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Neuropsychiatric Effects of *Nigella sativa* (Black Seed) – A Review

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Abstract

Nigella sativa (*N. sativa*) seed, commonly known as 'Black Seed' in English and 'Al-Habba Al-Sauda' in Arabic, had been frequently used as a folk medicine for a large number of diseases since ancient times. *N. sativa* seed, its oil, various extracts and active components are reported to possess very useful pharmacological effects to include: immune stimulation, anti-inflammatory, antioxidant, anticancer, hypoglycemic, antihypertensive, anti-asthmatic, antimicrobial and anti-parasitic, etc. Some authors have reviewed these pharmacological activities in general but their neuropsychiatric effects are not separately and adequately described. The literature search has revealed a lot of publications pertaining to the actions of *N. sativa* in neurological and psychiatric problems, e.g., the control of pain, epilepsy, Parkinsonism, anxiety and drug dependence, as well as improvement of learning and memory, alertness, elevation of mood and feeling of good health, etc. Besides, because of its antioxidant, anti-inflammatory and other useful actions, was shown to provide neuro-protection from spinal cord injury and prevent damage to brain cells from various nerve toxins in experimental animal models. Moreover, black seed showed promising prophylactic and therapeutic effects on murine toxoplasmosis and demonstrated excellent antimalarial activity against various Plasmodium species in in vivo experiments, including *Plasmodium falciparum* strains notorious for causing cerebral malaria. The present article is intended to briefly review the valuable efforts of scientists to investigate the pharmacological activities and therapeutic potential of this precious natural herb pertaining to the neuropsychiatric diseases. It is hoped that the present manuscript would be of particular interest to the neurologists and psychiatrists, and the information provided would also benefit general physicians, medical students and the community.

Keywords: *Nigella sativa*; Black seed oil; Extracts; Active components; Neurological and psychiatric problems

Introduction

Nigella sativa (*N. sativa*) is a small annual flowering plant, about 20-30 cm tall, commonly grown in Middle East, Middle Asia and North Africa. It has finely divided green leaves and beautiful, delicate, pale blue and white flowers. The inflated capsule of its ripe fruit is comprised of 3-7 united follicles, each containing numerous oval shaped black tiny seeds, about 1mm in diameter. *N. sativa* belongs to the botanical family of Ranunculaceae and its seeds are named as 'Black Seed' or 'Black Cumin' in English, 'Habba Al-Sauda' or 'Habba Al-Barakah' in Arabic, 'Kalonji' in Urdu, 'Siyah Daneh' in Persian and 'Corek Out' in Turkish language. The scientific name is a derivative of Latin 'niger' meaning 'black' [1].

N. sativa seed and its oil have been found in several sites from ancient Egypt, including Tutankhamun's tomb (1350 BD). Items entombed with a pharaoh were carefully selected to assist in the life hereafter [2]. *N. sativa* was included in the list, perhaps, because of its healing effect in many ailments. Interestingly, the cause of death for the boy king of ancient Egypt, who died at the age of 19 years only, has been suggested to be linked to cerebral malaria and temporal lobe epilepsy [3-5]; and recently some studies have reported the beneficial effects of *N. sativa* in malaria as well as epilepsy in *in vivo* experimental models [6,7]. *N. sativa* seed was a traditional condiment of the Old World and since classical times has been extensively used to flavor food [2]. Moreover, black seed and its oil have been employed for the treatment of a variety of conditions in the ancient systems of medicine: Unani, Ayurveda, Chinese and Arabic Medicines [8]. Ibne-Sina in his famous book 'Al-Qanoon fi el-Tibb' or 'The Canon of Medicine' has mentioned many medicinal uses of *N. sativa* including treatment of fever, common cold, headache, asthma, rheumatic diseases, 'Sartan' (cancer), scorpion and spider stings and bites of snake, cat and dog. Moreover, it is narrated that *N. sativa* seed stimulates body's energy and helps recovery from fatigue and dispiritedness [9].

N. sativa seed and its oil are known to contain many active components to include: thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine and alpha-hederin. Moreover, *N. sativa* is reported to possess numerous pharmacological properties, like immune stimulation, anti-inflammatory, hypoglycemic, antihypertensive, antiasthmatic, antimicrobial, antiparasitic, antioxidant and anticancer, which have been reviewed by Randhawa and Alghamdi, Ali and Blunden, Salem, Padhye et al. and Ahmed et al. [10-14). In acute and chronic toxicity studies conducted on the laboratory animals, *N. sativa* seed, its oil and thymoquinone, its major active component, were found to be quite safe, particularly when given orally [15-17]. In the review articles mentioned above more stress is laid upon the antiiflammatory, anticancer, antioxidant and cytoprotective properties of the *N. sativa* and its active components. The pharmacological actions and the therapeutic potential pertaining to the central nervous system, particularly effects on psychiatric and neurological dysfunctions, are not adequately described. Besides, a lot more studies have been reported since then regarding neuropsychiatric potential of *N. sativa*.

Therefore, the present manuscript is intended to review and update the scientific knowledge about this precious natural herbal remedy relevant to the neurological and psychiatric illnesses. It is hoped that the present work would be of particular interest to the neurologists and psychiatrists and also benefit general physicians, medical students and the community.

Methods

An online search was conducted for the published articles related to studies on the effects of *N. sativa* seed, its oil and active components on various clinical conditions concerning the central nervous system and abstracts and full articles in English were included for the preparation of this review. An effort was made to include all available information in the literature pertaining to neurological and psychiatric effects of *N. sativa*. However, it is not possible to cover everything in this short manuscript, therefore, authors apologize for any shortcomings. For the convenience of readers, various effects have been categorized as follows: Analgesic and antipyretic, anti-Parkinsonism, anticonvulsant, antidepressant, antianxiety and neuroprotective effects, as well as effects on tolerance and dependence, learning and memory and encephalitis.

Analgesic and antipyretic effects

Pain is an important symptom that draws the attention of a patient to get treatment. The sensation of pain has both peripheral and central mechanisms. The peripheral component involves the synthesis of chemical mediators like bradykinin, prostaglandins, thromboxanes and leukotrienes, etc., via activation of cyclooxygenase and lipooxygenase enzymes by the noxious stimuli. These chemical mediators then stimulate the pain receptors in the periphery. The central mechanisms involve the pain transmission via sensory neurons and then its perception in the sensory cortex. Some of the analgesic drugs, like non-steroidal anti-inflammatory drugs (e.g. aspirin), operate through decrease in the synthesis of chemical mediators in the periphery by the inhibition of the enzymes involved in their synthesis. Whereas, others like opioid analgesics (e.g. morphine), operate through the activation of pain modulation pathways, which are closely associated with the pain transmission pathways in the central nervous system. These analgesics act like natural opiopeptins on the spinal and supraspinal κ-, μ- and δ-opioid receptors, present at the level of sensory relay centers, thalamus and sensory cortex in the pain modulation system; thus decrease the pain transmission and perception by the sensory neurons [18,19].

In Saudi Arabia and neighboring countries *N. sativa* oil is used as a topical treatment for pain and stiffness in joints. Fixed oil of *N. sativa* and thymoquinone were demonstrated to block cycloxygenase and 5-lipooxygenase pathways of arachidonate metabolism in the rat peritoneal leukocytes via dose-dependent inhibition of the formation of thromboxane B2 and leukotriene B4, synthesized by the activation of these enzymes, thus confirming its traditional use in arthritic conditions [20]. Later, volatile oil of *N. sativa* and the aqueous suspension of its crushed seeds were shown to inhibit carrageenan induced pain and edema in the hind paw of rats and the analgesic and anti-inflammatory effects were comparable to those of indomethacin and aspirin [21,22]. The results of these studies indicate the participation of the peripheral mechanisms in the control of pain by the *N. sativa.*

Besides the involvement of peripheral mechanisms in the control of pain, some investigators have also reported the possible involvement of the central mechanisms for the antinociceptive effects of *N. sativa*. The oral administration of *N. sativa* oil (50-400 mg/kg) dose-dependently suppressed the nociceptive response in the hot-plate test, tail-pinch test, acetic acid-induced writhing test and in the early phase of the formalin test. The systemic administration (2.5-10 mg/kg oral and 1-6 mg/kg intraperitoneal) and the intracavernous injection (1-4 μg/mouse) of thymoquinone attenuated the nociceptive response in not only the early phase but also the late phase of the formalin test. Naloxone (μ, κ and δ receptor antagonist) injected subcutaneously (1 mg/kg) significantly blocked *N. sativa* oil- and thymoquinone-induced antinociception in the early phase of the formalin test. Moreover, the intracavernous injection of naloxone (10 μg/mouse), the μ1-opioid receptor antagonist, naloxonazine (1-5 μg/mouse), or the κ-opioid receptor antagonist, nor-binaltorphimine (1-5 μg/mouse), significantly reversed thymoquinone-induced antinociception in the early phase of the formalin test, whereas the δ-opioid receptor antagonist, naltrindole (1-5 ng/mouse, intracavernous), had no effect on either phase. The antinociceptive effect of morphine was significantly reduced in thymoquinone and *N. sativa* oil tolerant mice These experiments suggest that the black seed oil and its active component, thymoquinone, produced antinociceptive effects through the central pain modulation pathways (via activation of the supraspinal μ- and κ-opioid receptor subtypes), besides peripheral analgesic and anti-inflammatory effects [23].

Another study also demonstrated potent central nervous system and analgesic activities of the aqueous and methanolic extracts of *N. sativa* seed. The analgesic activity was tested using two different assay procedures, hot-plate test and pressure test. Both extracts induced significant effects on reaction time in the hot-plate and pressure tests. The methanolic extract showed an increase in analgesic activity with respect to time, reaching a maximum effect at 180 min after the intraperitoneal administration. The aqueous extract recorded its maximum effect at 60 min from the time of administration. The same trend was observed with the pressure test. Moreover, the aqueous and methanolic extracts caused a significant decrease of normal body temperature after 30 min (-3.15 ± 0.35 and -2.18 ± 0.41, respectively) [24].

However, in some studies investigators could not demonstrate the involvement of opioid receptors. For example in one study black seed essential oil was found to produce a significant analgesic effect in acetic acid-induced writhing, formalin and light tail flick tests, but the opioid antagonist, naloxone, could not reverse the analgesic effect observed in the formalin test. In the same study oral administration of essential oil at doses of 100, 200 and 400 μL/kg did not exert a significant anti-inflammatory effect in the carrageenan test, however, intraperitoneal injection at the same doses significantly ($p<0.001$) inhibited carrageenan-induced paw edema. The essential oil at doses of 10 and 20 μL/ear also reduced croton oil-induced edema. The authors concluded that mechanism(s) other than opioid receptors are involved in the analgesic effect of essential oil since naloxone could not reverse this effect [25].

Similarly, in another study, oral and intraperitoneal administration of *N. sativa* polyphenols (NSP) significantly suppressed, in a dose-dependent manner, the nociceptive response in the early and late phases of the formalin test, and the effect on the late phase was more pronounced. Pretreatment with naloxone failed to reverse the analgesic activity of NSP in this test. In the acetic acid-induced writhing test, oral

administration of NSP decreased the number of abdominal constrictions. NSP did not produce a significant analgesia in the light tail flick test in mice. Oral administration of NSP did not produce a significant reduction in carrageenan-induced paw edema, however, when injected intraperitoneally, NSP inhibited paw edema in a dose dependent manner. The authors concluded that NSP had analgesic and anti-inflammatory effects and the lack of analgesic effect of NSP in the light tail flick test and also the failure of naloxone to reverse the analgesia in the formalin test indicated that mechanisms other than stimulation of opioid receptors are involved [26].

The controversial results of the above mentioned studies, regarding the involvement of peripheral or central mechanisms for anti-nociceptive effects of *N. sativa*, are perhaps due to different types of extracts, chemical constituents, routes of administration and, particularly, doses used in these studies. Further investigations are required with more specific substances from the *N. sativa* seed and proper study designs to resolve these differences.

Anticonvulsant effects

Epilepsy is known from ancient times and since then *N. sativa* seed and its oil have been used for its treatment. As mentioned above in the introduction also, the selection of black seed amongst the burial goods in the tomb of Tutinkhamun is perhaps related to epilepsy and cerebral malaria as the possible causes of his death at a relatively younger age [3-5]. The black seed was perhaps prescribed for the treatment in his life time and was included in the burial goods to continue its use in the life hereafter (as it was believed). Many studies, mentioned below, have confirmed the use of black seed for the treatment of epilepsy in the folk medicine.

Thymoquinone, the major constituent of *N. sativa* seed, was investigated for the anticonvulsant effects using pentylenetetrazole (PTZ) - and maximal electroshock (MES) - induced seizure models. In PTZ-induced seizures, thymoquinone given intraperitoneally (40 and 80 mg/kg), prolonged the onset and reduced the duration of myoclonic seizures. The protective effect of thymoquinone against mortality was 71.4% and 100% in these doses, respectively. In MES model, thymoquinone failed to reduce the duration of seizure, whereas exhibited a complete protection against mortality [27].

Similarly, pretreatment with *N. sativa* oil was tested for its ability to suppress the convulsive and lethal effects of PTZ in kindled mice (anti-epileptogenic effect) and to attenuate the PTZ-induced oxidative injury in the brain tissue (antioxidant effect). Valproate, a major antiepileptic drug, was also tested for comparison. Both substances significantly decreased oxidative injury in the mouse brain tissue, however, *N. sativa* oil was found to be more effective than valproate in preventing PTZ-induced seizures [28]. Likewise, aqueous extract of *N. sativa* seeds suppressed penicillin- induced epileptic activity in rats, which was possibly due to selectively altering the monoamine level in different regions of brain [7].

Despite the availability and use of numerous antiepileptic drugs, nearly 15% of childhood epilepsy cases are reported to be resistant to treatment. Knowing that the *N. sativa* ameliorated fits in the experimental models of epilepsy, a double-blind crossover placebo controlled clinical trial was conducted in children (13 months to 13 years) with refractory epilepsy. The aqueous extract of black seed (40 mg/kg/8 h), or placebo were administered as an adjunct therapy for four weeks. The frequency of seizures significantly decreased in the children receiving *N. sativa* extract compared to placebo ($p<0.05$). The authors concluded that the water extract of *N. sativa* had antiepileptic effects in children with refractory seizures [29].

Later, in another study, aqueous extract, fixed oil and volatile oil of black seed and its major active constituents (thymoquinone, α-pinene and p-cymene) were investigated against PTZ and maximal electroshock (MES)-induced convulsions. The potential of these constituents to induce minimal neurological deficit (MND) was also evaluated by using chimney test. All of the black seed constituents protected mice effectively against PTZ-induced convulsions except fixed oil. Volatile oil and its component p-cymene effectively suppressed convulsions induced by MES. All of the *N. sativa* seed constituents induced varying degrees of MND in the chimney test. In the same study, exploration on the role of receptors suggested that picrotoxin and bicuculline-sensitive GABA receptors, most probably GABA-A receptors, mediated an increase in GABA-ergic response. Moreover, thymoquinone potentiated the effect of valproate in both PTZ and MES models [30].

Recently, antioxidant and antiepileptic effects of curcumin, *N. sativa* oil and valproate in the pilocarpine-induced animal model of chronic epilepsy were also studied. The animals were treated with curcumin, *N. sativa* oil or valproate for 21 days and there was reversal of the levels of malondialdehyde, nitric oxide and reduced glutathione and restoration of the activities of CAT, Na+, K+-ATPase and acetylcholinesterase in the hippocampus, as well as the reduction of seizures [31].

Antipakinsonism effects

Parkinson's disease is a slowly progressive neurodegenerative disorder caused by the damage to a small group of brain cells in the basal ganglia that control body movements. The available drug treatments for Parkinson's disease effectively control the symptoms but have many adverse effects. Natural herbal products could be better and safer substitutes for the management of Parkinsonism. Recently, some studies were carried out to evaluate the anti Parkinsonian activity of *N. sativa*. For example, the ethanolic extract of *N. sativa* seed (200 and 400 mg/kg orally) was investigated in chlorpromazine induced experimental animal model of catalepsy, besides the assessment of its effects on chemical parameters (TBARS, GSH, Nitrite and Total Protein). The cataleptic score was significantly reduced ($P<0.001$) with *N. sativa*. *N. sativa* also improved the depleted levels of reduced glutathione (GSH) ($P<0.001$) and total protein ($P<0.001$) and decreased the elevated levels of TBARS ($P<0.001$) and Nitrite ($P<0.001$) [32].

It is known that α-synuclein (αSN) induces synaptic toxicity and was reported to decrease the level of synaptophysin, a protein used as an indicator of synaptic density, in cultured hippocampal and hiPSC-derived neurons. Simultaneous treatment with αSN and thymoquinone protected neurons against αSN-induced synapstic damage, as revealed by immunostaining. Moreover, administration of thymoquinone efficiently induced protection in these cells against αSN-induced inhibition of synaptic vesicle recycling in hippocampal and hiPSC-derived neurons as well as against mutated P123H β-synuclein (βSN) neurons, as revealed by experiments using the fluorescent dye FM1-43. It was further demonstrated that thymoquinone reversed aSN-induced reduction in spontaneous firing activity. These results suggested that thymoquinone protected cultured rat primary hippocampal and hiPSC-derived neurons against αSN-induced synaptic toxicity and could be a promising therapeutic agent for patients with Parkinson's disease and dementia with Lewy bodies [33].

Thymoquinone was also shown to improve behavioral and cellular abnormalities and markers of oxidative stress in an experimental model of early Parkinson's disease in rats. In this study unilateral intrastriatal 6-hydroxydopamine (6-OHDA)-lesioned rats were pretreated with oral thymoquinone at doses of 5 and/or 10 mg/Kg three times daily for one week. Thymoquinone pretreatment significantly improved turning behavior, prevented loss of neurons in substantia nigra and lowered level of MDA. These results suggested that TQ could afford neuroprotection in neuro-degenerative disorders including Parkinson's disease [34].

Neuroprotective effects

Besides the protection of neurons related to Parkinsonism, mentioned above, *N. sativa* extracts and its active components were also shown to prevent damage to spinal cord and other brain cells from trauma and various nerve toxins.

Possible beneficial effects of *N. sativa* in comparison to methylprednisolone were investigated on experimental spinal cord injury (SCI) in rats. SCI was performed by placing an aneurysm clip extradurally at the level of T11-12. SCI significantly increased the spinal cord tissue malondialdehyde (MDA) and protein carbonyl (PC) levels, however, SCI decreased superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) enzyme activities compared to the control. Methylprednisolone and *N. sativa* treatment decreased tissue MDA and PC levels and prevented inhibition of SOD, GSH-Px and CAT enzymes in the tissues [35].

Toluene is a common industrial solvent and long term exposure can cause damage to brain cells. The neuroprotective effect of *N. sativa* and thymoquinone were investigated in hippocampus after chronic toluene exposure in rats. The rats were randomly allotted into one of four experimental groups: Control, toluene treated, toluene with *N. sativa* extract and toluene with thymoquinone. Chronic toluene exposure caused severe degenerative changes, shrunken cytoplasm, slightly dilated cisternae of endoplasmic reticulum, markedly swollen mitochondria with degenerated cristae and nuclear membrane breakdown with chromatin disorganization in neurons of the hippocampus. The distorted nerve cells were mainly absent in thymoquinone and *N. sativa* extract treated rats [36].

Propoxur is a broad spectrum carbamate, widely used as a household insecticide and can cause neurotoxicity. The black seed oil significantly reversed the abnormalities induced by propoxur in the lipid peroxidation, protein carbonyl content, acetylcholine esterase activity and demonstrated antioxidant activities in different parts of rat brain [37].

Cerebral ischemia or stroke is a common cause of mortality and requires development of new drugs for its prevention and treatment. Recently, aqueous, hydroalcoholic, chloroform and petroleum ether extracts of *N. sativa* seeds (400 mg/kg, orally for 7 days) were evaluated for their neuroprotective role in cerebral ischemia induced by middle cerebral artery (MCA) occlusion in rats. Pretreatment with various extracts of *N. sativa* improved locomotor activity and grip strength of animals as compared to controls. Changes in the level of thiobarbituric acid reactive substance (TBARS), glutathione (GSH), superoxide dismutase (SOD) and catalase levels produced by MCA occlusion were also reversed. Authors concluded that the neuroprotective effects of *N. sativa* were due to its antioxidant, free radical scavenging and anti-inflammatory properties [38, 39].

Moreover, metformin and thymoquinone were reported to have strong protective effect against ethanol-induced neuronal apoptosis in primary rat cortical neurons, as they inhibited the apoptotic cascade by increasing Bcl-2 expression, repressed the activation of caspase-9 and caspase-3, reduced the cleavage of PARP-1 and prevented morphological changes [40].

Antidepressant effects

In the folk medicine *N. sativa* seeds are reported to be used for the feeling of wellbeing, stimulate energy and recovery from fatigue and dispiritedness [9]. Recently, hydro-alcoholic extract of *N. sativa* and thymoquinone were investigated for their effect on lipopolysaccharide-induced depression like behavior in rats using forced swimming test and open field test. Forced swimming test was performed 3 times, on alternate days, for all groups: Passive controls (only saline), active controls (lipopolysaccharide 100 µg/kg, intraperitoneally 2 hours before test) and treated groups (receiving different doses of *N. sativa* extract or thymoquinone before lipopolysaccharide), and the immobility time was recorded each time. The immobility time in the active control (lipopolysaccharide group) was higher than that of control group (saline alone) in all 3 times, whereas, the groups pretreated with *N. sativa* extract or thymoquinone lowered immobility times as compared to active controls. In the open- field test, the peripheral crossing number was higher in active controls than passive controls; while in the animals pretreated with *N. sativa* extract or thymoquinone it was lower than active controls. Furthermore, the central crossing number was lower in the lipopolysaccharide group than that of saline controls, whereas, in animals pretreated with *N. sativa* extract or thymoquinone, the central crossing number was higher than that of active controls. The results indicated that hydro-alcoholic extract of *N. sativa* and its active principle, thymoquinone, could prevent lipopolysaccharide-induced depression like behavior in rats [41].

Considering the beneficial effects of *N. sativa* in the prevention of depression and relief of anxiety in animal studies a placebo controlled randomized clinical trial was carried out at a boarding school on young healthy adolescent human volunteers to investigate the effect of *N. sativa* seed on mood and anxiety. The assessment of mood was done with Bond-Lader scale and anxiety with State-Trait Anxiety Inventory, at the beginning and after four weeks of ingestion of 500mg once daily of either placebo (Group A) or *N. sativa* seed (Group B). Although the results for the evaluation of mood and anxiety were statistically not significantly different between group A and B, but there was statistically significant variation within group B and no variation within group A in both parameters. The authors suggested that the use of *N. sativa* seed as a nutritional supplement had positive effect on mood and anxiety [42].

Antianxiety effects

Hole-Board test is frequently used for the validation of the anxiety in small animals. The number of holes explored in a specified time is counted after the administration of the test drug. The increased exploration capacity is considered to be an index of anxiety, but it is difficult to separate it from motor activity. A significant decrease in exploratory conduct in the mice was produced by the aqueous and methanolic extracts of *N. sativa*, using hole-board test. The effect of these extracts on spontaneous motor activity and motor coordination was also observed. The spontaneous motility was recorded in a photoactometer, whereas the motor coordination was examined by

Rota-Rod test. Both of these extracts caused a significant decrease in the spontaneous motility as well as motor coordination [24].

Benzodiazepines are commonly used for the management of anxiety and they also produce muscle relaxation and motor incoordination in higher doses [43]. If proper dose-response curves are constructed for the antianxiety effect and for the motor in coordination with the *N. sativa* extract, perhaps, we can differentiate between the therapeutic doses relieving anxiety and the toxic doses causing motor incoordination.

In another study, antianxiety effect of *N. sativa* oil was studied in rats, using open field and elevated plus maze models. After the oral administration of *N. sativa* oil for 4 weeks, the rats exhibited an increase in open field activity. The oil also produced anti-anxiety effect in rats when tested in elevated plus maze. There was an increase in the brain levels of 5-HT (5-hydroxy-trytamine, or Serotonin), but the levels of brain 5-HIAA (hydroxyindole acetic acid), a metabolite of 5-HT, decreased significantly. Brain and plasma levels of tryptophan also increased significantly. Therefore, it was suggested that *N. sativa* oil is a useful choice for the treatment of anxiety [44]. Although by different mechanism, perhaps by decreasing the metabolism of 5-HT, these changes in 5-HT levels produced by *N. sativa* oil are similar to changes produced by Serotonin Specific Reuptake Inhibitors (SSRIs), used in the treatment of anxiety and depression.

Moreover, the toxicity of methanolic extract of *N. sativa* on cultured rat cortical neurons and its influence on the release of excitatory (glutamate and aspartate) and inhibitory (gamma-aminobutyric acid and glycine) neurotransmitters in these neurons was also investigated. The cultured neurons were exposed to different times and concentrations of dried methanolic extract and cell viability was then determined by a quantitative colorimetric method. The extract did not induce any toxic effects on these neurons. However, the extract modulated amino acid release in the cultured neurons: GABA was significantly increased whereas secretion of glutamate, aspartate, and glycine were decreased. These findings suggest that the sedative and antianxiety effects of methanolic extract of *N. sativa* are related to changes in the inhibitory and excitatory amino acids levels [45].

Furthermore, the antianxiety effect of thymoquinone and its effect on the modulation of GABA and NO levels in brain were investigated in both the unstressed and stressed mice (mice subjected to 6 h immobilization). Thymoquinone (10 and 20 mg/kg), methylene blue (1 mg/kg) and diazepam (2 mg/kg) were administered intraperitoneally, followed by behavioral testing using an elevated plus maze, the light/dark test and the social interaction test in both unstressed and stressed mice. The effects of the above-mentioned drugs on plasma nitrite, a stable metabolite of nitric oxide and brain GABA content were also studied. Thymoquinone (10 and 20 mg/kg) produced significant antianxiety effects in unstressed mice without altering nitrite levels, but only the higher dose (20 mg/kg) of thymoquinone increased the GABA content in unstressed mice. In stressed mice, TQ (20 mg/kg) showed anxiolytic effects, with a significant decrease in plasma nitrite and reversal of the decreased brain GABA content. Pre-treatment with methylene blue enhanced the antianxiety effect of thymoquinone in both unstressed and stressed mice. It was concluded that NO-cGMP and GABA-ergic pathways are involved in the anxiolytic-like activity of thymoquinone [46]. Benzodiazepines are well known to relieve anxiety by GABA-mimetic effects [43].

Effects on learning and memory

A long term administration of *N. sativa* increased 5-HT levels in brain and improved learning memory in rats [47]. Considering the experimental evidences for the beneficial effects of *N. sativa* seed on improvement of memory and learning, a randomized placebo controlled study was designed to investigate the effects of *N. sativa* seed on memory, attention and cognition in healthy elderly volunteers. The study group A received 500 mg *N. sativa* seed capsule twice daily for nine weeks and group B received placebo in the similar manner. There was significant difference ($p<0.05$) in the score of logical memory test-I and II, total score of digit span, 30 min delayed-recall, percent score in Rey-Osterrieth complex figure test, time taken to complete letter cancellation test, time taken in trail making test-A and test-B, score in part C of stroop test between two groups. The biochemical markers of cardiac, liver and kidney functions remained normal in both groups throughout the study period. It was concluded that *N. sativa* enhances memory, attention and cognition and thus could be considered as potential food supplement for the prevention of Alzheimer disease in elderly. However, for the treatment of Alzheimer disease and cognitive disorders further long term studies are recommended [48].

In another study, the effect of *N. sativa* on cognition in adolescent human males was examined at a boarding school in Bangladesh, besides the effect on mood and anxiety mentioned above. Two groups of recruited volunteers, A and B, received capsule of 500 mg placebo and 500 mg *N. sativa* seed, respectively, once daily for four weeks. All the volunteers were assessed for cognition with modified California verbal learning test-II (CVLT-II) at the beginning and after four weeks of either *N. sativa* or placebo ingestion. In the beginning of the study all parameters showed statistically similar results between group A and B. After 4 weeks of treatment CVLT II revealed that there was significant variation within group B in immediate short-term recall at trial 4 and 5 whereas this difference was found only in case of trial 5 between group A and B. However, long-term free recall and long-term cued recall had statistical differences between group A and B. The results indicated positive effect on the mood and anxiety [49].

Recently, a study was conducted to evaluate the effect of hydro-alcoholic extract of *N. sativa* on memory performance and its possible mechanisms in scopolamine-induced spatial memory impairment model using Morris water maze test. The time latency and path length in the Scopolamine treated group were significantly higher than in the passive controls receiving saline ($P<0.01$), while the *N. sativa* treated group showed a significantly shorter travel path and time latency compared to the active control receiving Scopolamine ($P<0.01$). Acetycholinestrase activity in the cortical tissues of the Scopolamine group was significantly higher than the passive control group ($P<0.01$), while Acetycholinestrase activity in the *N. sativa* treated groups was lower than the Scopolamine group ($P<0.01$). Moreover, pretreatment of the animals with *N. sativa* decreased the malondialdehyde concentration in hippocampal tissues and increased the thiol content compared to control group ($p<0.001$). The results revealed that the hydro-alcoholic extract of *N. sativa* prevented scopolamine-induced spatial memory deficits and decreased the Acetycholinestrase activity and oxidative stress induced by scopolamine in rats [50]. A similar study also demonstrated positive effects on learning and memory of rats after feeding of hydro-alcoholic extract of *N. sativa* during neonatal and juvenile growth [51].

Effects on tolerance and dependence

Drug dependence is one of the major social and psychiatric problems of society. The drug abusers usually report to seek the treatment of withdrawal effects of drugs of dependence. The withdrawal effects are mostly controlled with the replacement therapy of a similar drug, which is usually longer acting. For example, withdrawal effects due to opioid drugs (Morphine and heroin) are controlled with a longer acting opioid, methadone.

In a clinical study *N. sativa* was shown to control the withdrawal effects of opioid dependence and its long term use prevented its abuse as well. No changes were observed in the physiological parameters (Blood pressure, pulse rate and respiration, etc.). The appetite increased but there was no significant weight gain. Besides the control of opioid dependence, *N. sativa* also treated the infections and weakness, from which the majority of addicts suffered [52].

An animal study was conducted to elucidate the possible mechanism of action of protective effect of *N. sativa* oil against tramadol-induced tolerance and dependence in mice by the hot plate test. Naloxone, a potent opioid antagonist (5 mg/kg, intraperitoneally), was shown to precipitate the withdrawal manifestations. Black seed oil also inhibited nitric oxide overproduction and increase in brain malondialdehyde level induced by repeated administration of tramadol to mice or by administration of naloxone to tramadol-dependent mice. The inhibitory effect of *N. sativa* oil on tramadol-induced tolerance and dependence was enhanced by concurrent intraperitonial administration of the NMDA receptor antagonist, dizocilpine (0.25mg/kg). Also, the inhibitory effect of the oil on naloxone-induced biochemical alterations in tramadol-dependent mice was enhanced by concurrent administration of dizocilpine. Similarly, concurrent administration of the NO synthase inhibitor, L-N (G)-nitroarginine methyl ester (10mg/kg) or the antioxidant, N-acetylcysteine (50mg/kg) enhanced these inhibitory effects of *N. sativa* oil. On the other hand, these effects were antagonized by concurrent administration of NO precursor, L-arginine (300 mg/kg). These results provide evidence that *N. sativa* oil appears to have a therapeutic potential in tramadol tolerance and dependence through blockade of NO overproduction and oxidative stress induced by the drug [53].

Effect on encephalitis

Toxoplasmosis: *Toxoplasma gondii* is an intracellular protozoan parasite, with a worldwide distribution and causes opportunistic infection in humans and animals. Toxoplasmosis is a risk for newborns and for immunocompromized individuals, such as HIV carriers and patients receiving anticancer or immunesuppressant drugs. Infection of an immunocompetent human host leaves the subject with a life-long latent infection in the form of quiescent tissue cysts present mainly in the brain and muscles [54]. Combination of pyrimethamine and sulphadiazine is commonly used for treatment and prophylaxis of most clinical presentations of toxoplasmosis. However, this combination is not always suitable for prolonged treatment because of the appearance of adverse side effects.

Black seed oil (BSO) has been reported to show promising prophylactic and therapeutic effects against *T. gondii* in a murine model of infection. The mice were orally inoculated with *T. gondii* (Me49 strain) to cause brain cysts and divided into 3 groups: prophylactic group (given BSO for two weeks before *T. gondii* infection), treated group (given BSO on day 4 post infection) and control group (infected untreated). The effect of BSO was evaluated by the assessment of survival rate and brain cyst burden, brain histopathological lesions and immunohistochemical expression of inducible nitric oxide synthase (iNOS). In prophylactic or therapeutic groups BSO significantly enhanced protection of infected mice against death ($P=0.01$) and reduced brain cyst burdens at 5, 7 and 12 weeks post infection ($P<0.05$) compared to the infected untreated control. The brains of BSO prophylactic or therapeutic groups showed milder meningitis, encephalitis and perivascular cuffing compared to the infected untreated control ($P<0.05$). Moreover, expression of iNOS was significantly enhanced in both BSO prophylactic and therapeutic groups compared to the untreated infected control [55].

Malaria: Malaria is a significant public health problem in many countries of the world and causes substantial morbidity and mortality. *Plasmodium falciparum* is notorious to cause organ damage including brain and causes cerebral symptoms. The oxidative stress is an important factor in the pathophysiology of malaria, is mainly due to the generation of oxygen-nitrogen reactive species and causes further damage. The use of antioxidant supplements of synthetic or natural origin would constitute an effective adjuvant antimalarial therapy to prevent damage to the host and control cerebral symptoms [56]. *N. sativa* has been demonstrated to possess both the antimalarial and antioxidant properties and is a suitable potential remedy for cerebral malaria. *N. sativa* seeds extract when examined *in vitro* for antimalarial activity against schizonts maturation of *Plasmodium falciparum*, showed 100% inhibition of the parasite growth at a concentration of 50 mug/ml [57]. Methanolic extract of *N. sativa* seed was reported to possess both antimalarial and antioxidant activities against *Plasmodium yoelli nigeriensis* (*P. yoelli*) infection in mice. The extract, at a dose of 1.25 g/kg body weight not only suppressed *P. yoelli* infection in the mice, but also reversed the increase in levels of red cell and hepatic malondialdehyde (MDA) and the decrease in activities of superoxide dismutase, catalase, glutathione-S-transferase and the level of reduced glutathione in tissues of the mice [58]. In another study also the water extract of *N. sativa* seed was shown to have both anti-malarial and antioxidant effects and significantly reduced parasitaemia count and level of nitric oxide and increased survival rates of *Plasmodium berghei* NK65 infected mice [59]. Earlier, ethanol, chloroform and aqueous seed extracts of *N. sativa* were also demonstrated to possess anti-malarial properties against *Plasmodium berghei* in mice [6]. In a recent study, *N. sativa* seed or oil in feed in combination with chloroquine was shown to produce complete parasitaemia clearance and 100% survival rate in mice infected with chloroquine sensitive *Plasmodium berghei*, NK65 strain. Parasitaemia clearance and survival rate produced by these combinations were greater than *N. sativa* seed in feed or *N. sativa* oil extract in feed or chloroquine alone [60].

Conclusions

N. sativa is known to possess a wide variety of medicinal properties and has been used as a natural remedy for many diseases since ancient times. In the present article neuropsychiatric effects are reviewed separately for the first time. In many animal experiments and few clinical trials it was found to be effective in the control of pain, fever, epilepsy, Parkinsonism, anxiety, depression, toxoplasmosis, malaria and to improve memory, mood and feeling of good health. Further basic and clinical investigations are needed to confirm these observations. Isolation of the active principles and preparation of more remedies from their derivatives are, perhaps, the future targets for the development of new drugs for neurological and psychiatric diseases.

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