Neuroprotective effects of *Nigella sativa* extracts during germination on central nervous system

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ABSTRACT

Background: *Nigella sativa* Linn. which has many acclaimed medicinal properties is an indigenous herbaceous plant and belongs to the *Ranunculaceae* family, which grows in countries bordering the Mediterranean Sea, Pakistan and India. **Objective:** This study was designed to investigate the effects of *N. sativa* seed extracts of different germination phases on the central nervous system (CNS) responses in experimental animals. **Materials and Methods:** Anxiolytic, locomotor activity of extracts (1 g/kg of body weight) was evaluated in both stressed and unstressed animal models and antiepileptic effect was evaluated by maximal electroshock seizure model keeping diazepam (20 mg/kg) as a positive control. Antidepressant effect was evaluated by forced swim test and tail suspension test keeping imipramine (15 mg/kg) as a positive control. **Results:** All tested extracts of *N. sativa* during different phases of germination (especially 5th day germination phase) showed significant ($P < 0.001$) anxiolytic effect in comparison to control. Diazepam reduced locomotor activity in control (unstressed) rats but did not show affect in stressed rats while *N. sativa* extracts from germination phases significantly ($P < 0.001$) reduced locomotor activity in unstressed as well as stressed animals. All the extracts of *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). During antidepressant test, *N. sativa* extracts exhibited a slight reduction in the immobility of rats. **Conclusion:** During germination, especially in 5th day germination extract, *N. sativa* showed significant CNS depressant activity as compared to whole seeds that possibly may be due higher content of secondary metabolites produced during germination.

Key words: Antidepressant, anxiety, central nervous system, epilepsy, germination, *Nigella sativa*

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INTRODUCTION

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 sternly functionally overwhelming in nature.[1] Per estimate, up to 21% of the world's population is found to be affected by depressive disorders, one of the most prevalent psychiatric diseases.[2] It is a major cause of disability and death by suicide due to raised rates of physical disorders.[3] Neurodegenerative diseases represent a large group of neurological disorders with heterogeneous clinical and pathological expressions affecting specific subsets of neurons in specific functional anatomical systems. They arise from unknown reasons and progress in a relentless manner. Neurodegenerative disorders are a major cause or mortality and disability and as a result of increasing life spans represent one of the key medical research challenges.[3] 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.[4]

Nigella sativa L. is widely studied due to its strong traditional claims and beliefs of having a therapeutic role in almost every disease process.[5] The main active ingredients isolated from *N. sativa* seeds are thymoquinone, thymol, alkaloids like nigellidine, nigellimine, and nigellicine, vitamins, minerals and proteins.[6] Very modest literature

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was found on the neuroprotective effect of *N. sativa*. *In vitro* studies confirmed that pretreatment with *N. sativa* oil has significantly improved neuronal cell viability[7] and methanolic extract of *N. sativa* modulates the neuronal release of amino acid neurotransmitters including gamma-aminobutyric acid (GABA), glycine, aspartate and glutamate on cultured cortical neurons and also possesses a potent central nervous system (CNS) and analgesic activity.[8,9] *N. sativa* and thymoquinone have been recognized as neuroprotective agents.[10] The aim of the present study was to investigate *in vivo* neuroprotective effects of *N. sativa* seed during different germination phases on CNS in Wistar rat. This is the first study on neuroprotective effect during germination of *N. sativa* seed.

MATERIALS AND METHODS

Collection of *Nigella sativa* seed

Seeds of *N. sativa* were procured from a grocery shop in Lucknow in the month of February, 2012. A voucher specimen of the seeds was kept in the Museum of the Department for future reference.

Germination of seeds

Germination was done according to the method of Ahmad *et al*.[11] Seeds were surface sterilized with 0.1% $HgCl_2$ for 3 min. They were rinsed thoroughly with double distilled water and soaked in de-ionized water for 30 min. Seeds were grown in glass petri plates. They were placed on four folds of damp filter paper at 25°C and incubated in the dark till the initiation of sprouting (3rd day) after which they were placed at a light intensity of 100 μ mol/m^2/s and a 14/10 h (day/night) photoperiod until the complete plantlet with two leaves were obtained. The complete germination took 11 days with the 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 period.

Preparation of distilled extracts

Germination induces the formation of bioactive compounds.[12] Therefore, extracts of the different days of germination were taken for the study. The samples of seed and germinated phases (5th, 7th and 11th day) were shade-dried and ground to a fine powder. The powder (20 g) was extracted using soxhlet apparatus with 200 ml of methanol solvent for 48 h in order to extract bioactive compounds. The extracts were filtered using Whatman filter paper (No. 1) and methanol was evaporated using rotary distillation apparatus to obtain the pure extract. Oily fraction of extracts was stored at 4°C until use.

Animals

Male Wistar rats, weighing 150–200 g, were purchased from Central Drug and Research Institute, Lucknow, India and housed in a temperature controlled room (22°C ± 2°C) with a 12 h light-12 h 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 and 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.).

Drugs

Diazepam (Calmpose®; Ranbaxy Laboratories Ltd., India) and imipramine (Depsonil, S.G Pharmaceuticals, Vadodara).

Anxiolytic activity by elevated plus maze test

The plus maze apparatus consisted of two open arms (without walls), 16 × 5 cm, and two enclosed arms, 16 cm × 5 cm × 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 toward an open arm and time spent in the open arm during a 5 min observation period was noted.[13,14]

Locomotor activity

The effects of various treatments on the 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 were 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.[15]

Experimental protocol

The rats were divided into 12 groups containing six rats in each group. Stress was produced by immobilizing the rat for 6 h (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" × 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 (1 ml/kg of body weight) for 7 days. Group III received diazepam (20 mg/kg of body weight) 1 h before test on day 7 and group IV also received diazepam (20 mg/kg) 1 h before subjecting them to immobilization for 6 h. Groups V–XII received *N. sativa* extracts, 1 g/kg of body weight[16] from different germination phases (0th day, i.e. seed extract, 5th, 7th and 11th day extract) for 7 days. On day 7, unstressed groups of animals received extracts of *N. sativa* 1 h before testing them in various behavioral paradigms. The remaining groups of animals received extract 1 h before subjecting them to immobilization for 6 h.[17]

Anticonvulsant activity in maximal electroshock induced seizures model

Maximal electroshock (MES) model was used to evaluate the anticonvulsant activity of extracts. Seizures were induced in rats by delivering electroshock of 50 mA for 0.2 s by means of an electro-convulsiometer through a pair of ear clip electrodes.[18,19] All rats were divided into six different groups (Group I–VI). Group I (control group) and II (standard group) received distilled water (1 ml/kg of body weight) for 7 days; on day 7, group II received diazepam (20 mg/kg) as standard before 1 h of test. Group (III–VI) received *N. sativa* extracts from different germination phases (1 g/kg) from day 1 to 7. On day 7, after 1 h 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.

Antidepressant activity

Forced swim test

Forced swim test (FST), the most frequently used behavioral model for screening antidepressant-like activity in rats was first proposed by Porsolt in 1978.[20] Rats were moved from the animal house to the 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°C ± 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.

Group I and II received distilled water (1 ml/kg of body weight) for 7 days as control group, on day 7 Group II received imipramine (15 mg/kg) as standard before 1 h of test. Group (III–VI) received *N. sativa* extracts from different germination phases (1 g/kg of body weight) orally for 7 days. On day 7, after 1 h of treatment, each rat was forced to swim for a period of 6 min test. After an initial period of 2 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 4 min of the total test duration of 6 min by a blind observer.[21]

Tail suspension test

Tail suspension test (TST) used the uncontrollable, inescapable stressor of tail suspension to elicit immobility.[20] The rats were treated in the same manner as in FST for 7 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 6 min. Animals were considered to be immobile when it didn't show anybody movement, hung passively and completely motionless.[22]

RESULTS

Effect of different treatment on anxiolytic activity 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.

Six hours of acute immobilization induced a significant ($P < 0.001$) anxiogenic effects in animals as compared to vehicle-treated unstressed mice [Table 1]. Diazepam produced significant anti-anxiety effects in unstressed rats (21.10 ± 1.1 s time spent in open arm) as compared to the control group (9.40 ± 0.5 s) and in stressed rats (7.20 ± 0.4 s) as compared to immobilization-induced stressed rats (3.10 ± 0.3 s). All the extracts of *N. sativa* showed a 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 s time spent in open arm in unstressed and stressed animals respectively. Extract of 5th day germination phase showed the 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 1]. *N. sativa* produced significant anti-anxiety effects in germination phases compared with the control group (9.40 ± 0.5 s) and immobilization-induced stressed rats (3.10 ± 0.3 s).

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 degree in locomotor activity.

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 of 325.6 ± 12.3 and 115.1 ± 5.27 in unstressed and stressed animals respectively. Extract of 5th day germination phase again showed the best effect among 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 2] as compared to the unstressed control group (338.8 ± 10.6) and immobilization-induced group (134.3 ± 8.31).

Table 1: Effect of *Nigella sativa* extracts of different germination phases in elevated plus maze test

Group	Treatment	Category	Time spent in open arms (s)
Group I	Distilled water		9.40±0.5
Group II	Immobilized	Stressed	3.10±0.3[a]
Group III	Diazepam (20 mg/kg)	Unstressed	21.10±1.1[a]
Group IV	Diazepam (20 mg/kg)	Stressed	7.20±0.4[b]
Group V	Seed extract (0th day)	Unstressed	20.21±0.9[a]
Group VI	Seed extract (0th day)	Stressed	7.01±0.7[b]
Group VII	5th day extract	Unstressed	29.70±1.3[a]
Group VIII	5th day extract	Stressed	10.10±0.5[b]
Group IX	7th day extract	Unstressed	24.02±1.0[a]
Group X	7th day extract	Stressed	9.50±0.8[b]
Group XI	11th day extract	Unstressed	21.09±1.0[a]
Group XII	11th day extract	Stressed	8.29±0.6[b]

[a]*P*<0.001, compared with group I (control); [b]*P*<0.001, compared with group II

Table 2: Effect of different treatments of *Nigella sativa* extracts from different germination phases on locomotor activity counts in rats on actophotometer

Group	Treatment	Category	Locomotor activity counts
Group I	Distilled water		338.8±10.6
Group II	Immobilized	Stressed	134.3±8.31[a]
Group III	Diazepam (20 mg/kg)	Unstressed	295.5±11.2[a]
Group IV	Diazepam (20 mg/kg)	Stressed	169.2±6.29[a]
Group V	Seed extract (0th day)	Unstressed	325.6±12.3
Group VI	Seed extract (0th day)	Stressed	115.1±5.27[b]
Group VII	5th day extract	Unstressed	320.4±10.44[a]
Group VIII	5th day extract	Stressed	108.0±8.33[b]
Group IX	7th day extract	Unstressed	321.8±13.1[a]
Group X	7th day extract	Stressed	111.0±9.19[b]
Group XI	11th day extract	Unstressed	324.3±12.6
Group XII	11th day extract	Stressed	112.0±10.1[b]

[a]*P*<0.001, compared with group I (control); [b]*P*<0.001, compared with group II

Effect of different treatment on anticonvulsant (antiepileptic) activity during maximal electroshock induced seizures

All the extracts of *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 of body weight). There was also a significant reduction in the time required for the righting reflex (recovery) in the extract treated groups [Table 3].

A significant reduction in the time required for the recovery (righting reflex) was observed in this study [Table 3], which proves that *N. sativa* extracts from different germination phases provided a beneficial effect in controlling MES induced seizures. The convulsion was significantly reduced in extract treated groups as well as standard group II (3.1 ± 0.13 s) when compared to control (9.5 ± 0.12 s). Extract of 5th day germination phase of *N. sativa* strongly reduced convulsion (2.9 ± 0.09 s) followed by 7th (3.0 ± 0.20 s), 11th (3.1 ± 0.21 s) and seed extract (3.1 ± 0.30 s). Hind limb extension was not observed in diazepam treated and 5th day extract treated groups. Clonus time and recovery time was also reduced in *N. sativa* extracts treated groups [Table 3].

Effect of different treatment, of *Nigella sativa* on antidepressant effects during forced swim test and tail suspension test

Nigella sativa extracts from different germination phases exhibited a reduction in the immobility of rats during FST and TST, in comparison with the reference standard Imipramine 15 mg/kg of body weight. Furthermore, extracts of 5th and 7th day germination phases showed a significant reduction in immobility in rats.

A significant reduction in immobility during both tests was not observed, except in 5th, day germination phase extract [Table 4]. Imipramine significantly ($P < 0.001$)

Table 3: Effect of *Nigella sativa* extracts of different germination phases on maximal electroshock induced seizures in rats

Group	Treatment	Convulsion (s)	Hind limb extension (s)	Clonus (s)	Recovery (s)
Group I	Distilled water	9.5±0.12	13.4±0.14	15.2±0.13	120±2.6
Group II	Diazepam (20 mg/kg)	3.1±0.13[a]	0±0.00	5.32±0.20[a]	110±3.1[a]
Group III	Seed extract (0th day)	3.1±0.30[a]	5.2±0.33[a]	7.11±0.31[a]	106±2.2[a]
Group IV	5th day extract	2.9±0.09[a]	0.2±0.01[a]	5.20±0.13[a]	35±2.4[a]
Group V	7th day extract	3.0±0.20[a]	2.1±0.12[a]	5.21±0.22[a]	59±1.6[a]
Group VI	11th day extract	3.1±0.21[a]	2.9±0.10[a]	5.20±0.12[a]	61±2.1[a]

[a]*P*<0.001, compared with group I (control)

Table 4: Effects of *Nigella sativa* extracts from germination phases on immobility period of rats in FST and TST

Group	Treatment	Duration of immobility (s) in FST	Duration of immobility (s) in TST
Group I	Distilled water	108.23±4.2	125±5.1
Group II	Imipramine (15 mg/kg)	34.66±2.3[a]	49.33±3.66[a]
Group III	Seed extract (0th day)	95.21±3.3	110.0±4.3
Group IV	5th day extract	89.12±2.4[a]	102.25±2.3[a]
Group V	7th day extract	93.32±2.7	109.0±3.5
Group VI	11th day extract	95.36±3.9	110.32±3.8

[a]*P*<0.001, compared with group I (control). FST: Forced swim test; TST: Tail suspension test

reduced immobility (34.66 ± 2.3 s and 49.33 ± 3.66 s) in rats when compared to control group (108.23 ± 4.2 s and 125 ± 5.1 s) 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.[23]

DISCUSSION

In the present study, *N. sativa* extracts of different germination phases showed significant anxiolytic activity in unstressed rats as well as stressed rats compared to whole seed extract or nongerminated *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*.[24] The anxiolytic effect of *N. sativa* was comparable to that of diazepam (20 mg/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 change locomotor function of treated rats (unstressed) as compared to control mice. This confirms the assumption that the anti-anxiety-like effect of these drugs is specific. 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 h as a stressor in rat and found that stress-exposed rats showed more anxious behavior when compared with unstressed mice. These findings are in agreement with earlier reports that acute (6 h) stress activates nitric oxide synthase (NOS) and enhances anxiety in rodents.[25-28] Acute immobilization stress, as used in the present study, is reported to increase expression of inducible NOS in the brain cortex and leads to production of the stable NO metabolites (nitrite and nitrate) in both plasma and brain.[29]

Study of Gilhotra *et al.,* (2011) reported that diazepam served to increase brain GABA levels in both unstressed and stressed mice as it produced significant anxiolytic effects in unstressed mice but was unable to exert significant anti-anxiety effects under stressful conditions. In the present study, diazepam produced significant anxiolytic effects in unstressed rats [Table 1] but not in stressed rats.[30] The pragmatic lack of anti-anxiety effect of diazepam in stressed rats may be sufficiently explained by two sets of interpretation: 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 NO cyclic guanosine monophosphate 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.[31]

In addition, 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.[32] Immobilization stress of 6 h, as used in the present study, has been shown to produce subsensitivity of central GABA receptors.[33] In addition, inducible NOS-derived NO activates an endogenous NO-sensitive guanylyl cyclase, resulting in increased levels of cyclic

guanosine monophosphate (cGMP).[34,35] There is evidence suggesting that the role of the NO/cGMP signaling pathway is the effect of NO on anxiety.[36] Inhibition of the NO–cGMP pathway by inhibition of NOS has been reported to produce anti-anxiety effects.[37] 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.[30,38]

In the 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.[39] Tahira *et al*., (2009) reported that that administration of *N. sativa* oil increased tryptophan and 5-HT level and decreased the level of 5-Hydroxyindoleacetic acid.[40] Similar results were also reported following the administration of anxiolytic drugs.[41] Serotonin (5-HT) is an inhibitory neuro-transmitter involved in the regulation of mood, sleep, anxiety, arousal and aggression. Serotonin agonists, precursors, and neuronal uptake inhibitors are reported to enhance narcoleptic catalepsy.[42] The increase in the serotonergic transmission raises the threshold of MES induced seizures in many animal test systems, thereby protecting against MES induced convulsions. Administration of *N. sativa* significantly increased the brain levels of serotonin, dopamine, and noradrenaline, which could be attributed to the significant protection offered against MES induced seizures as well as anxiety.[40] *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 3], which proves that *N. sativa* was providing a beneficial effect in controlling MES induced seizures. It was reported *N. sativa* interact with GABA receptors, most probably GABA-A receptors and increased in GABAergic response that reduced epileptic responses.[43]

Extracts of *N. sativa* also possess anti-depressant effect, and 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[44,45] and produce antidepressant effects.[46] *N. sativa* oil increased brain 5-HT levels and decreased 5-HT turnover. Levels of tryptophan increased significantly in brain and plasma following repeated administration of *N. sativa* oil. Thus, *N. sativa* oil showed a potential antidepressant-like effect.

These effects may be due to decidedly 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 sterols, alkaloids, saponins, phenols, flavonoids, terpenoids and cardiac glycosides.[47] In addition, *N. sativa* extracts, especially 5th day and other germination phases showed significant anti-anxiety antiepileptic and antidepressant activity in rats through possible modulation of 5-HT, tryptophan, NO and GABA level. Our study also an agreement for the study of Al-Naggar (2003) who reported that the methanolic extract of *N. sativa* possess CNS depressant activity.[8]

CONCLUSION

The extracts of *N. sativa* from different germination phases showed significant anxiolytic, antiepileptic and antidepressant effects. Anxiolytic effect of *N. sativa* was observed in both unstressed as well as stressed animal model. In addition, extracts from germination phases of *N. sativa* especially 5th germination extract followed by 7th day extract showed significant anti-anxiety like activity as well as antiepileptic 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 compared to nongerminated seed.

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