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Nigella sativa as an anti-inflammatory and promising remyelinating agent in the cortex and hippocampus of experimental autoimmune encephalomyelitis-induced rats

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Abstract Experimental autoimmune encephalomyelitis (EAE) is a well-established animal model of multiple sclerosis. This study aimed to investigate the protective and therapeutic effects of *Nigella sativa* (*N. sativa*) seeds (2.8 g/kg body weight) in EAE-induced rats. EAE-induced animals were divided into: (1) EAE-induced animals (''EAE'' group). (2) ''*N. sativa* + EAE'' group received a daily oral administration of *N. sativa* 2 weeks prior to EAE induction until the end of the experiment. (3) ''EAE + *N. sativa*'' group received a daily oral administration of *N. sativa* after the appearance of the first clinical signs until the end of the experiment. All animals were sacrificed at the 28th day post EAE-induction. Disease pathogenesis was monitored using a daily clinical scoring, body weight, open field test, histopathological and ultrastructural examination and determination of some oxidative stress parameters in the cortex and hippocampus. *N. sativa* ameliorated the clinical signs and suppressed inflammation observed in EAE-induced rats. In addition, *N. sativa* enhanced remyelination in the hippocampus. However, protection of rats with *N. sativa* administered 2 weeks prior to EAE induction and its continuation until the end of the

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; CFA, complete Freund's adjuvant; CNS, central nervous system; DA, dark astrocyte process; DF, demyelinated fiber; DO, dark oligodendrocyte process; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; EAE, experimental autoimmune encephalomyelitis; GSH, reduced glutathione; GST, glutathione-S-transferase; H&E, hematoxylin and eosin; LA, light astrocyte process; LO, light oligodendrocyte process; M, myelinated fiber; MDA, malondialdehyde; MO, medium oligodendrocyte process; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NO, nitric oxide; *N. sativa*, *Nigella sativa*; PBS, phosphate buffered saline; PD, partially demyelinated fiber; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates; ROS, reactive oxygen species; TBA, thiobarbituric acid; TCA, trichloroacetic acid; OFT, open field test; R, remyelinated fiber; TEM, transmission electron microscope; TQ, thymoquinone; U, unmyelinated fiber

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experiment resulted in a significant increase in the cortical lipid peroxide level with reference to control and "EAE" rats. In conclusion, *N. sativa* seeds could be used as a protective agent or an adjunct treatment for EAE even when the treatment started after the appearance of the first clinical signs. However, the dose and duration of *N. sativa* must be taken into consideration to avoid its probable pro-oxidant effect.

Introduction

Multiple sclerosis (MS) is an immune-mediated, demyelinating neurodegenerative disease of the central nervous system (CNS) of young adults with a female predominance (Compston and Coles, 2002, 2008). It is a chronic progressive, potentially disabling disorder with considerable social impact and economic consequences (Sadovnick and Ebers, 1993). Experimental autoimmune encephalomyelitis (EAE) is a well-established animal model of MS in which the immune system attacks the myelin protein of the CNS and leads to inflammatory demyelination and oligodendrocyte loss (Frohman et al., 2006; Compston and Coles. 2008; Moore, 2010).

Experimental autoimmune encephalomyelitis is induced in laboratory animals by immunization with myelin-derived antigens; and believed to be mediated by activation of myelin-reactive CD4+ T cells. Expression of high levels of proinflammatory cytokines and chemokines (small chemotactic cytokines) in the brain is thought to contribute to the initiation and maintenance of EAE (Godiska et al., 1995; Ransohoff et al., 1996). Myelin oligodendrocyte glycoprotein (MOG) is a strong encephalitogen, comprising less than 0.05% of all myelin proteins and located exclusively on the surface of CNS myelin sheaths. MOG is a unique myelin autoantigen as it induces not only an encephalitogenic T-cell response in susceptible species, but also a demyelinating autoantibody response (Gold et al., 2006).

Zamvil and Steinman (1990) reported that EAE development is characterized by the infiltration of reactive leukocytes into the CNS. Recruited reactive macrophages/microglia are effector cells in EAE impairing oligodendrocyte axon function (Raivich and Banati, 2004). Astrocytes are involved in multiple and complex actions, including the regulation of the production of pro- and anti-inflammatory cytokines (De Groot et al., 1999; Ambrosini et al., 2002, 2005). In addition, a number of studies reported that reactive oxygen species (ROS) play a key role in myelin damage, contributing to several of the processes underlying MS pathogenesis (Miller et al., 2010; Van Horssen et al., 2011).

The use of natural products as drugs has increased substantially over the last decade to treat many pathological conditions instead of the use of synthetic drugs because of their safety, availability, and ease of administration. *Nigella sativa* (*N. sativa*), also known as black seed or black cumin, belongs to the family of the Ranunculaceae and it is native to Mediterranean countries. Many beneficial biological properties of *N. sativa* extracts have been reported such as anti-inflammatory (Houghton et al., 1995; Alemi et al., 2013; Pichette et al., 2012), antioxidative (Burits and Bucar, 2000) and neuroprotective (Kanter et al., 2006). In addition, *N. sativa* has been widely used in neurodegenerative diseases like Parkinson and Alzheimer because of its antioxidant potential (Hajra, 2011). Therefore, the aim of the present study was to investigate the protective and therapeutic effects of *N. sativa* in the cortex and the hippocampus of EAE-induced female Wistar rats.

Methods

Experimental animals, induction of EAE and clinical scoring

Adult female Wistar rats (age between 6 and 8 weeks) were used in this experiment. They were maintained under fixed appropriate conditions of housing and handling and were given food and water *ad libitum*. All animals received humane care in compliance with the guidelines of the Ethics Committee of the National Research Center, Egypt that followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication Nos. 85–23, revised 1985).

The EAE was induced according to Adelmann et al. (1995) with some modifications. Under light Halothane anesthesia, EAE was induced by a single subcutaneous injection in the tail base with an emulsion of 100 μg $MOG_{35–55}$ dissolved in 100 μl phosphate buffered saline (PBS), mixed with 100 μl complete Freund's adjuvant (CFA) containing 1 mg/ml of *Mycobacterium tuberculosis*.

For the evaluation of the disease course, all rats were weighed and examined daily from the sensitization until the 28th day after immunization. An adapted EAE scoring scale was used (Al-Izki et al., 2012). Animals were scored 0 = normal. 0.5 = incomplete flaccid tail. 1 = fully flaccid tail. 1.5 = semi-impaired righting reflex. 2 = impaired righting reflex. 2.5 = weakness of hind limb. 3 = hind limb paresis. 4 = complete hind limb paralysis. 5 = moribund/death. Control rats were handled in the same manner as the rats of other EAE-induced groups to affront the same degree of stress.

Chemicals

Rat synthetic myelin oligodendrocyte glycoprotein $peptide_{35–55}$ ($MOG_{35–55}$) was purchased from Titan Biotech Limited, Bhiwadi, India. Phosphate buffered saline (PBS) was obtained from the Bio Basic Inc., USA. Complete Freund's adjuvant (CFA), Thiobarbituric acid (TBA) and reduced glutathione were purchased from Sigma Aldrich, Germany. 1-Chloro-2,4-dinitrobenzene (CDNB) was purchased from Sigma Aldrich, St. Louis, USA. Trichloroacetic acid (TCA) was obtained from SDFCL (SD Fine-Chem Limited), Egypt. Potassium phosphate buffer pH 7.4 (50 mM/L, Triton × 0.1%, EDTA 0.5 mμ), potassium phosphate buffer pH 6.5 (100 mM/L) and kits for the determination of oxidative stress parameters were obtained from Bio Diagnostic Co., Giza, Egypt.