CHAPTER-6

The Effect of Nigella sativa L. in Various Germination Phases on Various Activities of Central Nervous System

“We can judge our progress by the courage of our questions and the depth of our answers, our willingness to embrace what is true rather than what feels good.”
~Carl Sagan
6.1 Rationale

In the 21st century cognitive dysfunction is a major health trouble and many neuropsychiatric disorders and neurodegenerative disorders such as Alzheimer's disease dementia, depression, schizophrenia, seizure disorders, cerebrovascular impairment, head injury and parkinsonism, can be severely function overwhelming in nature (Borgesius et al., 2011). Up to 21% of the world’s population has been estimated to be affected by depressive disorders, one of the most prevalent psychiatric diseases (Murray et al., 1997). It is a major cause of disability and death by suicide due to raised rates of physical disorders (Paykel, 2006). Neurodegenerative diseases represent a large group of neurological disorders with heterogeneous clinical and pathological expressions upsetting specific subsets of neurons in specific functional anatomical systems.

Neurodegenerative disorders are a major cause of mortality and disability. Although, several classes of neuroprotective drugs presently being used, due to clinical confines and adverse effects there is critical interest in development of proficient and secure drugs for treatment of neurodegenerative disorders (Paykel, 2006). Because the mechanism of neurodegenerative disorders is quite complex, many currently available synthetic neuroprotective drugs/chemicals have low rates of response and remission and even severe adverse effects (Sarko, 2000). The majority of patients are often reluctant to take synthetic neuroprotective in their appropriate doses due to their anticipated side effects including inability to drive a car, dry mouth, constipation, and sexual dysfunction. In course of time, a number of neurotransmitters and signaling molecules have been identified which have been considered as therapeutic targets. Accordingly, plants may be important sources of new neuroprotective drugs and the safety of such plant extracts may be better than that of synthetic drugs (Schulz, 2006).

Phytoconstituents obtained from medicinal plants play a very important role in maintaining the brain's chemical equilibrium by influencing the function of receptors for the major inhibitory neurotransmitters. Several plants have been
reported to treat cognitive disorders in traditional practice of medicine. Phytochemical substances from herbs like fatty acids, phenols, alkaloids, flavonoids, saponins, terpenes etc. contain many neuroprotective activities. Presence of receptors or transporters for polyphenols or other phytochemicals in brain tissues remains to be ascertained. Compounds with multiple targets appear as a potential and promising class of therapeutics for the treatment of diseases with a multifactorial etiology. In traditional practice of medicines, various plants have been used for neuroprotection. An ethnopharmacological approach has provided which leads to identify potential of new drugs from plant sources, including those for neurodegenerative disorders.

The aim of present study was to investigate in vivo neuroprotective effects of *Nigella sativa* L. seed during different germination phases on CNS. This is the first study on neuroprotective effects during germination of *N. sativa* L. Seed.

6.2 REVIEW OF LITRATURE

6.2.1 Neuronal Pathways and their neurotransmitters associated with neurodegenerative disorders

Neuroscience is the study of the nervous system, including the brain, spinal cord and peripheral nerves. Neurons are the basic cells of the brain and nervous system which exerts its functional role through various neurotransmitters and receptor systems. The activity of neurons depends on the balance between the number of excitatory and inhibitory process affecting it, both process occurring individually and simultaneously. The functional balance of different neurotransmitters such as Acetylcholine (Ach), Dopamine (DA), Serotonin (5-HT), Nor epinephrine (NE) Epinephrine (LPI), Glutamate and Gamma amino butyric acid (GABA) regulates the growth, division and other vital functions of a normal cell / organism (Sudha et al., 1998).

Any change in the cell environment causes imbalance in cell homeostasis and function. Pollution/neurotoxins cause a change in the neurotransmitters and their
receptor function leading to early occurrence of neurodegenerative disorders such as hypoxia, Alzheimer’s and Huntington's disease early in life.

The major amino acid neurotransmitters in the brain are γ-aminobutyric acid (GABA), an inhibitory transmitter and glutamic acid, an excitatory transmitter. GABA is widely distributed in the mammalian brain and has been shown to contribute to over 40% of the synapses in the cortex alone. Evidence GABAergic involvement in modulating anxiety is that certain classes of drugs such as the benzodiazepines, barbiturates and alcohol all bind to GABA receptors to increase its post-synaptic inhibitory effect and reduce anxiety. Benzodiazepines bind allosterically to the GABA receptor and have their own binding site. Additionally, benzodiazepine inverse agonists such as flumazenil decrease effects of GABA and cause anxiety. Anxiety may be brought in non-anxious subjects through the administration of bicuculline, a competitive antagonist of GABA and Picrotoxin a non-competitive GABA antagonist (Hoehn-Saric, 1982).

The most predictable anxiolytic effects of neurotransmitters are linked to the activation of a gamma-aminobutyric acid (GABA)-ergic subsystem associated with specific benzodiazepine receptors. Recent studies have indicated that subtypes of benzodiazepine receptors may be associated specifically with anxiolytic actions. Animal studies suggest that some forms of anxiety are mediated through the noradrenergic system, but a recent study testing this hypothesis confirmed it only partially. Currently the role of other neurotransmitters, such as dopamine, histamine, acetylcholine, and peptides, appears to be minimal. Clinical responses to drugs suggest that existence of at least two types of anxiety disorders representing perhaps different psychobiologic mechanisms (Hoehn-Saric, 1982).

Stress and anxiety can lead to mental and physical health (e.g., depression, nervous breakdown, and heart diseases). Nitric oxide (NO), an intercellular messenger in the brain generated from L-arginine by different isoforms of nitric oxide synthase (nNOS, iNOS and eNOS) plays an important role in various physiological and pathological processes (Thomas et al., 1997; Gow et al., 2004).
Nitric oxide synthase (NOS) is localized in brain regions involved with anxiety, such as hypothalamus, amygdala and hippocampus (Vincent, 1994; McHugh et al., 2004; McNaughton et al., 2004). Acute stress induces a generalized increase in the production of NO and causes anxious behavior in rodents (Sevgi et al., 2006). Role of 5-HT (serotonin) in anxiety related behavior is well documented (Handley and McBalney, 1993). It has been reported that reduced level of 5-HT and increases in 5-HIAA levels cause anxiety (Collinge et al., 1983) while the synthesis of serotonin in brain depends upon the availability of precursor amino acid tryptophan to serotonergic neurons (Frenstrom and Wurtman, 1971; Leathwood, 1987; Haleem, 1990). Increased brain tryptophan contributes in enhancement of brain 5-HT metabolism (Haleem et al., 1998).

Anxiety is a normal part of the response to a challenging or threatening situation. Anxiety symptoms include palpitations, sweating, trembling and feeling of fear and panic. It is a pervasive and significant negative effect that is a central feature of many psychological problems, including those that were frequently called "neuroses". Anxiety is one of the most prominent and pervasive emotions and large number of people are distressed by inappropriate or excessive anxiety (Rachman, 1998). There is not just one anxiety symptom; a whole range of reactions can be associated with it (Hamilton, 1959).

**Table 6.1:** Major symptoms of anxiety.

<table>
<thead>
<tr>
<th>Anxious mood</th>
<th>Worrying, apprehension, anticipation of the worst irritability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fears of</td>
<td>The dark, being left alone, traffic, strangers, large animals</td>
</tr>
<tr>
<td>Intellectual (cognitive) Symptoms</td>
<td>Difficulty in concentration, poor memory</td>
</tr>
<tr>
<td>Depressed mood</td>
<td>Loss of interest, depression, diurnal swing, lack of pleasure in hobbies, early waking</td>
</tr>
<tr>
<td>General body sensations</td>
<td>Tinnitus, hot and cold flushes, prickling sensations, blurred vision, feelings of weakness</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td>Pressure or constriction in chest, tightness of breath, feelings of choking, sighing</td>
</tr>
<tr>
<td>Genitourinary symptoms</td>
<td>Frequency of urination, suppressed periods, frigidity, premature ejaculation, impotence, urgency of urination, loss of erection</td>
</tr>
<tr>
<td>Physiological symptoms</td>
<td>Tremor of hands, strained face, swallowing, sweating, furrowed brow, facial pallor</td>
</tr>
<tr>
<td>Tension</td>
<td>Feelings of tension, inability to relax, easily moved to tears, feelings of restlessness, fatigue, startled response, trembling</td>
</tr>
<tr>
<td>Insomnia</td>
<td>Difficulty in falling asleep, unsatisfying sleep, fatigue on waking, night terrors, broken sleep, dreams, nightmares</td>
</tr>
<tr>
<td>General somatic symptoms</td>
<td>Muscular aches and pains, muscular twitching, muscular stiffness, Grinding teeth</td>
</tr>
<tr>
<td>Cardio-vascular symptoms</td>
<td>Tachycardia, pain in chest, feelings of faintness, palpitations, throbbing of vessels skipped heartbeats</td>
</tr>
<tr>
<td>Gastrointestinal Symptoms</td>
<td>Difficulty in swallowing, indigestion, heartburn, looseness of bowels, feelings of bloating loss of weight</td>
</tr>
<tr>
<td>Autonomic nervous system symptoms</td>
<td>Dry mouth, pallor, giddiness, flushing, tendency to sweat, raising of hair</td>
</tr>
</tbody>
</table>

Epilepsy is a progressive disorder comprising of many seizure types and syndromes. A significant percentage of patients with epilepsy continue to experience seizures although violent treatment with one or more anti-epileptic drugs has been used. As a result, in attendance continues to be an unmet clinical need for more effective and less toxic anti-epileptic drugs (Feldman et al., 1997; Barton et al., 2003; Leonard, 2003; Rang et al., 2003). Glutamate is the major excitatory
neurotransmitter in the CNS and on release from pre-synaptic terminals, it binds to G-protein linked receptors either positively coupled to inositol phosphate metabolism or negatively coupled to adenylate cyclase (Webster and Jordan, 1989). Ionotopic receptors such as N-methyl D-aspartate (NMDA), kainate, and alpha -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) are based on their activation by specific agonists. Therefore, the activation of these metabotropic and ionotropic receptors is important for normal CNS functions such as synaptogenesis, synaptic plasticity and the development of functional neural circuits. However, excessive levels of glutamate may be responsible for such pathological CNS processes as seizure generation or neurodegeneration following stroke and ischaemia. Therefore, the development of glutamate receptor agonists and glutamate antagonists has been viewed as a potentially important therapeutic strategy for the treatment of many neurological disorders (Barton et al., 2003). The agreed clinical classification of epilepsy recognizes two categories, namely partial seizures and generalized seizures, although there are some overlaps and many varieties of each.

Approximately 1% of the world’s population has epilepsy, the second most common neurological disorder after stroke (Porter and Meldrum, 2001). The type of seizure depends on the site of focus in the brain. Epileptic attack can be caused by biochemical insults to the brain, such as during hypoglycaemia, anoxia, hypocalcaemia, hyperventilation, water intoxication and sudden withdrawal of certain drugs such as barbiturates or alcohol (Bienvenu et al., 2002). Epilepsy can also be caused by previous active pathology, such as birth trauma to the brain, during or following meningitis, trauma to the skull and brain later in life, cerebral abscesses, cerebral infarction, cerebral haemorrhage or subarachnoid haemorrhage (Biller, 1997; Bienvenu et al., 2002). It is evident that a reduction in GABAergic activity is associated with seizures and most anticonvulsant drugs either directly or indirectly facilitate GABAergic transmission, GABA also has a fundamental role in the brain by shaping, integrating and refining information transfer generated by the excitatory transmitters (Leonard, 2003).
The GABAergic system has long been implicated in epilepsy with defects in GABA neurotransmission being linked to epilepsy in both experimental animal models and human syndromes. However, to date no human epileptic syndromes may be directly attributed to an altered GABAergic system (DeLorey, 1999). The observed defects in GABA neurotransmission in human epileptic syndromes may be the indirect result of a brain besieged by seizures (DeLorey, 1999). Drugs have been developed to modulate GABA function; the inhibitors of GABA transaminases, which metabolize GABA, have been shown to be effective anticonvulsants. These are derivatives of valproic acid that do not only inhibit the metabolism of GABA, but also act as antagonist of GABA auto receptor and thereby enhance the release of the neurotransmitter (Leonard, 2003). Apart from these recent advances in the field of neurology, it has come through the examination of claims for herbal remedies. Most of the areas of concern of neurology will potentially benefit from herbal therapeutics, and indeed the science of psycho-pharmacology itself is largely based on chemicals discovered in plants.

6.2.2 Neuroprotective effects of *Nigella sativa* L.

*Nigella sativa* L. has been used to promote health and fight diseases for centuries. Very modest literature was found on the neuroprotective effect of *N. sativa*. Pharmacological studies have been conducted on the aqueous and methanolic extracts of defatted *N. sativa* seeds to evaluate their effects on the central nervous system (CNS) and on analgesic activity. The observations suggest that the two extracts of *N. sativa* possesses a potent CNS and analgesic activity and depressant action especially in the case of the methanolic extract (Al-Naggar et al., 2003). Kanter (2008) reported that *N. sativa* therapy causes morphologic improvement on neurodegeneration in frontal cortex and brain stem after chronic toluene exposure in rats and believed that further preclinical research into the utility of *N. sativa* may indicate its usefulness as a potential treatment on neurodegeneration after chronic toluene exposure in rats.
Increasing evidence demonstrates that oxidative stress plays an important role in brain injury in experimental models of brain ischemia. Thymoquinone, the main constituents of the volatile oil from \textit{N. sativa} seeds, is reported to possess strong antioxidant properties. Thymoquinone is effective in protecting rats against transient forebrain ischemia-induced damage in the rat hippocampus. This spectacular protection makes thymoquinone a promising agent in pathologies implicating neurodegeneration such as cerebral ischemia (Al-Majed \textit{et al.}, 2006). \textit{N. sativa} treatment might be beneficial in spinal cord tissue damage and therefore shows potential for clinical implications (Kanter \textit{et al.}, 2006). In another study, it was reported that oral administration of \textit{N. sativa} oil decreased 5-HT turnover and possess anti-anxiety effect (Perveen \textit{et al.}, 2009; Gilhotra \textit{et al.}, 2011). Present study is the first study to evaluate neuroprotective effects of \textit{N. sativa} during germination phases of seed.

\textbf{6.3 MATERIALS AND METHODS}

\textbf{6.3.1 Collection of \textit{N. sativa} seeds}

Seeds of \textit{N. sativa} were procured in September, 2010 from a herbal shop in Lucknow, India and authenticated by a botanist at National Botanical Research Institute, Lucknow. A voucher specimen of the seeds was kept in the museum of the Department for future reference.

\textbf{6.3.2 Germination of \textit{N. sativa} seeds}

Seed lots used for different experiments showed germination capacities ranging from 80 to 98%. The seeds were surface sterilized with 0.1% HgCl\textsubscript{2} for 3 min. They were rinsed thoroughly with double distilled water and soaked in de-ionized water for 30 min. For germination of seeds, they were placed on four folds of damp filter paper at 25°C and incubated in dark till the initiation of sprouting after
which they were placed at a light intensity of 100 µmol m$^{-2}$ s$^{-1}$ (that was measured by LI-190SA quantum Sensor, Li-COR Co., USA) and a 14/10 h (day/night) photoperiod till the complete plantlet with two leaves were obtained. The complete germination took eleven days with emergence of epicotyl, hypocotyl, roots and green leaves. Germination, defined as 1 mm radicle emergence, was followed for 11 days. No contamination by microorganisms was observed during this time period.

6.3.3 Harvest of germinated seeds

The germinated seeds of different days were harvested with a sterilized forceps and were kept on blotting sheet to remove excess water. The germinated seeds collected for different experiments were used immediately for preparing extracts. Seeds were considered to be germinated after the radical emerged from the testa. All the samples were stored at -80°C in a deep freezer until used further.

6.3.4 Preparation of distilled extracts

The samples of seed and germinated phases 5$^{th}$, 7$^{th}$ and 11$^{th}$ day were shade-dried and ground to a fine powder. The powder (20gm) was extracted with 200 ml methanol solvent for 48 h in order to extract bioactive compounds using soxhlet apparatus (AOAC method 1980). The extracts were filtered using Whatman filter paper (No.1) and methanol was evaporated using rotary distillation apparatus to obtained pure extract. Oily fraction of extracts was stored at 4°C until use.

6.3.5 Animals

Male Wistar rats, weighing 150 - 200 g, were purchased from Central Drug And Research Institute (CDRI), Lucknow, India and housed in a temperature controlled room (22±2°C) with a 12 hour light-12 hour dark cycle and allowed free access to a standard rat chow and filtered tap water for 7 days for acclimatization. The study received the approval of the Institutional Animal ethics Committee of Era’s Lucknow Medical College & Hospital. Animals were cared for in accordance
with the internationally accepted principles for laboratory animal use and care and the procedures followed were in accordance with the standards set forth in the Guide for the Care and Use of Laboratory Animals (published by the National Academy of Science, National Academy Press, Washington, D.C.). They were housed under controlled conditions of temperature of 23±2°C, relative humidity of 30–70% and 12 h light–12 h dark cycle. The animals were housed individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment. All animals were fed with sterile commercial pelleted rat chow supplied by Hindustan Lever Ltd. (Mumbai, India) and had free access to water. Animals were kept under fasting for overnight and weighed before the experiment.

6.3.6 Drugs

Diazepam (Calmove®, Ranbaxy Laboratories Ltd., India) and imipramine (Depsonil, S.G Pharmaceuticals, Vadodra).

6.3.7 Anxiolytic activity

6.3.7.1 Elevated plus maze

The plus maze apparatus consisted of two open arms (without walls), 16 × 5 cm and two enclosed arms 16 × 5 × 12 cm arranged opposite to each other. The maze was elevated to a height of 25 cm. Each mouse was placed individually at the center of the elevated plus maze with its head facing towards an open arm and time spent in open arm during a 5 min observation period was noted (Pellow et al., 1985; Kulkarni, 1999). Data from control, diazepam and test extracts treated groups were compared.

6.3.7.2 Locomotor activity

The effects of various treatments on spontaneous locomotor activity of animals were measured using an actophotometer (INCO, Ambala, India). The cognitive effect was measured by placing the animals in the actophotometer and the readings were recorded for 10 min. The data was presented as the number of counts recorded by the apparatus as the light beam was interrupted between the light source
and photo sensors in response to animal movements. The locomotor activity was expressed in terms of total photo beam interruption counts/min/animal (Turner, 1965).

6.3.7.3 Experimental protocol

The rats were divided into twelve groups containing six rats in each group. Stress was produced by immobilizing the rat for six hours (9 a.m. – 3 p.m.) in a cage. The cage was an indigenous one which was designed to suit the experiment. It was framed to provide adequate immobilization without giving any physical harm to the animal. It was small, made up of steel wire, measuring 9”x 2.75” and light weighted. Animals subjected to immobilization were considered as stressed mice. Animals not subjected to immobilization were considered as unstressed mice.

All treatments were administered orally in all experimental groups (I-XII). Animals of Control (Group I), immobilized (Group II) and standard groups (Group III and IV) received distilled water (1ml/kg b.w.) for seven days. Group III received diazepam (20mg/kg b.w.) 1h before test on day seven and group IV also received diazepam (20mg/kg) 1h before subjecting them to immobilization for 6 hrs. Groups V-XII received *N. sativa* extracts (1g/kg) from different germination phases (0th day i.e. seed extract, 5th, 7th and 11th day extract) for seven days. On day seven unstressed groups of animals received extracts of *N. sativa* 1h before testing them in various behavioral paradigms. The remaining groups of animals received extract 1h before subjecting them to immobilization for 6 hrs (Kumari *et al*., 2007).

6.3.8 Antiepileptic/Anticonvulsant activity

6.3.8.1 Maximal electroshock (MES) induced seizures

Maximal electroshock seizure model was used to evaluate the anticonvulsant activity of extracts. Seizures were induced in rats by delivering electroshock of 150mA for 0.2 seconds by means of an electro-convulsimeter through a pair of ear clip electrodes (Barton, 2003; Kumar, 2008). All rats were divided into six different
groups (Group I-VI). Group I (control group) and II (standard group) received distilled water (1ml/kg b.w.) for seven days, on day seven group II received diazepam (20mg/kg) as standard before one hour of test. Group (III-VI) received *N. sativa* extracts from different germination phases (1g/kg b.w. orally) from day one to seven. On day seven, after one hour of treatment all animals were ready for MES induced seizure. Various phases of epilepsy like seizure, extension in limbs, clonus and recovery time were observed in MES-induced animals.

### 6.3.9 Antidepressant activity

Antidepressant activity was evaluated by forced swim and tail suspension test.

#### 6.3.9.1 Forced Swim Test (FST)

Forced swim test, the most frequently used behavioral model for screening antidepressant-like activity in rats was first proposed by Porsolt *et al.*, (1978). Rats were moved from the animal house to laboratory in their own cages and allowed to adapt to the laboratory conditions for 1-2 h. Rats were forced to swim in an open cylindrical container (diameter 20 cm, height 45 cm), containing 38 cm of water at 25 ± 1°C. All rats were divided into six different groups (Group I-VI). The rats were tested in two sessions: an initial 15 min training session latter after 24 h by a 6 min test session. Following the training session rats were removed from the cylinder, towel dried and then returned to the home cage for testing them again after 24 h latter.
Plate 6.2 Rat in cylindrical container during forced swim test.

Group I and II received distilled water (1ml/kg b.w.) for seven days as control group, on day seven Group II received imipramine (15 mg/kg) as standard before one hour of test. Group (III-VI) received *N. sativa* extracts from different germination phases (1g/kg b.w.) orally for seven days. On day seven, after one hour of treatment each rat was forced to swim for a period of six min test. After an initial period of two min which is a period of vigorous activity, each animal assumed a typical immobile posture. A rat was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above the water. The total duration of immobility was recorded during the next four min of the total test duration of six min by a blind observer (Detke *et al*., 1995).

6.3.9.2 Tail Suspension Test (TST)

The tail suspension test used the uncontrollable, inescapable stressor of tail suspension to elicit immobility (Porsolt *et al*., 1978). The rats were treated in same manner as in forced swim test for seven days. Each rat was individually suspended to
the edge of a table, 50 cm above the floor, by adhesive tape placed approximately 1 cm from the tip of the tail. The total period of immobility was recorded manually for six minutes. Animals were considered to be immobile when it didn’t show any body movement, hung passively and completely motionless (Sharma et al., 2009).

6.4 RESULTS AND DISCUSSION

6.4.1 Anxiolytic activity

Anxiolytic effect of *N. sativa* extracts during germination phases was measured using the elevated plus maze test and locomotor activity.

6.4.1.1 Effect of different treatments on anxiolytic effect during elevated plus maze test

In the elevated plus maze test significant increase in the time spent in the open arms indicate an anxiolytic effect of *N. sativa* germination extracts both in unstressed and stressed conditions. All the tested extracts showed significant anxiolytic activity (P<0.001) when compared with control unstressed group.
**Table 6.2:** Effect of *N. sativa* extracts of different germination phases on time spent by rats during elevated plus maze test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Category</th>
<th>Time spent in open arms (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>(Dist. Water)</td>
<td></td>
<td>9.40±0.5</td>
</tr>
<tr>
<td>Group II</td>
<td>Immobilized</td>
<td>Stressed</td>
<td>3.10±0.3^a</td>
</tr>
<tr>
<td>Group III</td>
<td>Diazepam (20mg/kg)</td>
<td>Unstressed</td>
<td>21.10±1.1^a</td>
</tr>
<tr>
<td>Group IV</td>
<td>Diazepam (20mg/kg)</td>
<td>Stressed</td>
<td>7.20±0.4^b</td>
</tr>
<tr>
<td>Group V</td>
<td>Seed extract (0^{th} day)</td>
<td>Unstressed</td>
<td>20.21±0.9^a</td>
</tr>
<tr>
<td>Group VI</td>
<td>Seed extract (0^{th} day)</td>
<td>Stressed</td>
<td>7.01±0.7^b</td>
</tr>
<tr>
<td>Group VII</td>
<td>5^{th} day extract</td>
<td>Unstressed</td>
<td>29.70±1.3^a</td>
</tr>
<tr>
<td>Group VIII</td>
<td>5^{th} day extract</td>
<td>Stressed</td>
<td>10.10±0.5^b</td>
</tr>
<tr>
<td>Group IX</td>
<td>7^{th} day extract</td>
<td>Unstressed</td>
<td>24.02±1.0^a</td>
</tr>
<tr>
<td>Group X</td>
<td>7^{th} day extract</td>
<td>Stressed</td>
<td>9.50±0.8^b</td>
</tr>
<tr>
<td>Group XI</td>
<td>11^{th} day extract</td>
<td>Unstressed</td>
<td>21.09±1.0^a</td>
</tr>
<tr>
<td>Group XII</td>
<td>11^{th} day extract</td>
<td>Stressed</td>
<td>8.29±0.6^b</td>
</tr>
</tbody>
</table>

^aP<0.001, Compared with Group I (control); ^bP<0.001, compared with Group II.
Figure 6.1: Effect of *N. sativa* extracts of different germination phases on time spent by the rats during elevated plus maze test.

Six hours of acute immobilization induced a significant (P<0.001) anxiogenic effects in animals as compared to vehicle-treated unstressed mice (Table 6.1). Diazepam produced significant anti-anxiety effects in unstressed rats (21.10±1.1 sec time spent in open arm) as compared to the control group (9.40±0.5 sec) and in stressed rats (7.20±0.4 sec) as compared to immobilization-induced stressed rats (3.10±0.3 sec). All the extracts of *N. sativa* showed significant anxiolytic effect on unstressed as well as stressed animals that was higher in stressed animals. Extracts from different germination phases showed different degree of anxiolytic activity. Seed of *N. sativa* showed 20.21±0.9 and 7.01±0.7 sec time spent in open arm in unstressed and stressed animals respectively. Extract of 5th day germination phase showed best anxiolytic activity among the all tested extracts in both unstressed and stressed animal model 29.70±1.3 and 10.10±0.5 respectively followed by 7th and 11th day germination extracts (Table 6.1). *N. sativa* produced significant anti-anxiety effects in germination phases when compared with the control group (9.40±0.5 sec) and immobilization-induced stressed rats (3.10±0.3 sec, Figure 6.1).
6.4.1.2 Effect of different treatments on locomotor activity

Locomotor activity in rats after treatment with *N. sativa* extracts from different germination phases was measured using actophotometer in unstressed as well as stressed animal model. All the tested extracts and standard drug diazepam showed different degrees in locomotor activity.

**Table 6.3:** Effect of *N. sativa* extracts of different germination phases on locomotor activity in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Category</th>
<th>Locomotor activity counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Dist. Water</td>
<td></td>
<td>338.8 ± 10.6</td>
</tr>
<tr>
<td>Group II</td>
<td>Immobilized</td>
<td>Stressed</td>
<td>134.3 ± 8.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>Diazepam (20mg/kg)</td>
<td>Unstressed</td>
<td>295.5 ± 11.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>Diazepam (20mg/kg)</td>
<td>Stressed</td>
<td>169.2 ± 6.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>Seed extract (0&lt;sup&gt;th&lt;/sup&gt; day)</td>
<td>Unstressed</td>
<td>325.6 ± 12.3</td>
</tr>
<tr>
<td>Group VI</td>
<td>Seed extract (0&lt;sup&gt;th&lt;/sup&gt; day)</td>
<td>Stressed</td>
<td>115.1 ± 5.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VII</td>
<td>5&lt;sup&gt;th&lt;/sup&gt; day extract</td>
<td>Unstressed</td>
<td>320.4 ± 10.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VIII</td>
<td>5&lt;sup&gt;th&lt;/sup&gt; day extract</td>
<td>Stressed</td>
<td>108.0 ± 8.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX</td>
<td>7&lt;sup&gt;th&lt;/sup&gt; day extract</td>
<td>Unstressed</td>
<td>321.8 ± 13.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group X</td>
<td>7&lt;sup&gt;th&lt;/sup&gt; day extract</td>
<td>Stressed</td>
<td>111.0 ± 9.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group XI</td>
<td>11&lt;sup&gt;th&lt;/sup&gt; day extract</td>
<td>Unstressed</td>
<td>324.3 ± 12.6</td>
</tr>
<tr>
<td>Group XII</td>
<td>11&lt;sup&gt;th&lt;/sup&gt; day extract</td>
<td>Stressed</td>
<td>112.0 ± 10.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>P<0.001, Compared with Group I (control); <sup>b</sup>P<0.001, compared with Group II.
Figure 6.2: Effect of *N. sativa* extracts of different germination phases on locomotor activity counts in rats.

Immobilization significantly decreased the locomotor activity of rats as compared to unstressed control group. Diazepam reduced locomotor activity in unstressed rats (295.5 ± 11.2) as compared to the unstressed control group (338.8 ± 10.6) but did not affect stressed rats (169.2 ± 6.29) as compared to immobilization-induced stressed rats (134.3 ± 8.31). All the extracts of *N. sativa* significantly reduced locomotor activity in unstressed as well as stressed animals that was higher in stressed animals. Seed of *N. sativa* showed locomotor activity count 325.6 ± 12.3 and 115.1 ± 5.27 in unstressed and stressed animals respectively. Extract of 5th day germination phase again showed best effect amongst the all tested extracts in both unstressed and stressed animal model having 320.4 ± 10.44 and 108.0 ± 8.33 locomotion count respectively followed by 7th and 11th day germination extracts (Table 6.2 and Figure 6.2) as compared to the unstressed control group (338.8 ± 10.6) and immobilization-induced group (134.3 ± 8.31).
6.4.2 Antiepileptic activity

6.4.2.1 Effect of different treatments on maximal electroshock induced seizures

All the extracts of \textit{N. sativa} from different germination phases exhibited significant ($P<0.001$) reduction in various phases of epileptic seizure on comparison with the reference standard diazepam (20 mg/kg). There was also a significant reduction in the time required for the righting reflex (recovery) in the extract treated groups (Table 6.3).

\textbf{Table 6.4} Effect of \textit{N. sativa} extracts of different germination phases on maximal electroshock induced seizures in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Convulsion (sec)</th>
<th>Hind limb extension (Sec)</th>
<th>Clonus (Sec)</th>
<th>Recovery (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Dist. Water</td>
<td>9.5±0.12</td>
<td>13.4±0.14</td>
<td>15.2±0.13</td>
<td>120±2.6</td>
</tr>
<tr>
<td>Group II</td>
<td>Diazepam-20mg/kg</td>
<td>3.1±0.13$^a$</td>
<td>0.0±0.00</td>
<td>5.32±0.20$^a$</td>
<td>110±3.1$^a$</td>
</tr>
<tr>
<td>Group III</td>
<td>Seed extract (0$^{th}$ day)</td>
<td>3.1±0.30$^a$</td>
<td>5.2±0.33$^a$</td>
<td>7.11±0.31$^a$</td>
<td>106±2.2$^a$</td>
</tr>
<tr>
<td>Group IV</td>
<td>5$^{th}$ day extract</td>
<td>2.9±0.09$^a$</td>
<td>0.2±0.01$^a$</td>
<td>5.20±0.13$^a$</td>
<td>35±2.4$^a$</td>
</tr>
<tr>
<td>Group V</td>
<td>7$^{th}$ day extract</td>
<td>3.0±0.20$^a$</td>
<td>2.1±0.12$^a$</td>
<td>5.21±0.22$^a$</td>
<td>59±1.6$^a$</td>
</tr>
<tr>
<td>Group VI</td>
<td>11$^{th}$ day extract</td>
<td>3.1±0.21$^a$</td>
<td>2.9±0.10$^a$</td>
<td>5.20±0.12$^a$</td>
<td>61±2.1$^a$</td>
</tr>
</tbody>
</table>

$^a$P<0.001 compared with Group I (control).
Figure 6.3 Effect of *N. sativa* extracts of different germination phases on MES induced seizures in rats.

Figure 6.4 Effect of *N. sativa* extracts of different germination phases on recovery time during MES induced seizures in rats.
A significant reduction in the time required for the recovery (righting reflex) was observed in this study (Table 6.3) which proves that *N. sativa* extracts from different germination phases provided a beneficial effect in controlling MES-induced seizures. Convulsion was significantly reduced in extract treated groups as well as standard group II (3.1±0.13 sec) when compared to control (9.5±0.12 sec). Extract of 5\textsuperscript{th} day germination phase of *N. sativa* strongly reduced convulsion (2.9±0.09 sec) followed by 7\textsuperscript{th} (3.0±0.20 sec), 11\textsuperscript{th} (3.1±0.21 sec) and seed extract (3.1±0.30 sec). Hind limb extension was not observed in diazepam treated and 5\textsuperscript{th} day extract treated groups. Clonus time and recovery time was also reduced in *N. sativa* extracts treated groups (Table 6.3, Figure 6.3 & 6.4).

### 6.4.3 Antidepressant activity

#### 6.4.3.1 Effect of different treatments of *N. sativa* on immobility period of rats in forced swim test (FST) and Tail suspension test (TST)

*N. sativa* extracts from different germination phases did not exhibited significant reduction in immobility of rats during FST and TST, in comparison with the reference standard Imipramine with a dose of 15 mg/kg b.w..

**Table 6.5:** Effects of *N. sativa* extracts of germination phases in forced swim test (FST) and Tail suspension test (TST).

<table>
<thead>
<tr>
<th>Group *</th>
<th>Treatment</th>
<th>Duration of immobility (sec) in FST</th>
<th>Duration of immobility (sec) in TST</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>Dist. Water</td>
<td>108.23 ±4.2</td>
<td>125±5.1</td>
</tr>
<tr>
<td>Group II</td>
<td>Imipramine (15mg/kg)</td>
<td>34.66 ±2.3\textsuperscript{a}</td>
<td>49.33±3.66\textsuperscript{a}</td>
</tr>
<tr>
<td>Group III</td>
<td>Seed extract (0\textsuperscript{th} day)</td>
<td>95.21 ±3.3</td>
<td>110.0±4.3</td>
</tr>
<tr>
<td>Group IV</td>
<td>5\textsuperscript{th} day extract</td>
<td>89.12 ±2.4\textsuperscript{a}</td>
<td>102.25±2.3\textsuperscript{a}</td>
</tr>
<tr>
<td>Group V</td>
<td>7\textsuperscript{th} day extract</td>
<td>93.32 ±2.7\textsuperscript{a}</td>
<td>109.0±3.5\textsuperscript{a}</td>
</tr>
<tr>
<td>Group VI</td>
<td>11\textsuperscript{th} day extract</td>
<td>95.36 ±3.9</td>
<td>110.32±3.8</td>
</tr>
</tbody>
</table>

\textsuperscript{a}P<0.001, compared with Group I (control).
Figure 6.5 Effects of *N. sativa* extracts of germination phases on immobility period of rats during forced swim test (FST)

Figure 6.6: Effects of *N. sativa* extracts of germination phases on immobility period of rats during tail suspension test (TST)
A significant reduction in immobility during both tests was not observed, except in 5th day germination extracts (Table 6.4, Figure 6.5 and 6.6). Imipramine significantly (P<0.001) reduced immobility (34.66 ±2.3 sec and 49.33±3.66 sec) in rats when compared to control group (108.23 ±4.2 sec and 125±5.1sec) respectively in FST and TST. Imipramine is an anti-depressant medication, a tricyclic antidepressant of the dibenzazepine group. Imipramine is mainly used in the treatment of major depression and enuresis (inability to control urination). It has also been evaluated for use in panic disorder (Lepola et al., 2003). During the test for antidepressant effect of N. sativa showed slight antidepressant property only in 5th day germination extract.

In the present study N. sativa extracts of different germination phases showed (1g/kg) significant anxiolytic activity in unstressed rats as well as stressed rats as compared to non-germinated seed extract of N. sativa. Diazepam produced a significant anxiolytic effect in unstressed mice, but the anti-anxiety effect of diazepam was observed to be compromised in stressed mice. This is in agreement with the study of Gilhotra et al., (2010). The anxiolytic effect of N. sativa was comparable to that of diazepam (20mg/kg) in unstressed rats.

The anti-anxiety like effect of N. sativa and diazepam seem not to be associated with any motor effects because these drugs did not significantly changed locomotor function of treated rats (unstressed) as compared to control group. This confirms the assumption that these drugs are specific in their anti-anxiety like effect. Forced immobilization is one of the best explored models of stress in rodents. This model combines touching stress (escape reaction) and physiological stress (muscle work), resulting in both limited mobility and violent behavior. In this study, we used physical immobilization for 6 hrs as a stressor in rat and found that stress-exposed rats showed more anxious behavior when compared with unstressed mice. This finding is in agreement with earlier reports that acute (6 hrs) stress activates nitric oxide synthase (NOS) and enhances anxiety in rodents (Sevgi et al., 2006; Goyal et al., 2007; Gilhotra et al., 2009; Gilhotra et al., 2010a; Gilhotra et al., 2010b). Acute
immobilization stress, as used in the present study, is reported to increase expression of inducible nitric oxide synthase (iNOS) in the brain cortex and leads to production of the stable nitric oxide metabolites (nitrite and nitrate) in both plasma and brain (Madrigal et al., 2002b). Furthermore, physical or psychological stress-induced changes in the brain correlate with the production of NO metabolites in both peripheral (plasma) and central (brain) compartments (Madrigal et al., 2002a).

Study of Gilhotra et al., (2011) reported that diazepam served to increase brain GABA levels in both unstressed and stressed mice, it produced significant anxiolytic effects in unstressed mice but was unable to exert significant anti-anxiety effects under stressful conditions. In present study diazepam produced significant anxiolytic effects in unstressed rats (Table 6.1) but not in stressed rats. Our study is in agreement with previous study of Gilhotra et al., (2011). There are two reasons of the pragmatic lack of anti-anxiety effect of diazepam in stressed rats: first the immobilization stress-induced disturbances in GABAergic receptors and benzodiazepine coupling to these receptors; and second the immobilization stress-induced strong anxiogenic nitriergic power and ensuing nitric oxide cyclic guanosine monophosphate (NO\textsuperscript{cGMP}) enhanced endogenous anxiety accompanied by decreased GABAergic influence. It is well known that behavioral effects of drugs acting at the GABA-benzodiazepine-barbiturate complex may vary between stressed and unstressed animals (Boix et al., 1988).

Furthermore, immobilization stress is accompanied by an increase in the level of endogenous anxiety and induces demanding changes in the GABA-benzodiazepine-barbiturate complex in the brain of stressed animals (Weizman et al., 1990). Immobilization stress of 6 hrs, as used in the present study, has been shown to produce subsensitivity of central GABA receptors (Sun et al., 1990). Study of Mosaddeghi et al., (1993) reported chronic mild restraint stress that produces a decrease in benzodiazepine receptor binding sites. Moreover, immobilization stress for 6 hrs has previously been reported to act as a nitriergic stimulus and to enhance endogenous anxiety (Madrigal et al., 2002b; Gilhotra et al., 2009; Gilhotra et al
2010a). In addition, iNOS-derived NO activates an endogenous NO-sensitive guanylyl cyclase, resulting in increased levels of cGMP (Nagao et al., 2003; André et al., 2005). There is evidence suggesting that the role of the NO/cGMP signaling pathway is the effect of NO on anxiety (Eroglu et al., 1997; Wexler et al., 1998). Inhibition of the nitric oxide–cGMP pathway by inhibition of NOS has been reported to produce anti-anxiety effects (Spolidório et al., 2007).

In a previous study of Gilhotra et al., (2011) diazepam has no effect on NO under stressed conditions; as a result, the effect of diazepam is suppressed under stressed conditions. Therefore, the inability of diazepam to modify the stress-induced increase in nitriergic influence may be responsible for the compromised effect of diazepam in stressed mice. They evaluated the involvement of nitriergic and GABAergic systems in the anti-anxiety effect of thymoquinone (active constituent of N. sativa) under both unstressed and stressed conditions. Another study of Raza et al., (2006) reported anxiolytic effect of thymoquinone in unstressed mice. Thymoquinone significantly attenuated the immobilization-induced increase in plasma nitrite levels and immobilization-induced decrease in GABA content in stressed mice, suggesting that a decrease in NO and increase in GABA may be responsible for the anti-anxiety effect of thymoquinone in stressed mice (Gilhotra et al., 2011).

In unstressed rats, thymoquinone and diazepam induced increases in GABA are accompanied by a significant anxiolytic effect, which may further be attributed to the absence of a strong nitriergic influence in unstressed rats, as evident by the insignificant change in plasma nitrite levels produced by these drugs. The absence of nitriergic influence in unstressed mice has also been demonstrated in other reports (Beijamini et al., 2006; Gilhotra et al., 2010). The observed pattern of behavioral and biochemical effects of N. sativa extracts from germination phases and diazepam under unstressed and stressed conditions further suggests that the nitriergic stimulus in stressed rats is sufficient to disturb benzodiazepine-GABA receptor function. These observations are strengthened by earlier reports of disturbance in
benzodiazepine-GABA receptor function by stressful stimuli, including immobilization (Weizman et al 1990). Thus, the inability of diazepam to show anxiolytic effects under stressed conditions presented here show a stress-induced disturbance in the GABA-benzodiazepine-barbiturate complex as well as strong nitriergic influence, although the exact mechanism behind this inability has yet to be explored fully.

In modulation of various behaviors serotonin, 5-hydroxytryptamine (5-HT) plays an important role. Evidence supporting the involvement of central 5-HT in anxiety related behavior and in the mechanism of action of anxiolytic is well documented (Handley and McBlaney, 1993). Studies on the benzodiazepine (BZ) antagonist flumazenil showed that the anxiolytic activity of thymoquinone may involve BZ receptors (Raza et al., 2006). Pharmacological actions of BZ are mediated via BZ recognition site and subsequent facilitation of GABAergic neurotransmission (Raza et al., 2006).

Parveen et al., (2009) reported that that administration of *N. sativa* oil increased tryptophan and 5-HT level and decreased the level of 5HIAA. 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 precursor amino acid tryptophan to serotonergic neurons (Frenstrom and Wurtman, 1971; Leathwood, 1987; Haleem, 1990). Increased brain tryptophan contributes in enhancement of brain 5-HT metabolism (Haleem et al., 1998; Parveen et al., 2009). 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). Anxiolytic effect of *N. sativa* observed in the present study possibly mediated increase in tryptophan level and decrease in 5-HT turnover.

*N. sativa* extracts from germination phases also reduced various phases of epileptic seizure on comparison with the control group. A significant reduction in the time required for the recovery (righting reflex) was observed in this study (Table 6.3,
Figure 6.3 and 6.4), which proved that *N. sativa* was providing a beneficial effect in controlling MES induced seizures. It is well known that anticonvulsants/antiepileptic drugs act to prevent the spread of the neuronal excitation by mechanisms that are not fully understood, but which can be roughly divided into those which involve stabilizing effect on excitable cell membranes, and those which involve enhanced functional activity of neurotransmitters, such as GABA, which then act to inhibit spread of seizure activity by blocking synaptic transmission at some point. Status epilepticus is potentially fatal, and is a medical emergency requiring swift and effective treatment to minimize the risk of brain damage (Grammes-Smith and Aronson, 1984).

Administration of *N. sativa* significantly increased the brain levels of serotonin, dopamine and noradrenalin, which could be attributed to the significant protection offered against MES induced seizures as well as anxiety (Parveen *et al*., 2009). Serotonin (5-HT) is an inhibitory neurotransmitter involved in the regulation of mood, sleep, anxiety, arousal and aggression. Serotonin agonists, precursors and neuronal uptake inhibitors were reported to enhance narcoleptic catalepsy (Bhattacharya *et al*., 1984). The increase in the serotonergic transmission raises the threshold of MES induced seizures in many animal test systems, thereby protecting against MES induced convulsions (Hosseinzadeh *et al*., 2004; Jennifer *et al*., 2011). Brain and plasma levels of tryptophan also increased significantly following oral repeated administration of *N. sativa* oil (Parveen *et al*., 2009). Based on this, it may be suggested that *N. sativa* oil is a useful choice for the treatment of anxiety and epilepsy.

Our study is conformity of previous study of Gilhotra *et al*., (2011) and Raza *et al*., (2006) who reported anxiolytic effect of *N. Sativa* and their active constituent thymoquinone. In present study, a significant anxiolytic effect of *N. sativa* extracts from different germination phases was observed. Extract from 5th day germination showed significant anxiolytic as well as antiepileptic effect as compared to whole seed extract. This may be due to the enhanced production of secondary metabolites
and active constituent during germination. Germination is a phenomenon during which rapid changes in metabolic activities and the inter-conversions of metabolites take place. The qualitative analyses of phytochemicals present in the methanolic extracts of *N. sativa* seed during germination showed the presence of higher amount of sterols, alkaloids, saponins, phenols, flavonoids, terpenoids and cardiac glycosides, this was reported in our previous study (Islam *et al.* 2013a). Furthermore, *N. sativa* extracts especially 5th day and other germination phases showed significant anti-anxiety and antiepileptic activity in rats through possible modulation of 5-HT, tryptophan, NO and GABA level.

On the other hand in present study extracts of *N. sativa* posses significant anti-depressant effect it may be due to increased 5-HT levels. Higher 5-HT levels produce antidepressant effects. Administration of tryptophan, precursor of 5-HT, has been shown to increase concentration of brain 5-HT (Young *et al.*, 1981; Fernstrom, 1985) and produce antidepressant effects (Haleem *et al.*, 1998). *N. sativa* oil increased brain 5-HT levels and decreased 5-HT turnover (5-HT/5-HIAA ratio). Levels of tryptophan increased significantly in brain and plasma following repeated administration of *N. sativa* oil (Parveen *et al.*, 2010). Thus *N. sativa* oil showed a potential antidepressant-like effect. *N. sativa* showed very good anti-inflammatory and analgesic effect during germination phases especially in 5th day germination phase that was reported in our previous study (Islam *et al.*, 2013b). Study of Barber (2011) reported that tramadol which is a centrally acting synthetic opioid with analgesic efficacy comparable to codeine attributed to low but effective affinity for the mu-opioid receptor (μ). These medications are proven effective antidepressants and this shared monoaminergic action resulted in the research of tramadol as a potential treatment for depression (Barber *et al.*, 2011). On the basis of this report we can say that *N. sativa* also showed slight anti-depression effect due to their analgesic properties.

From these results it is wrapped up that *N. sativa* extracts, especially 5th day and other germination phases showed significant CNS depressant effect in rats. Our
study is in agreement with the study of Al-Naggar, (2003) who reported that methanolic extract of *N. sativa* possess CNS depressant activity (Al-Naggar *et al.*, 2003). During the germination *N. sativa* extracts (5\(^{th}\) day) also possess significant antiepileptic effects as compared than standard drug.

### 6.5 CONCLUSION

It is concluded that, the extracts of *N. sativa* from different germination phases showed significant anxiolytic and antiepileptic effects. Anxiolytic effect of *N. sativa* was observed in both unstressed as well as stressed animal model. Diazepam was unable to induce anxiolytic effect in stressed rats. This incapability of diazepam to show anxiolytic effects under stressed conditions may be due to disturbance in the GABA-benzodiazepine-barbiturate complex as well as strong nitriergic influence by stress. Furthermore, extracts from germination phases of *N. sativa* especially 5\(^{th}\) germination extract followed by 7\(^{th}\) day extract showed significant anti-anxiety like activity as well as antiepileptic and antidepressant effect in rats. It may be due to possible modulation in serotonin (5-HT), NO and GABA level. Hence, it was concluded that during germination *N. sativa* have significant neuroprotective effects as compare to non-germinated seed.
6.6 REFERENCES


