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Black Seed Oil Improves Motor and Anxiety-Like Behaviours and Cerebellar Cyto-Architectonic in Adult Male Wistar Rats

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ABSTRACT

The objectives of this study were to investigate the potential efficacy of black seed oil (BSO) in motor activity, anxiety-like behaviour, cerebellar architecture and Purkinje morphometry in Wistar rats. Saline and black seed oil were administered to adult rats at 1 ml/kg orally for 14 days, and the rats were then subjected to behavioural tests to evaluate locomotor activity and anxiety-like behaviour using the open field and elevated plus mazes paradigms respectively. The cerebella were removed, processed and stained for cerebellar architectonic and Purkinje morphometry respectively. BSO significantly ($P \leq 0.05$) increased frequency of line crossing, rearing frequency and total alternation, which are measures of motor activities. It increased open arm explorations, head dip frequency, decreased freezing period and closed arm entry, which are measures of anxiety-like behaviours and did not affect either the architectonics of the cerebellar cortices or the Purkinje morphometry when compared with the control. These results suggest that BSO may reduce anxiety related behaviours in Wistar rats.

Keywords: *Black seed oil, Anxiety, Psychomotor disorders, Purkinje morphometry, Cerebellar architectonic*

INTRODUCTION

Anxiety related disorders are among the most common and prevalent forms of psycho-social dysfunctions, existing as a normal emotional response to a fearful condition resulting in abnormal hypersensitivity in sufferers (Andrews et al. 2000). Depression is a closely related psychosocial dysfunction, with no age boundary, characterized by a combined state of stress, sadness, despair, confusion, fear, loneliness and irritability among others (Andrews et al. 2000). This may lead to a more complicated psychomotor disorder, with physical symptoms such as slow walking. Treatment of these disorders has been challenging as only about 5% to 15% recover partially or completely after drug or psychotherapy while about 20% to 35% did not respond at all (Coulehan et al. 1997; Fava and

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Davidson 1996). The search for new substances as therapeutic alternatives for the management of anxiety and anxiety related health problems is thus a paramount need, due to their constant progress and spread (Irie et al. 2004 and Klodzinska et al. 2004).

The use of natural products as complementary alternative medicines has increased substantially over the last decade to treat many pathological conditions because of their perceived safety, widespread availability, cost effectiveness and ease of administration. Herbal and natural plant extracts have recently gained wide attention in their usage in the treatment and/or management of neurological, psychiatric and degenerative diseases, due to the absence of or fewer occurrences of side-effects (Yadav et al. 2009; Kumar et al. 2012).

Nigella sativa is a plant commonly called black cumin, fennel flower, or nutmeg flower. Other names include *Kalonji* seeds (Qidwai et al. 2009) and *Ajaji*, black *caraway* seed, and *Habbatu Sawda*. It is a fairly well regarded medicinal herb, and regarded in the Islamic tradition as the 'remedy for all diseases except death' (Ahmad et al. 2013).

The oil of *Nigella sativa* also known as black seed oil (BSO) is a high value medicinal solvent, widely used traditionally in the treatment of many diseases. Compelling evidences show that BSO exhibits pharmacological activities including antioxidant (Ahmad et al. 2013), anti-inflammatory (Pichette et al. 2012; Ahmad and Beg 2013; Alemi et al. 2013), neuroprotective (Javanbakht et al. 2013), immunomodulatory and anti-tumour (Majdalawieh et al. 2010; Salim 2010; Woo et al. 2012; Bai et al. 2013), men infertility improvement (Kolahdooz et al. 2014), efficacy in neurodegenerative diseases (Alhebshi et al. 2013; Dariani et al. 2013) and memory enhancing effects (Farimah et al. 2016; Imam et al. 2016).

It is also reported to inhibit liver and lung fibrosis (El-Khouly et al. 2012; Bai et al. 2013), attenuate diabetic nephropathy (Sayed 2012), prevent *E. coli* induced tissue damage in bacterial prostatitis (Inci et al. 2013) and prevent dextran sulphate sodium-induced colitis (Lei et al. 2012).

In this study, we examined the potential effects of the BSO on anxiety-like behaviours and motor activities in rats using the elevated plus (EPM) and the open field mazes (OFT) respectively. In addition, its effects on the cerebellar organization of rats were also evaluated.

MATERIALS AND METHODS

Animals

Twelve adult male Wistar rats weighting 200 ± 20 g about 10 weeks old at the time of acquisition and acclimatization were used in this study. They had free access to food and water, were housed six in a cage, and kept at controlled temperature (22 ± 2°C) under a 12/12 hr light-dark cycle. The guidelines of the Institutional Animal Care and Use Committee (IACUC) were strictly followed throughout in the handling of the animals. Ethical approval was granted by the University of Ilorin Ethical Committee on the Use of Experimental Animals and Laboratory Research.

Experimental Design

The rats were randomly distributed into two groups (n = 6) as follows:

1. Control: received 1 ml/kg of saline, consecutively for 14 days
2. Experimental: received 1 ml/kg of black seed oil, consecutively for 14 days

All exposure were via oral route and animals were subjected to behavioural assessments in the open field maze (OFM) and elevated plus maze (EPM) paradigms to evaluate the motor activities and anxiety-like behaviours respectively.

Drugs

Normal saline (NaCl 0.9%) at 1 ml/kg was given as the physiological control drug while BSO (100% pure natural oil) was gotten from Masra Warda, Kingdom of Saudi Arabia, and given at 1 ml/kg.

Open Field Test (OFT)

To assess the effects of the BSO on motor exploratory activity, experimental animals were evaluated in the open-field paradigm. Animals were individually placed in an open field apparatus made up of Perspex plastic with dimensions (40×60×50 cm) and the floor was divided into 25 equal squares by lines. The numbers of squares crossed with all paws (frequent line crossing) were counted in a 5 min session (Engeland et al. 2006) and the following were recorded: (1) Frequency of line crossing, (2) the freezing period, and (3) rearing counts (vertical postures of the rat with its hind paws on the floor and forepaws on the wall) (Pitychoutis et al. 2009). All groups of animals were monitored in a balanced design during experiments.

Elevated Plus Maze Test (EPM)

For the assessment of anxiolytic-like activity of Black seed oil in rats, the elevated plus maze (EPM) apparatus was used. EPM consisted of two open arms (OA; 16 x 5 cm) and two closed arms (CA; 16 x 5 cm) and was elevated (60 cm) above from the floor. A 5-min session was used for each rat to determine (1) number of entries in an OAE (%) and (2) total time spent in an OA (sec.) (3) number of entries in the CAE (%), (4) total number of entry (OAE+CAE), (5) total rearing and (6) head dip frequency. Rats were individually placed at the centre of the EPM with heads facing the OA i.e., fear-inducing environment (Lister 1987). All behavioural procedures were done in a balanced design.

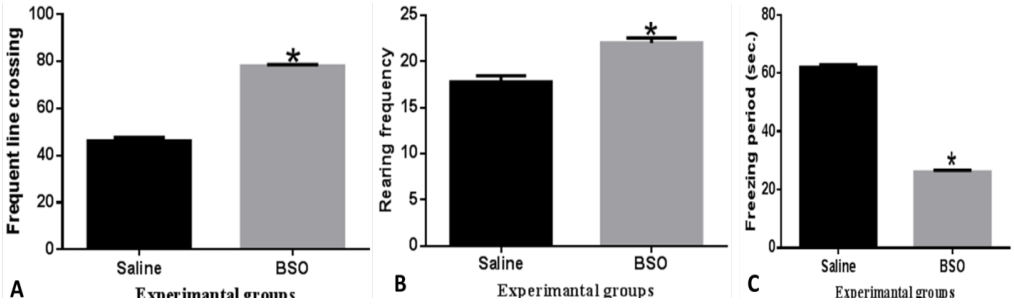

Fig. 1: The effects of black seed oil and saline on exploratory (a and b) and anxiety related behaviours (c) measured from the Open field maze paradigm. BSO treated rats display less anxiety-like behaviours (a) BSO treated rats shows significantly (* = P ≤ 0.05) increased line crossing compared with saline treated control group (P<0.05, student t test) (b) Freezing period was significantly (* = P≤0.05) reduced in BSO treated rats compared with control.

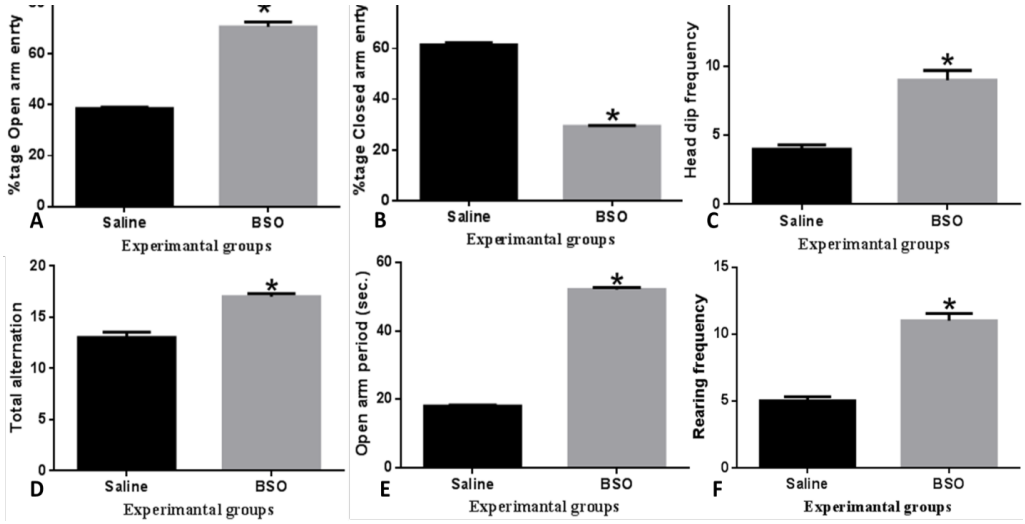

Fig. 2: The effects of black seed oil and saline on exploratory (d and f) and anxiety related behaviours (a, b, c and e) recorded from the Elevated plus maze paradigm. Showing data in bar chats with significant (* = P ≤ 0.05) increase in percentage open arm entry, open arm period, head dip frequency, total alternation, rearing frequency and decrease in percentage closed arm entry in the BSO treated animals when compared with the saline treated animals.

Tissue Preparations and Microscopy

The animals were euthanized 24 hours after the last treatment and about 18 hours after the behavioural tests, transcardially perfused with 4% paraformaldehyde (PFA), the brains were immediately removed and fixed in buffered 4% PFA until use. The cerebella were removed, wax embedded, serially sectioned at 5 microns (μ) and stained in Haematoxylin and Eosin (H&E) for general cerebellar architecture and Cresyl fast violet (CFV) for Purkinje cytoarchitecture. Images of the general cerebellar architecture were captured under ×5 and ×10 objective lenses respectively. While the Purkinje cytoarchitecture was captured under ×40 objective lens using the Zeiss Axiostar Plus Light microscope.

Image Analysis

The general cerebellar histoarchitecture was examined under the Axioscope. The Purkinje cyto-morphometry was done using Image J software calibrated to micrometer as unit of measurement per pixel, and Purkinje soma area (μm2) and soma diameter (μm) were measured.

Statistical Analysis

In this investigation, the results were expressed as mean ± standard deviation (n = 6). The data from EPM, OFT, Purkinje soma area (μm2) and soma diameter were subjected to student t - test. P<0.05 was considered statistically significant in all cases. The software package GraphPad Prism (5th edition) was used for analysis and graphical representation of data.

RESULTS

Effects of Black Seed Oil (BSO) on Motor and Activity Related Behaviours

In this experiment, we subjected rats in saline group and those in BSO group to a battery of test to assess their motor activities, such as exploratory and locomotors activities. BSO treated rats show a significant (P ≤ 0.05) increase in the frequency of line crossing, total alternations and rearing frequencies in both OFT and EPM paradigms respectively (Fig. 1a, 1b and 2d, 2f) when compared with the saline-treated control animals. These results suggest positive effects of BSO in locomotors or exploratory activities.

Effect of Black Seed Oil on Psychosocial or Anxiety-Related Behaviour

We assessed whether black seed oil could affect anxiety-like behaviours using both EPM and OFT paradigms. BSO exhibited anxiolytic and positive efficacy in psychosocial behaviours on all measures of fear, restraining and other anxiety-like behaviours in rats. BSO treated rats significantly (P≤0.05) visited the open arms and also displayed increased head dip frequency. Thus, BSO treated rats were less anxious.

Moreover, a significant (P ≤ 0.05) reduction in freezing period and closed arm exploration was observed in BSO treated rats, which are indications of reduced anxiety-like behaviours, when compared with the control animals (Figure 1c and 2a, 2b, 2c, 2e).

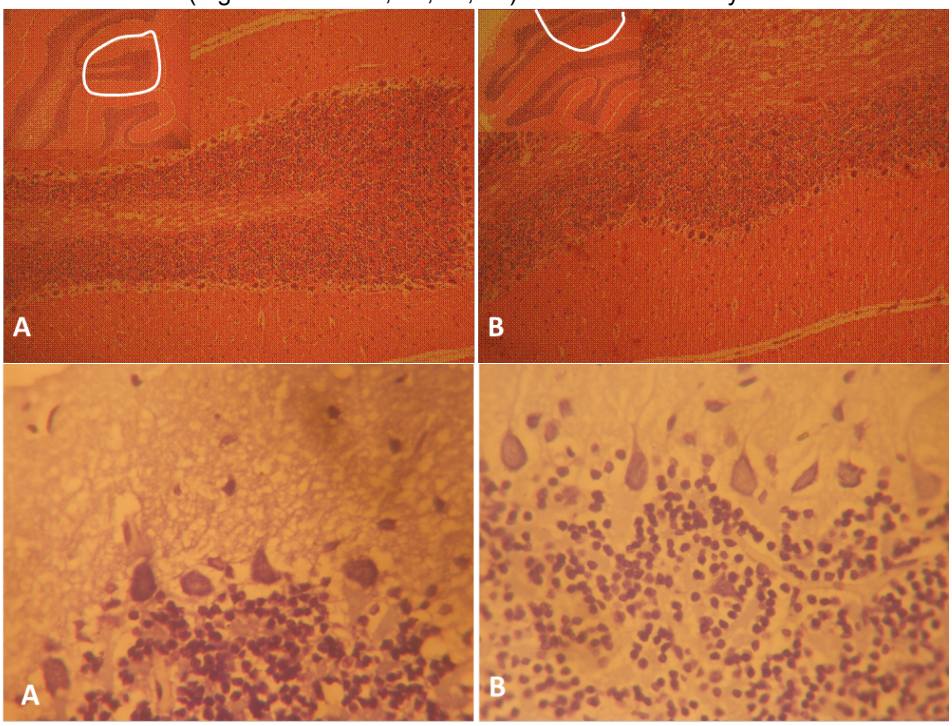

Fig. 3: The effects of black seed oil and saline on cerebella architectonic. Observable are the normal outlined architecture of the molecular, Purkinje and granular cell layers in the cerebella cortices of both saline (A) and BSO (B) treated animals. H&E ×100

Fig. 4: The effects of black seed oil and saline on Purkinje architectonics on a more focused Purkinje cell layer. Observed are undistorted Purkinje cells with distinct soma and projections. The apical dendritic projections and its arborisations were more distinct and visible in the BSO treated animals (B) when compared with the saline treated (A). CFV ×400

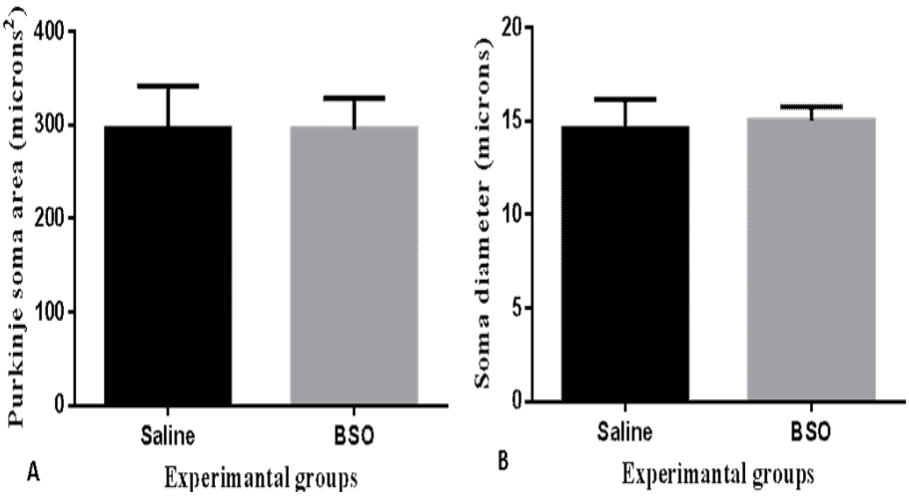

Fig. 5: The effects of black seed oil and saline on Purkinje morphometric. BSO treatment has no detrimental effect on cerebellar neurons morphometry (a and b), there is no significant (P≤0.05) difference in soma area and diameter in both BSO treated and control rats.

Effects of Black Seed Oil on Cerebellar Cytoarchitecture and Purkinje Morphometry

To understand the impact of BSO on the topography and integrity of cerebella neurons, we did histological analysis of cerebella thin slices. The results from the cyto-architectural investigation showed normal cellular and general architecture in all the cerebella cortical layers (molecular, granular and Purkinje cell layers) of the BSO treated rats when compared with the control rats (Figure 3). The Purkinje neurons cyto-architecture appeared well with normal soma, dendritic projections and arborisations in the molecular layer (Figure 4). The BSO did not affect the Purkinje neural morphometry including the Purkinje soma area and diameters as compared with the control animals (Figure 5).

DISCUSSION

Psychomotor related disorders like anxiety and depression have become regular in the social life of man, and have been complicated with stress, fear, financial loss, emotional disturbance, competition for survival, pain and drugs, among other social daily activities of man.

BSO is referred to as a miracle herb because of its high therapeutic efficacy in almost all diseases (Ahmad et al. 2013) and implicated as a potent neuropharmacological agent in many neurological disorders (Ahmad et al. 2013), including neuropsychiatric dysfunctions (Randhawa and Alenazi 2016).

This study examined various locomotor activities and anxiety related behaviour, using standard behavioural models such

as the elevated plus maze (Lister 1987) and the open field test (Boguszewski and Zagrodzka 2002; Engeland et al. 2006; Pitychoutis et al. 2009). These two test batteries are classical in the screening of the phenotypic manifestations of central neuronal activities, especially psychological and motor related activities like depression, stress and anxiety (Maria et al. 2007). And both paradigms are widely standardized measurements of exploratory, anxiety related, and locomotor behaviour (File 1993; Frye and Madeline 2008)

In this study, BSO was recorded to improve psychomotor activities in the treated animals. The various behavioural markers of anxiety related activities, such as the open arm explorations and head dip frequencies were high in the BSO treated animals, and such activities point to the anxiolytic efficacy of the substance. This claim follows the general knowledge that anxiogenic compounds cause animals' aversion to the open arms and reduce exploration, while anxiolytic agents does otherwise (Maria et al. 2007). BSO also caused marked reduction in the freezing period in the free exploratory open field paradigm, and complemented such with more exploratory activities as noted in the high rearing frequencies in both OFT and EPM. This result thus strengthens the previous claim of the anxiolytic efficacy of BSO inferred from the high open arm explorations.

These inferences are supported by its abilities in stimulating energy and recovery from fatigue and dispiritedness (Al-jishi 2000), inducing short recall (an indication of good mood and anxiolytic effect) (Bin Sayeed et al. 2014) and modulating nitric oxide and GABA, which are implicated in the anxiolytic activities of thymoquinone, the active ingredient of BSO (Gilhotra and Dhingra 2011).

Locomotor or motor activities which are central to social behaviours were also evaluated in this study and are found to improve with BSO treatment. Such activities as total exploratory activities in the EPM, measured as total alternations was recorded high in the BSO treated animals, an observation strengthened by an increase in the primary measure of locomotors activities, the frequency of line crossing (Pitychoutis et al. 2009). The frequency of line crossing in the OFT paradigm was also recorded to be significantly high with BSO treatment, indicative of high locomotors or exploratory activities in the animals (Tahira et al. 2009). The implicated anxiolytic and positive effects on exploratory activities can be complementarily validated by its efficacies in improving memory and recall in both adult and developing rats (Farzaneh et al. 2015; Imam et al. 2016).

The cerebellum plays a key role in motor co-ordination, and also increasingly appreciated in its role in cognitive and emotional behaviours such as stress and anxiety (Aldinger and Doherty 2016). The cerebella cortices architectecture as investigated in this study shows a normal and non-distorted architecture in the composing layers, which includes the molecular layers that receives the dendritic arborisations, the Purkinje cells layer containing the soma of the large cells and the highly populated granular cell layer. The isolated observation of the Purkinje cell layer revealed high projections of the apical dendrites, their well establish arborisation and outlined soma in the BSO treated animals when compared with the control. Such positive effects of BSO have been previously reported in the prefrontal and frontal cortical pyramidal neurons, dentate gyrus large granular cells, hippocampal CA pyramidal neurons, brain stem and spinal cord following degenerative exposures to scopolamine, toluidine, autoimmune encephalomyelitis and spinal cord injury respectively (Kanter et al. 2008; Heba et al. 2015; Aldinger and Doherty 2016; Farimah et al. 2016; Imam et al. 2016).

Further supporting the reported effects of BSO on the cerebella and Purkinje architectonic are its protective and ameliorative effects against amyloid-peptide induced toxicity in cerebella granule neurons (Kanter et al. 2006) and lead induced cerebella and hippocampal neuronal degeneration and axonal demyelination (Ismail et al. 2008; Khaled et al. 2014).

CONCLUSION

It can be concluded from this present study that BSO could improve anxiety-like behaviours, locomotors activity and Purkinje or cerebellar architectonic in the Wistar rats, thus suggesting its possible potential anxiolytic properties.

Conflict of Interest

None declared.

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REFERENCES

Ahmad, A., Husain, A., Mujeeb, M., Khan, S.A., Najmi, A.K., Siddique, N.A., Damanhouri, Z.A. and Anwar, F. (2013) A review on therapeutic potential of Nigella sativa: a miracle herb. Asian Pacific Journal of Tropical Biomedicine. 3:337-352.

Ahmad, S. and Beg Z.H. (2013) Elucidation of mechanisms of actions of thymoquinone-enriched methanolic and volatile oil extracts from Nigella sativa against cardiovascular risk parameters in experimental hyperlipidemia. Lipids in Health and Disease. 12(1):86.

Aldinger, K.A. and Doherty, D. (2016) The genetics of cerebellar malfunctions. Seminars in Fetal and Neonatal Medicine. pii: S1744-165X(16)30008-7. doi: 10.1016/j.siny.2016.04.008

Alemi, M., Sabouni, F., Sanjarian, F., Haghbeen, K. and Ansari, S. (2013) Anti-inflammatory effect of seeds and callus of Nigella sativa L. extracts on mix glial cells with regard to their thymoquinone content. AAPS PharmSciTech. 14(1):160-167.

Alhebshi, A.H., Gotoh, M. and Suzuki, I. (2013) Thymoquinone protects cultured rat primary neurons against amyloid β-induced neurotoxicity. Biochemical and Biophysical Research Communications. 19;433(4):362-367.

Al-jishi, S.A.A. (2000) Study of Nigella sativa on blood hemostatic functions. MSc Thesis, King Faisal University, Dammam, Saudi Arabia.

Andrews, G., Sanderson, K., Slade, T. and Issakidis, C. (2000) Why does the burden of disease persist? Relating the burden of anxiety and depression to effectiveness of treatment. Bulletin of the World Health Organization. 78(4):446-454.

Bai, T., Lian, L.H., Wu, Y.L., Wan, Y. and Nan, J.X. (2013) Thymoquinone attenuates liver fibrosis via PI3K and TLR4 signaling pathways in activated hepatic stellate cells. International Immunopharmacology. 15(2):275-281.

Bin Sayeed, M.S., Shams, T., Fahim Hossain, S., Rahman, M.R., Mostofa, A., Fahim Kadir, M., Mahmood, S. and Asaduzzaman, M. (2014) Nigella sativa L. seeds modulate mood, anxiety and cognition in healthy adolescent males. Journal of Ethnopharmacology. 152:156-162.

Boguszewski, P. and Zagrodzka, J. (2002) Emotional changes related to age in rats--a behavioral analysis. Behavioural Brain Research. 133:323-332.

Coulehan, J.L., Schulberg, H.C., Block, M.R., Madonia, M.J. and Rodriguez, E. (1997) Treating depressed primary care patients improves their physical, mental, and social functioning. Archives of Internal Medicine. 157:1113-20.

Dariani, S., Baluchnejadmojarad, T. and Roghani, M. (2013) Thymoquinone attenuates astrogliosis, neurodegeneration, mossy fiber sprouting, and oxidative stress in a model of temporal lobe epilepsy. Journal of Molecular Neuroscience. 51(3):679-686.

El-Khouly, D., El-Bakly, W.M., Awad, A.S., El-Mesallamy, H.O. and El-Demerdash, E. (2012) Thymoquinone blocks lung injury and fibrosis by attenuating bleomycin-induced oxidative stress and activation of nuclear factor Kappa-B in rats. Toxicology. 16;302(2-3):106-113.

Engeland, C.G., Kavaliers, M. and Ossenkopp, K.P. (2006) Influence of the estrous cycle on tolerance development to LPS-induced sickness behaviors in rats. Psychoneuroendocrinology. 31:510-525.

Farimah, B., Mahmoud, H., Farzaneh, V., Mohammad, N.S. and Mohammad, S. (2016) Feeding of Nigella sativa during neonatal and juvenile growth improves learning and memory of rats. Journal of Traditional and Complementary Medicine. 6:146-152.

Farimah, B., Mahmoud, H. and Majid, K. (2016) Neuropharmacological effects of Nigella sativa. Avicenna Journal of Phytomedicine. 6(1):124-141.

Farzaneh, V., Mahmoud, H., Zahra, H., Mohammad, A.E., Hamid, R.S., Masoumeh, S. (2015) The effects of Nigella sativa hydro alcoholic extract on memory and brain tissues oxidative damage after repeated seizures in rats. Iranian Journal of Pharmaceutical Research. 14(2):547-557.

Fava, M. and Davidson, K.G. (1996) Definition and epidemiology of treatment-resistant depression. Psychiatric Clinics of North America. 19:179-200.

File, S.E. (1993) The interplay of learning and anxiety in the elevated plus-maze. Behavioural Brain Research. 58:199- 202.

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

Gilhotra, N. and Dhingra, D. (2011) Thymoquinone produced antianxiety-like effects in mice through modulation of GABA and NO levels. Pharmacological Reports. 63:660-669.

Heba, M.F., Neveen, A.N., Faten, F.M., Anwar, A.E. and Nasr, M.R. (2015) Nigella sativa as an anti-inflammatory and promising remyelinating agent in the cortex and hippocampus of experimental autoimmune encephalomyelitis-induced rats. International Journal of Clinical and Experimental Pathology. 8(6):6269-6286.

Imam, A., Ajao, M.S., Ajibola, M.I., Amin, A., Abdulmajeed, A.I., Lawal, A.Z., Alli-Oluwafuyi, A., Akinola, O.B., Oyewopo, A.O., Olajide, O.J. and Adana, M.Y. (2016) Black seed oil reversed scopolamine-induced Alzheimer and cortico-hippocampal neural alterations in male Wistar rats. Bulletin of Faculty of Pharmacy, Cairo University. 54(1):1-106.

Inci, M., Davarci, M., Inci, M., Motor, S., Yalcinkaya, F. R., Nacar, E., Aydin, M., Sefil, N. K. and Zararsiz, I. (2013) Anti-inflammatory and antioxidant activity of thymoquinone in a rat model of acute bacterial prostatitis. Human and Experimental Toxicology. 32(4):354-361.

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

Ismail, N., Ismail, M., Latiff, L. A., Mazlan, M. and Mariod, A. A. (2008), Black cumin seed (Nigella sativa linn.) oil and its fractions protect against beta amyloid peptide-induced toxicity in primary cerebellar granule neurons. Journal of Food Lipids. 15(24: 519–533.

Javanbakht, J., Hobbenaghi, R., Hosseini, E., Bahrami, A.M., Khadivar, F., Fathi, S. and Hassan, M.A. (2013) Histopathological investigation of

neuroprotective effects of Nigella sativa on motor neurons anterior horn spinal cord after sciatic nerve crush in rats. Pathologie Biologie. 61:250-253.
Kanter, M., Coskun, O., Kalayci, M., Buyukbas, S., Cagavi, F. (2006) Neuroprotective effects of Nigella sativa on experimental spinal cord injury in rats. Human and Experimental Toxicology. 25(3):127–133.
Khaled, R., Khaled, H., Mubarak, A., Rudolf, M. and Wolf-Dieter, R. (2014) Thymoquinone ameliorates lead-induced brain damage in Sprague Dawley rats. Experimental and Toxicologic Pathology. 66(1):13-17.
Klodzinska, A., Tatarczynska, E., Chojnacka-Wojcik, E., Nowak, G., Cosford, N.A. and Pilc, A. (2004) Anxiolytic-like effects of MTEP: A potent and selective mGlu5 receptor agonist does not involve GABAA signaling. Neuropharmacology. 47:342-350.
Kolahdooz, M., Nasri, S., Modarres, S.Z., Kianbakht, S. and Huseini, H.F. (2014) Effects of Nigella sativa L. seed oil on abnormal semen quality in infertile men: A randomized, doubleblind, placebo controlled clinical trial. Phytomedicine. 21:901-905.
Kumar, H., More, S.V., Han, S.D., Choi, J.Y., Choi, D.K. (2012) Promising therapeutics with natural bioactive compounds for improving learning and memory review of randomized trials. Molecules. 17:10503-10539.
Lei, X., Liu, M., Yang, Z., Ji, M., Guo, X. and Dong, W. (2012) Thymoquinone prevents and ameliorates dextran sulfate sodium-induced colitis in mice. Digestive Diseases and Sciences. 57(9):2296-2303.
Lister, R.G. (1987) The effects of repeated doses of ethanol on exploration and its habituation Psychopharmacology. 92:78–83.
Majdalawieh, A.F., Hmaidan, R. and Carr, R.I. (2010) Nigella sativa modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. Journal of Ethnopharmacology. 131:268–275.
Maria, I.G.S., Manuel Rufino, D.A.N., Paulo, F.T.N., Brinell, A.M., Jeferson, F.D.A., Damião, P.D.S., Silvânia, M.M.V. and Francisca, C.F.D.S. (2007) Central nervous system activity of acute administration of isopulegol in mice. Pharmacology Biochemistry and Behaviour. 88(2):141- 147.
Randhawa, M.A. and Alenazi, S.A. (2016) Neuropsychiatric effects of Nigella sativa (black seed) a review. Alternative and Integrated Medicine. 5:209.
Pichette, A., Marzouk, B., Legault, J. (2012) Antioxidant, antiinflammatory, anticancer and antibacterial activities of extracts from Nigella sativa (black cumin) plant parts. Journal of Food Biochemistry. 36:539–546.
Pitychoutis, P.M., Nakamura, K., Tsonis, P.A. and Papadopoulou-Daifoti, Z. (2009) Neurochemical and behavioral alterations in an inflammatory model of depression: sex differences exposed. Neuroscience. 159:1216-1232.
Qidwai, W., Hamza, H.B., Qureshi, R. and Gilani, A., (2009) Effectiveness, safety, and tolerability of powdered Nigella sativa (kalonji) seed in capsules on serum lipid levels, blood sugar, blood pressure, and body weight in adults: results of a randomized, double-blind controlled trial. Journal of Alternative and Complementary Medicine. 15(6):639-644.
Salim, E.I. (2010) Cancer chemopreventive potential of volatile oil from black cumin seeds, Nigella sativa L., in a rat multi-organ carcinogenesis bioassay. Oncology Letters. 1:913-924.
Sayed, A.A. (2012) Thymoquinone and proanthocyanidin attenuation of diabetic nephropathy in rats. European Review for Medical and Pharmacological Sciences. 16(6):808-815.
Tahira, P., Saida, H., Sumera, K. and Darakhshan, J. H. (2009) Repeated administration of Nigella sativa decreases 5-HT turnover and produces anxiolytic effects in rats. Pakistan Journal of Pharmaceutical Sciences. 22(2):139-144
Woo, C.C., Kumar, A.P., Sethi, G. and Tan, K.H. (2012) Thymoquinone: potential cure for inflammatory disorders and cancer. Biochemical Pharmacology. 15;83(4):443-451.
Yadav, R.S., Sankhwar, M.L., Shukla, R.K., Chandra, R., Pant, A.B., Islam, F. and Khanna, V.K. (2009) Attenuation of arsenic neurotoxicity by curcumin in rats. Toxicology and Applied Pharmacology. 240:367-376.