http://pmjournal.ir Review Article
Spring 2019, Volume 4, Issue 13 (14-18)

Evaluation of Personalized Medicine for Patients with Brain and Spinal Cord Injury using the *Nigella sativa L.* Seed: An Updated Comprehensive and Critical Review

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DOI: 10.21859/pmj04015

Submitted: 2019/01/04
Accepted: 2019/02/05

Keywords:
Nigella Sativa
Thymoquinone
Neuroprotective
Treatment
Clinical

Abstract

Introduction: *Nigella sativa* (NS) is a widely used medicinal plant and appears to have a general health protective effect. As has been reported previously, pharmacological actions of NS have been explored including antidiabetic, anticancer, immunomodilative, analgesic, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, antioxidant properties, gastroprotective, antihistaminic, antibacterial, neuroprotective and antioxidative effects and etc.

Methods: The present review aimed to give a personalized care for patients using detailed survey of the literature on and neuroprotective activities of the plant. Pubmed, Science Direct, Google scholar and Springer databases were searched from 1983 till January 2015. Key words were included: *N. sativa*, black seed, neuropathy, neuroprotective, brain and spinal injury, thymoquinone and posttrauma. Searching was limited to articles with English language. Review articles, case reports, abstract in symposium and congress, studies on *N. sativa* mixed with other plants were excluded. This study thus launches a huge resource for understanding the role of NS in in brain and spinal cord tissue damage after trauma with broad relevance for personalized medicine.

Results: Results indicated that based on personalized medicine many of the herbal attributes of the herbal are due to the thymoquinone presence as its bioactive component, and therapy with NS notably decreased post-traumatic degenerative neurons and distorted nerve cells were not primarily treated in NS rats. In conclusion, NS can improve neuropathic status and neurological dysfunctions in the brain and spinal injury models. However, more clinical trials are necessary to clarify beneficial effects of NS its effective type and dosage for neuropathies management and its complications.

Conclusions: Finally, NS treatment might be effective in detrimental the cerebral and spinal cord after trauma as personalized care for patients, and therefore shows potential for clinical implications.

INTRODUCTION

Personalized medicine generally refers to a medical method that suggests the customization of healthcare, therefore,personalized medicine approach can establish with the primary step of the empiric culture of brain lesions followed by medication sensitivity testing using relevant techniques/models [1, 2]. In parallel, Plants have used for diseases in many centuries as herbal medicines. Many researchers are focusing on medicinal plants for their medicinal properties and mechanism of action. *Nigella sativa* (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background that has been used for medicinal proposes also it was pressed into oil, in Asia, Middle East and Africa.

N. sativa seed contain more than 30% fixed oil and 0.4–0.45% volatile oil. The fixed oil is composed of unsaturated fatty acids [3]. Thymoquinone is known as the major chemical component of the *N. sativa* oil. [4]. *N. sativa* has been studied for its therapeutic potential and it was described to have many therapeutic effects such as bronchodilatation, immunomodilative [5] antibacterial [6], and hypotensive [7].

Antidiabetic [8, 9], hepatoprotective [8, 9], gastroprotective [10], antihistaminic, antioxidative [10, 11] and neuroprotective effects [5, 10]. As matter of fact, the thymoquinone (TQ) of this plant was known to have therapeutic properties.

Several studies of neuroprotective effects have evaluated the protection of drug factors against lipid antioxidant activity in NS. Pharmacological researches have been expressed that water and methanol extracts of NS seeds have a strong CNS and pain relief activity. Moreover, it has depressant action in the case of the methanolic extract. Moreover, it was shown that NS seeds have narcotic analgesic activity mediated possibly through opioid receptors [12].

The oil from the seeds exhibited central nervous system (CNS) depressant and potentialanalgesic effects. The previous studies indicated increased secretion of neurotransmitters on cultured cortical neurons. Moreover, it can modulate amino acid release in cultured neurons. And also neuroprotective effects of NS were previously reported by different studies. Therefore, the present review aimed was aimed to collect any information about the neuroprotective effects of NS.

METHODS

For collection of any information about *N. sativa* a web based search was done from the literature on PubMed, Scopus, Science Direct, Web of Knowledge, and Google Scholar, and most of the national search engines such as scientific information database, we included all types of study about the neuroprotective effects of NS.

Neuro-pharmacolgical Activities

Anxiolytic Effects

As has been reported previously, an oral administration of NS oil can increase brain levels of 5-HT but the levels of brain 5-HIAA (hydroxyindole acetic acid) decreased. Current evidence suggests the central 5HT involvement in anxiety linked behavior and in the anxiolytic mechanism of action [13]. It can be proposed that NS oil is a effective option for treating anxiety. It has been found that a long term administration of *N. sativa* increases 5-HT levels in rat brain [14]. Previous studies had shown that the water and methanol extracts of *N. sativa* L. seeds possess a potent CNS. Furthermore, the level of tryptophan in the brain and plasma also after repeated injection of *N. sativa* oil (NSO) increased [15]. Ilhan et al. (2005) evaluated the anticonvulsant and antioxidant activities of NSO on pentylenetetrazol (PTZ) kindling seizures in mice [16]. They reported that oxidative injury decreased by both substances in the mouse brain tissue in comparison with the PTZ-kindling group. NS oil was most effective in prevention of PTZ-induced seizures relative to valproate. The obtained data suggested this hypothesis that NSO has neuroprotective action that may relate with its ability to inhibit not only excessive reactive oxygen species (ROS) formation but also seizure generation [17].

The Protective Effects of NS on the Neuronal Injury

The pharmacological effects of *N. sativa* have been investigated in the last decades. The concept of secondary injury has been investigated and important progress achieved to date. Javanbakht et al., 2013 reported in a study on the safety effects of NS on nerve damage in the rats sciatic nerve, this reseach found that NS treatment significantly decraeded the post-traumatic degenerative neurons and mutilated nerve cells typically in rats with untreat of NS. Moreover, they observed that the neurons number in the untreated rats was lower than the control group and was associated with neuronal nerve cells of the NS rat spinal cord [3]. Kanter et al. (2006) reported such findings as minimal degenerative changes in the cytoplasma and especially the nucleus of spinal cord tissues in the NS-treated rats [11]. Kanter (2008) reported that NS remedy can be beneficial in ameliorate the morphology in the brain stem neurodegeneration and frontal cortex tissues after chronic toluene exposure and formaldehyde in rats also NS can be effective in preventing of neuronal damage caused by formaldehyde in the frontal cortex tissues of rats [5]. In a other study, NS protective effects on cell death in rats hippocampal nerve after global ischemic / reperfusion complications in rats was investigated. NS extraction could prevent the intracellular cerebral edema of interneurons in 50 mg/kg group significantly compared with sham group [18]. Other study indicated that chloroform and ether petrochemical extracts of NS-

preventing rats are effective in improving motor activity and in gaining strength and reducing infarction rates compared to cerebral needle-induced rats. [19]. Previous data have been proposed that TQ plays a protective role against ethanol-induced neuronal apoptosis in rat neurons [20]. Mousavi et al., (2010) indicates that *N. sativa* and TQ pretreatment ameliorates SGD-induced cell toxicity in cultured PC12 cells, against serum/glucose deprivationinduced cytotoxicity via attenuation of oxidative stress they believes that neuroprotective effects of NS and TQ may suggest the potential application of *N. sativa* and TQ in clinical to prevent and treat the neurological insults [21].

Protective effects of Thymoquinone Produced (TQ)

Secondary damage due to degradability phenomena such as lipid enzyme hydrolysis, peroxidation damage of free radical lipids induced [22] by hydroxyl radicals [23], and inflammation with neuronophagia by polymorphonuclear leukocytes [24]. Neurons are commonly not mighty of mitotic mitochondrial damage, and in any way, such as free radicals, is damaging, particularly destructive and can motive constant lesions [11, 25]..Therefore, different conducted studies had evaluated the neuroprotective efficacy of pharmacological material with lipid antioxidant activity in SN *Nigella sativa* phytochemically antioxidant properties can suppress reactive oxygen and nitrogen species formation [26-29]; Therefore, this can play important role in protecting the antioxidant defense system [8, 11]. It is worth noting that TQ from *N. sativa* seeds has antioxidant effects (Fig. 1) [30].

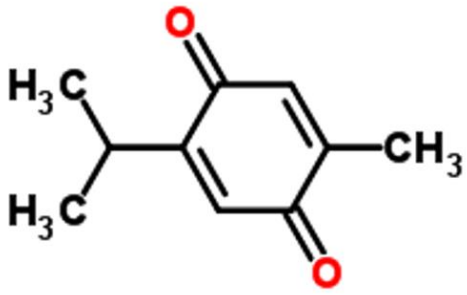

Figure 1: Chemical Structures of Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone)

TQ protects organs against oxidative damage induced by different free radical including doxorubicin induced cardiotoxicity [31], carbon tetrachloride evoked hepatotoxicity [32], nephropathy produced by cisplatin [29], autoimmune, allergic encephalomyelitis [33, 34] and gastric mucosal injury induced by ischemia reperfusion [35]. Badary et al., (2003) has been reported that TQ and synthetic structurally-related tert-butylhydroquinone (TBHQ) have through scavenging ability of different free radicals [29]. In a research authenticated that TQ is as a strong superoxide radical that reduces the power of superoxide dismutase versus superoxidase. [31].

In a reporting by Gilhotra and Dhingra, 2011 the role of the GAB Aergic and Nitriergic molecules in the antimustion of TQ in mice under stressless and severe conditions is investigated. TQ was seen to generate anti-anxiety effects in stress-free mice without changes in nitrite levels (10 and 20 mg / kg), but it was shown that a premier dose of TQ (20 mg / kg) growths GABA amount in stressless mice. Furthermore, TQ (20 mg / kg) revealed anxiolytic effects with a notable reduction in plasma nitrite and a decrease in the rate of GABA reduction in the brain in stressed mice. This result indicates involvement of NO-cGMP and GABAergic pathways in the anxiolytic-like activity of TQ. Krishek et al. (1996) suggest that NS prevented seizures from picotroxin and protects against picrotoxin against bicuculline (a competitive GABAA antagonist) by injecting 150 mg / kg more than 300 mg / kg sodium valproate. Moreover, TQ as major constituent of NS has sowed to lower the ED_{50} for sodium valproate, indicating that the $GABA_A$ receptor can be enhanced by TQ [36]. TQ enhance $GABA_A$ signaling, it may be somewhat indirectly opioidergic signaling, nitrinergic signaling, nitric oxide pathways in the brain, or both of them [37, 38].

Sedaghat et al (2014) has been evaluated TQ effects on behavioral and cellular irregularity and oxidative stress markers in an exploratory model of primary Parkinson's Disease (PD) in rats. They observed that before remedy of TQ notably improved rotational behavior, inhibiting the loss of SNC neurons and reducing the level of MDA. TQ can protect the nerve against the neurological 6-OHDA, somewhat due to a decrease in lipid peroxidation, and it is noteworthy that this may have advantages in neurogenic distubances such as PD [39]. Furthermore, prior to TQ therapy, the main components of the main elements of the nerve resulting from NS-decompensated ischemia, clearly reducing the number of hippocampal neurons in rats, show that the protective effect of TQ on ischemic-reperfusion [40]. NS oil and TQ have been demonstrated to prevent oxidative injury during cerebral ischemia-reperfusion injury in rat [26]. Abdulhakeem et al., 2006 reported that oral administration of TQ protected rats from ischemia-induced brain injury. They believed that this protection may be due to the reduction of oxidative stress.

Studies findings have been suggested that TQ plays a protective role against ethanol-induced neuronal apoptosis in rat brain neurons [20]. Mousavi et al., (2010) indicates that *N. sativa* and TQ pretreatment ameliorates SGD-induced cell toxicity in cultured PC12 cells, against serum/glucose deprivation induced cytotoxicity via attenuation of oxidative stress [21] they believed that neuroprotective effects of NS and TQ may indicate the potential application of *N. sativa* and TQ in

prevention and treatment of neurological insults. TQ offered protection and curative effect against chronic relapsing experimental autoimmune encephalomyelitis based on an animal model for multiple sclerosis [5]. Nigella sativa and TQ were neuroprotective in chronic toluene blue exposure, an experimental spinal cord injury model, a forebrain ischemia model [40], as well as in an experimental allergic encephalomyelitis model [41], Neuroprotective effects of TQ against rotenone toxicities was shown based on in vitro study [27].

Protective Effect of *Nigella sativa* oil against Tramadolinduced Tolerance

Regarding to this topic, a research by Abdel-Zaher et al., (2011) reported the NS inhibitor effects were incearsed on the development of tramadol tolerance and the dependence of mice on coexistence with NAC. Furthermore, this study increased the effect of oil suppressant on naloxone-induced biochemical changes in tramadol-dependent mice using NAC simultaneously, indicating the role of oxidative stress and NO in over development in the improving of tolerance and dependence on tramadol [42]. The oil may cause these effects through inhibition. Regarding to this obtained result provide evidence that , *N sativa* oil appears to be an alternative to treat tramadol tolerance through blockade of NO overproduction and oxidative stress induced by the drug [43].

Ameliorating Effects of *Nigella sativa* in middLe Cerebral Artery in Rat

Neuropathic effects of watery and hydroalcoholic extracts of NS on cerebrovascular (MCAO) rats. This study was shown that that motor activity and animal gain can be improved in aquatic and hydroalcoholic extracts. The neuroprotective effects could be due to its antioxidant, free radical scavenging, and anti-inflammatory properties. Therefore, further studies are needed to confirm the anti-ischemic effect of *Nigella sativa* by using both extracts as pretreatment and post therapy via several drug administration. It is worth noting that the protective neuroprotective effects shown by both NS extract against ischemia in rat are more or less than those produced by aspirin with antioxidant properties and free radical properties [19].

In conclusion, *N. sativa* can improve neuropathic status and neurological dysfunctions in the brain and spinal injury models. However, more clinical trials are necessary to clarify beneficial effects of *N. sativa*, its effective type and dosage for neuropathies management and its complications.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for profit sectors.

Conflict of Interest

None of the authors have any conflict of interest.

REFERENCES

1. Redekop WK, Mladsi D. The faces of personalized medicine: a framework for understanding its meaning and scope. Value Health. 2013;16(6 Suppl):S4-9. doi: 10.1016/j.jval.2013.06.005 pmid: 24034312
2. Garrino L, Curto N, Decorte R, Felisi N, Matta E, Gregorino S, et al. Towards personalized care for persons with spinal cord injury: a study on patients' perceptions. J Spinal Cord Med. 2011;34(1):67-75. doi: 10.1179/107902610x12883422813741 pmid: 21528629
3. Javanbakht J, Hobbenaghi R, Hosseini E, Bahrami AM, Khadivar F, Fathi S, et al. Histopathological investigation of neuroprotective effects of Nigella sativa on motor neurons anterior horn spinal cord after sciatic nerve crush in rats. Pathol Biol (Paris). 2013;61(6):250-3. doi: 10.1016/j.patbio.2013.03.007 pmid: 23751360
4. Worthen DR, Ghosheh OA, Crooks PA. The in vitro anti-tumor activity of some crude and purified components of blackseed, Nigella sativa L. Anticancer Res. 1998;18(3A):1527-32. pmid: 9673365
5. Kanter M. Nigella sativa and derived thymoquinone prevents hippocampal neurodegeneration after chronic toluene exposure in rats. Neurochem Res. 2008;33(3):579-88. doi: 10.1007/s11064-007-9481-z pmid: 17929168
6. Hanafy MS, Hatem ME. Studies on the antimicrobial activity of Nigella sativa seed (black cumin). J Ethnopharmacol. 1991;34(2-3):275-8. doi: 10.1016/0378-8741(91)90047-h pmid: 1795532
7. Zaoui A, Cherrah Y, Lacaille-Dubois MA, Settaf A, Amarouch H, Hassar M. [Diuretic and hypotensive effects of Nigella sativa in the spontaneously hypertensive rat]. Therapie. 2000;55(3):379-82. pmid: 10967716
8. Kanter M, Meral I, Yener Z, Ozbek H, Demir H. Partial regeneration/proliferation of the beta-cells in the islets of Langerhans by Nigella sativa L. in streptozotocin-induced diabetic rats. Tohoku J Exp Med. 2003;201(4):213-9. doi: 10.1620/tjem.201.213 pmid: 14690013
9. Kanter M, Coskun O, Korkmaz A, Oter S. Effects of Nigella sativa on oxidative stress and beta-cell damage in streptozotocin-induced diabetic rats. Anat Rec A Discov Mol Cell Evol Biol. 2004;279(1):685-91. doi: 10.1002/ar.a.20056 pmid: 15224410
10. Kanter M, Demir H, Karakaya C, Ozbek H. Gastroprotective activity of Nigella sativa L oil and its constituent, thymoquinone against acute alcohol-induced gastric mucosal injury in rats. World J Gastroenterol. 2005;11(42):6662-6. pmid: 16425361
11. Kanter M, Coskun O, Kalayci M, Buyukbas S, Cagavi F. Neuroprotective effects of Nigella sativa on experimental spinal cord injury in rats. Hum Exp Toxicol. 2006;25(3):127-33. doi: 10.1191/0960327106ht608oa pmid: 16634331
12. Krishek BJ, Moss SJ, Smart TG. A functional comparison of the antagonists bicuculline and picrotoxin at recombinant GABAA receptors. Neuropharmacology. 1996;35(9-10):1289-98. pmid: 9014144
13. Handley SL, McBlane JW. 5HT drugs in animal models of anxiety. Psychopharmacology (Berl). 1993;112(1):13-20. pmid: 7871002
14. Perveen T, Abdullah A, Haider S, Sonia B, Munawar A, Haleem DJ. Long-term administration of Nigella sativa effects nociceotion and improves learning and memory in rats. Pak J Biochem Mol Biol. 2008;41(3):141-3.
15. Perveen T, Haider S, Kanwal S, Haleem DJ. Repeated administration of Nigella sativa decreases 5-HT turnover and produces anxiolytic effects in rats. Pak J Pharm Sci. 2009;22(2):139-44. pmid: 19339222
16. Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M. Antiepileptogenic and antioxidant effects of Nigella sativa oil against pentylenetetrazol-induced kindling in mice. Neuropharmacology. 2005;49(4):456-64. doi: 10.1016/j.neuropharm.2005.04.004 pmid: 15913671

17. Ilhan A, Aladag MA, Kocer A, Boluk A, Gurel A, Armutcu F. Erdosteine ameliorates PTZ-induced oxidative stress in mice seizure model. Brain Res Bull. 2005;65(6):495-9. doi: 10.1016/j.brainresbull.2005.02.027 pmid: 15862921
18. Hobbenaghi R, Javanbakht J, Sadeghzadeh S, Kheradmand D, Abdi FS, Jaberi MH, et al. Neuroprotective effects of Nigella sativa extract on cell death in hippocampal neurons following experimental global cerebral ischemia-reperfusion injury in rats. J Neurol Sci. 2014;337(1-2):74-9. doi: 10.1016/j.jns.2013.11.019 pmid: 24314720
19. Akhtar M, Maikiyo AM, Khanam R, Mujeeb M, Aqil M, Najmi AK. Ameliorating effects of two extracts of Nigella sativa in middle cerebral artery occluded rat. J Pharm Bioallied Sci. 2012;4(1):70-5. doi: 10.4103/0975-7406.92740 pmid: 22368403
20. Ullah I, Ullah N, Naseer MI, Lee HY, Kim MO. Neuroprotection with metformin and thymoquinone against ethanol-induced apoptotic neurodegeneration in prenatal rat cortical neurons. BMC Neurosci. 2012;13:11. doi: 10.1186/1471-2202-13-11 pmid: 22260211
21. Mousavi SH, Tayarani-Najaran Z, Asghari M, Sadeghnia HR. Protective effect of Nigella sativa extract and thymoquinone on serum/glucose deprivation-induced PC12 cells death. Cell Mol Neurobiol. 2010;30(4):591-8. doi: 10.1007/s10571-009-9484-1 pmid: 20054635
22. Iwasa K, Ikata T, Fukuzawa K. Protective effect of vitamin E on spinal cord injury by compression and concurrent lipid peroxidation. Free Radic Biol Med. 1989;6(6):599-606. pmid: 2753391
23. Liu D, Yang R, Yan X, McAdoo DJ. Hydroxyl radicals generated in vivo kill neurons in the rat spinal cord: electrophysiological, histological, and neurochemical results. J Neurochem. 1994;62(1):37-44. pmid: 8263539
24. Means ED, Anderson DK. Neuronophagia by leukocytes in experimental spinal cord injury. J Neuropathol Exp Neurol. 1983;42(6):707-19. pmid: 6631457
25. Reiter R, Poeggeler B, Dun-xian T, CHEN L-D, Manchester L, Guerrero J. Antioxidant capacity of melatonin: a novel action not requiring a receptor. Neuroendocrinol Lett. 1993;15(1-2):103-16.
26. Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T. Effect of thymoquinone and Nigella sativa seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine. 2007;14(9):621-7. doi: 10.1016/j.phymed.2006.12.005 pmid: 17291733
27. Radad K, Moldzio R, Taha M, Rausch WD. Thymoquinone protects dopaminergic neurons against MPP+ and rotenone. Phytother Res. 2009;23(5):696-700. doi: 10.1002/ptr.2708 pmid: 19089849
28. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res. 2000;14(5):323-8. pmid: 10925395
29. Badary OA, Taha RA, Gamal el-Din AM, Abdel-Wahab MH. Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol. 2003;26(2):87-98. doi: 10.1081/DCT-120020404 pmid: 12816394
30. Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med. 1995;61(1):33-6. doi: 10.1055/s-2006-957994 pmid: 7700988
31. Nagi MN, Mansour MA. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection. Pharmacol Res. 2000;41(3):283-9. doi: 10.1006/phrs.1999.0585 pmid: 10675279
32. Nagi MN, Alam K, Badary OA, al-Shabanah OA, al-Sawaf HA, al-Bekairi AM. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. Biochem Mol Biol Int. 1999;47(1):153-9. pmid: 10092955
33. Mohamed A, Shoker A, Bendjelloul F, Mare A, Alzrigh M, Benghuzzi H, et al. Improvement of experimental allergic encephalomyelitis (EAE) by thymoquinone; an oxidative stress inhibitor. Biomed Sci Instrum. 2003;39:440-5. pmid: 12724933
34. Mohamed A, Afridi DM, Garani O, Tucci M. Thymoquinone inhibits the activation of NF-kappaB in the brain and spinal cord of experimental autoimmune encephalomyelitis. Biomed Sci Instrum. 2005;41:388-93. pmid: 15850137
35. El-Abhar HS, Abdallah DM, Saleh S. Gastroprotective activity of Nigella sativa oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. J Ethnopharmacol. 2003;84(2-3):251-8. doi: 10.1016/s0378-8741(02)00324-0
36. Raza M, Alghasham AA, Alorainy MS, El-Hadiyah TM. Potentiation of Valproate-induced Anticonvulsant Response by Nigella sativa Seed Constituents: The Role of GABA Receptors. Int J Health Sci (Qassim). 2008;2(1):15-25. pmid: 21475467
37. Gilhotra N, Dhingra D. Thymoquinone produced antianxiety-like effects in mice through modulation of GABA and NO levels. Pharmacol Rep. 2011;63(3):660-9. pmid: 21857076
38. Duan Y, Haugabook SJ, Sahley CL, Muller KJ. Methylene blue blocks cGMP production and disrupts directed migration of microglia to nerve lesions in the leech CNS. J Neurobiol. 2003;57(2):183-92. doi: 10.1002/neu.10262 pmid: 14556284
39. Sedaghat R, Roghani M, Khalili M. Neuroprotective effect of thymoquinone, the nigella sativa bioactive compound, in 6-hydroxydopamine-induced hemi-parkinsonian rat model. Iran J Pharm Res. 2014;13(1):227-34. pmid: 24734075
40. Al-Majed AA, Al-Omar FA, Nagi MN. Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus. Eur J Pharmacol. 2006;543(1-3):40-7. doi: 10.1016/j.ejphar.2006.05.046 pmid: 16828080
41. Ozugurlu F, Sahin S, Idiz N, Akyol O, Ilhan A, Yigitoglu R, et al. The effect of Nigella sativa oil against experimental allergic encephalomyelitis via nitric oxide and other oxidative stress parameters. Cell Mol Biol (Noisy-le-grand). 2005;51(3):337-42. pmid: 16191402
42. Abdel-Zaher AO, Abdel-Rahman MS, Elwasei FM. Protective effect of Nigella sativa oil against tramadol-induced tolerance and dependence in mice: role of nitric oxide and oxidative stress. Neurotoxicology. 2011;32(6):725-33. doi: 10.1016/j.neuro.2011.08.001 pmid: 21855572
43. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed. 2013;3(5):337-52. doi: 10.1016/S2221-1691(13)60075-1 pmid: 23646296