *et al*., 2007). Our findings also indicate that animals treated with *N. sativa* oil pass significantly greater time in open arm than control rats. Anxiolytic effect of N.Sativa constituent such as thmoquinone, α pinene and ρ-cymene has been reported previously (Raza *et al*., 2006). These finding supported the results observed in the present study.

5-HT plays an important role in modulation of various behaviors. Evidence supporting the involvement of central 5HT in anxiety related behavior and in the mechanism of action of anxiolytic is well documented (Handley and McBlaney, 1993). Studies on the Benzodiazapine (BZ) antagonist Flumazenil show that the anxiolytic activity of thymoquinone may involve BZ receptors (Raza *et al*., 2006). Pharmacological action of BZ are mediated via BZ recognition site and subsequent facilitation of GABAnergic neurotransmission (Trulson *et al*., 1982). Increased 5HT levels and decreased levels of 5HIAA observed in the present study following the administration of *N. sativa* oil suggests a decreased 5-HT turnover (fig. 3). Similar results were also reported following the administration of anxiolytic drugs (Collinge *et al*., 1983). The synthesis of serotonin in brain depends upon the availability of precorsor amino acid tryptophan to serotonergic neurons (Leathwood 1987, Haleem 1990, Frenstrom and Wurtman, 1971). Increased brain tryptophan contibute in enhancement of brain 5-HT metabolism (Haleem *et al*., 1998 and Saida *et al*., 2006). Increase in brain tryptophan levels observed in the present study was accompanied by increase blood tryptophan levels. Increased brain tryptophan concentration following the administration of anxiolytic drug has been reported previously (Pralt *et al*., 1979, Haleem and Batool, 1996). It is reported previously that anxiolytic drugs may increase the availability of tryptophan to brain by increasing the plasma free tryptophan (Haleem and Batool, 1996). An increase in brain tryptophan concentration observed in present study may be explain in term of increase concentration of plasma tryptophan.

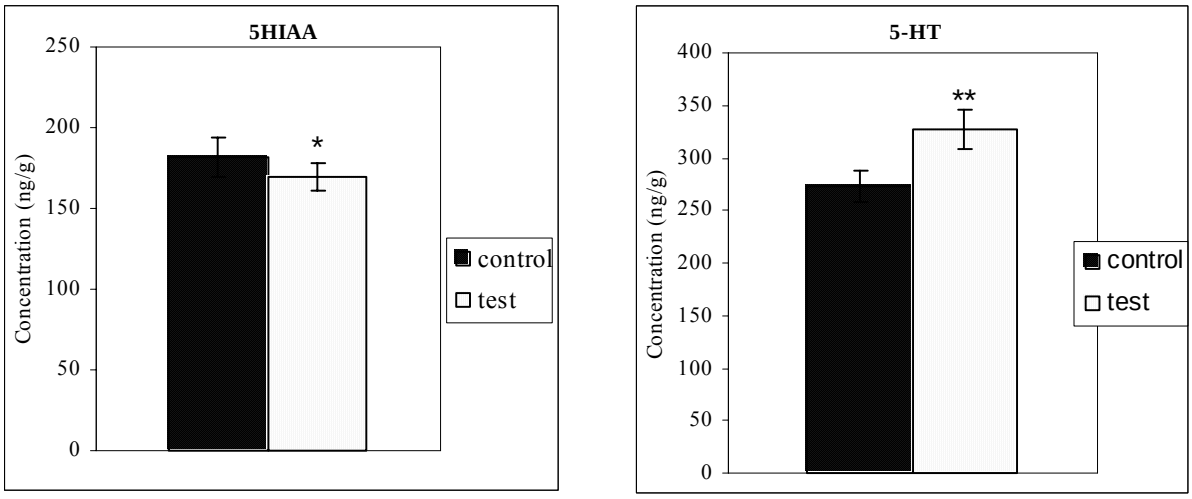

Fig. 3: Effect of repeated administration of *N. sativa* oil (0.1ml/day) for four weeks on 5HIAA and 5HT levels in rats. Values are means + SD (n=6). Significant differences by Student's t-test. **p<0.01 & *p<0.05 from respective controls.

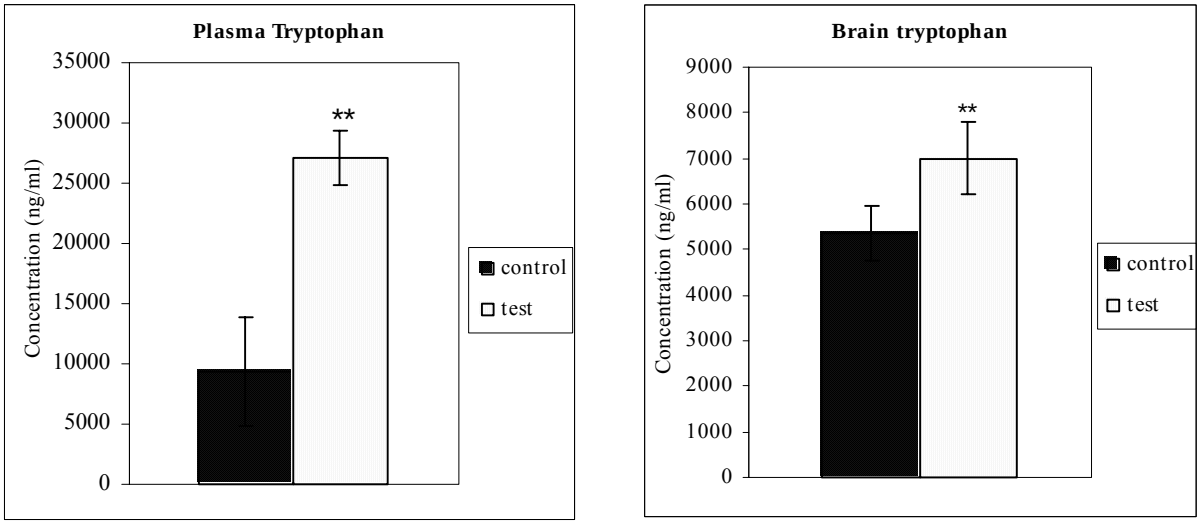

Fig. 4: Effect of repeated administration of *N. sativa* oil (0.1ml/day) for four weeks on plasma and brain tryptophan levels in rats. Values are means ± SD (n=6). Significant differences by Student's t-test. **p<0.01from respective controls.

In conclusuion it is suggested that anxiolytic effect of *N. sativa* observed in present study possibly mediated by a decreased in 5-HT turnover.

REFERENCES

Al-Naggar TB, Gómez-Serranillos MP, Carretero ME and Villar AM (2003). Neuropharmacological activity of *N. sativa* L. *Journal of Ethnopharmacology*, **88**(1): 63-68.

Al-Rowais NA (2002). Herbal medicine in the treatment of diabetes mellitus. *Saudi. Med. J.*, **23**: 1327-1331.

Andrews G, Sanderson K, Slade T and Issakidis C (2000). Why does the burden of disease persist? Relating the burden of anxiety and depression to effectiveness of treatment. *Bulletin of the World Health Organization*, **78**: 446-454.

Bhattacharya SK, Clow A, Przyborowska A, Halket J, Glover V and Sandler M (1991). Effect of aromatic amino acids, pentylenetetrazole and yohimbine on isatin and tribulin activity in rat brain. *Neurosci. Lett*, **132**: 44-46.

Cícero BF (1987). Piplartine, an amide alkaloid from Piper tuberculatum, presents anxiolytic and anti-

depressant effects in mice. *Phytomedicine,* **14**(9): 605-612.

Collinge J, Pycock CJ and Taberner PV (1983). Studies on the interaction between cerebral 5 hydroxytryptamine and y-aminobutyric acid in the mode of action of DZ in the rat. *Br. J. Pharmac,* **79**(3): 637-643.

E1-Kadi A and Kandil O (1987). The black seed *(N. sativa)* and immunity – its effects on human T-cell subsets. *Fed. Proc*, **46**: 1222-1226.

El-Tahir KEH, Ashour MMS and Al-Harbi MM (1993). The respiratory effects of the volatile oil of the black seed *(N. sativa)* in guinea pigs – elucidation of the mechanism(s) of action. *General Pharmacol,* **24**: 1115-1122.

File SE (1993). The interplay of learning and anxiety in the elevated plus-maze. *Behav. Brain Res.,* **58**: 199-202.

Frenstorm JD and Wurtman RJ (1971). Brain serotonin content: Physiologicaldependence on plasma TRP levels. *Science*, **173**: 149-152.

Frye CA and Madeline E Rhodes (2008). Infusions of 3α, 5α-THP to the VTA enhance exploratory, anti-anxiety, social, and sexual behavior and increase levels of 3α, 5α-THP in midbrain, hippocampus, diencephalon, and cortex of female rats. *Behavioural Brain Research*, **187**(1): 88-99.

Haider S, Roohi M, Khaliq S, Perveen T and Haleem DJ (2006). Oral administration of Phytolacca Baccis (poke rote) Extract increases brain serotonin metabolism and decreases food intake in rats. *Pak. J. Bot.,* **38**(3): 745-750.

Haleem DJ, Naz H, Parveen T, Haider S, Ahmed SP and Khan NH (2002). Serotonin and serotonin 1-A receptors in the failure of ethanol treated rats to adapt to a repeated stress schedule. *J. Stud. Alcohol.,* **63**: 389-396.

Haleem DJ, Jabeen B and Parveen T (1998). Inhibition of restraint-induced anorexia by injected tryptophan. *Life Sci.,* **63**(14): 205-12

Haleem DJ and Farhat Batool. (1996). Regionally specific effects of diazepam on brain serotonin metabolism in rats: Sustained effects following repeated administration. *Life Sciences,* **59**(15): 239-246.

Haleem DJ (1993). Function specific supersensitivity of m-chlorophenyl piperazine-induced serotonergic neurotransmission in female compared to male rats. *Life Sci*., **52**(25): 79-84.

Haleem DJ, Kennett GA and Curzon G (1990). Hippocampal 5-HT synthesis is greater in females than in males and is more decreased by 5-HT 1A agonist 8-OH-DPAT. *J. Neural Transm.,* **79**: 93-101.

Haleem DJ, Kennett G and Curzon G (1988). Adaptation of female rats to stress: Shift to male pattern by inhibition of corticosterone synthesis. *Brain Res.,* 23, **458**(2): 339-347.

Handley SL and McBlane GW (1993). 5HT drugs in animal model of anxiety. *Psycopharmacology*, **112**: 13-21.

Huffman MA and Hirata S (2003). Biological and ecological foundations of primate behavioral traditions. In: D.M. Fragaszy and S. Perry (editors). *The Biology of Traditions*, Cambridge University Press, Cambridge pp.267-296.

Irie Y, Itokazua N, Anjiki N, Ishige A, Watanabe K and Keung WM (2004). Eugenol exhibits antidepressant-like activity in mice and induces expression of metallothionein-III in the hippocampus. *Brain Res.,* **1011**: 243-246.

Kennett GA, Dickinson SL and Curzon G. (1985). Central serotonergic responses and behavioural adaptation to repeated immobilisation: the effect of the corticosterone synthesis inhibitor metyrapone. *Eur. J. Pharmacol.,* **119**(3): 143-152.

Klodzinska A, Tatarczynska E, Chojnacka-Wojcik E, Nowak G, Cosford NA and Pilc A (2004). Anxiolytic-like effects of MTEP: A potent and selective mGlu5 receptor agonist does not involve GABAA signaling. *Neuropharmacology,* **47**: 342-350.

Laetitia P and Catherine B (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, **463**(1-3): 3-33.

Leathwood PD (1987). Tryptophan availability and serotonin synthesis. *Proc. Nutr. Soc.,* **46**: 143-156.

Maria IGS, Manuel Rufino DAN, Paulo FTN, Brinell AM, Jeferson FDA, Damião PDS, Silvânia MMV and Francisca CFDS (2007). Central nervous system activity of acute administration of isopulegol in mice. *Pharmacology Biochemistry and Behavior*, **88**(2): 141-14.

Maxwell SRJ (1995). Prospects for the use of antioxdant therapies. *Drugs*, **49**: 345-361.

Novas ML, Wolfman C, Medina JH and Robertis ED (1988). Proconvulsant and anxiogenic effects of n-butyl-h-carboline-3-carboxylate, on endogenous benzodiazepine binding inhibitor from brain. *Pharmacol Biochem Behav.,* **30**: 331-336.

Olsen RW (1981) GABA-benzodiazepine-barbiturate receptor interactions. *J. Neurochem.,* **37**(1): 1-13

Pratt J, Jenner P, Reynolds EH and Marsden CD (1979). Clonazepam induces decreased serotoninergic activity in the mouse brain. *Neuropharmacology*, **18**: 791-799.

Perveen T, Abdullah A, Haider S, Sonia B, Munawar AS and Darakhshan J Haleem (2008). Long-term administration of *N. sativa* effects nociceotion and improves learning and memory in rats. *Pak. J. Biochem. Mol. Biol.,* **41**(3): 141-143.

Raza M, El-Hadiah TM and Al-Shabanah O (2006). A. Journal of herbs, spices and medicinal plants. **12**(1-2): 153-164.

Salem ML (2005). Immunomodulatory and therapeutic properties of the *N. sativa* L. seed. *International Immunopharmacology*, **5**(13-14): 1749-1770.

Sharma KP, Kushwaha and Sharma SS (1993). A placebo-controlled trial on the efficacy of Mentat in managing depressive disorders. *Probe* (XXXIII), **1**: 26-31.

Trulson ME, Preussler DW, Howell GA and Frederickson CJ (1982). Raphe unit activity in freely moving cats: Effects of benzodiazepines. *Neuropharmacology,* **21**: 1045-1050.