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Effects of 8-Week Aerobic Training with Nigella Sativa Extract on Neurotrophic Factors, Tissue Damage, and Muscle Weakness in a Multiple Sclerosis Cuprizon Model using Male C57BL/6 Mice

Aziz zinvand Lorestani [1], **Rahim Mirnasouri** [1]✉, **Masoud Rahmati** [1], **Marzieh Darvishi** [2]

[1] Department of Physical Education and Sport Sciences, Faculty of Literature and Human Sciences, Lorestan University, Khorramabad, Iran
[2] Anatomical Sciences Department, School of medicine, Ilam University of Medical Sciences, Ilam, Iran

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✉ ***Correspondence to:***
Rahim Mirnasouri
Department of Physical Education and Sport Sciences, Faculty of Literature and Human Sciences, Lorestan University, Khorramabad, Iran

Email:
mirnasuri.r@lu.ac.ir

A B S T R A C T

Introduction: Multiple sclerosis (MS) is an autoimmune, inflammatory, chronic, and progressive disease targeting neurons in the brain and spinal cord, leading to myelin sheath and axonal damage. Regular physical activity is recognized as a complementary and moderating treatment for MS. Nigella sativa is known for its antioxidant, anti-inflammatory, and anti-apoptotic effects.

Materials & Methods: Sixty-four adult male C57BL/6 mice (20-26 grams) were randomly divided into eight groups. MS was induced by adding 0.2% cuprizone to rodent food powder. The 8-week training program involved forced treadmill running (15-20 meters/minute), six days a week, twice a day, with 15-minute sessions and a minimum 2-hour interval. Balance was assessed using repeated measurements, BDNF levels through immunohistochemistry, and memory/spatial learning using the Morris blue maze test.

Results: After 8 weeks, BDNF increased in the experimental (37.69±2.04), Nigella sativa (34.96±1.84), and combined groups (43.17±3.2), with a significant difference between the Nigella sativa group and the others (P< 0.05). Clinical examination showed zero scores for sham, NS, EX, and NS+EX groups, indicating no movement disorders or limb weakness. In the Cuprizone group, scores increased, while treatment groups (Cup+NS, Cup+EX, and Cup+NS+EX) demonstrated a significant decrease compared to the Cup group (P< .05).

Conclusion: *Nigella sativa* and aerobic training appear to delay MS onset, reduce symptom severity, and contribute to the repair of damaged myelin areas, emphasizing their potential as therapeutic interventions. The condensed text is now approximately 249 words.

Keywords: Aerobic training, Nigella sativa, Brain-derived neurotrophic factor, Demyelination, Multiple sclerosis

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Introduction

Multiple sclerosis (MS) is an autoimmune, inflammatory, chronic, and progressive disease in which the immune system attacks nerve cells in the brain and spinal cord, causing damage to the myelin sheath and axons of these cells (1). This disease is one of the most common diseases of the central nervous system (CNS) in young people, so that the age of its onset is usually between the ages of 20 to 40 years and its prevalence in women is almost twice that of men (2). The reason for the higher prevalence of this disease in women than men may be due to differences in the immune system or nervous system in men and women or the effects of gonadal hormones and lifestyle changes (3).

One of the pathological symptoms of MS is the infiltration of immune cells into the CNS and the destruction of myelin and axonal damage that, during the process of tissue repair, immune cells produce brain-derived neurotrophic factor (BDNF). BDNF is a member of the neurotrophin family, which plays an important role in synaptic flexibility, neural survival, and differentiation, and has beneficial effects on CNS function in animal models. BDNF secretion improves the regeneration of various tissues. Various studies have shown that in people with MS, the level of this protein decreases. One of the most important factors that increase the level of BDNF is training (4). The secretion of neurotrophic factors following training seems to be one of the possible mechanisms to increase axonal protection and brain health of patients (5).

The neuroprotective and inhibitory potential of training by inducing neurogenesis in the hippocampus, an important brain structure for learning and memory, has been demonstrated in several animal studies. In addition, training is known to improve neurocognitive function in the elderly (6). Training has been reported to lead to a positive regulation of BDNF, which can contribute to animal brain health (7). A large number of studies show the positive effects of training on functional capacity, symptoms, and quality of life in patients with MS. However, its effects on the pathophysiology of MS including immune cells, cytokines, and neurotrophic factors are less known (8). Cytokines and their associated pathways are considered to be the most important regulators of the immune system, which may be important in the development of MS lesions and disease activity (9).

It appears to be an environmental stimulus of the immune system that leads to demyelination and disruption of CNS processes in genetically predisposed individuals. The result of CNS demyelination is associated with impaired neural conduction and reduced motor and cognitive abilities (3). Such immune-mediated processes include disruption of the blood-brain barrier (BBB) as well as the lack of nerve support for myelin repair (10).

The BBB controls the entry of beneficial substances into the CNS and restricts the passage of harmful substances. Dysfunction of the BBB, both acute and chronic, leads to the entry of harmful substances such as unexpected cytokines such as tumor necrosis factor-alpha (TNF-α) and immune cells into the CNS. This seems to be an important process in the pathophysiology of MS, because immune cells can enter the CNS during periods of cerebral blood vessel dysfunction and repair the cascade of events that lead to demyelination and disruption (11). Training is associated with functional improvement in several disease outcomes such as walking, balance, and fatigue. Likewise, other symptoms such as depression and cognition can be improved with training.

Despite increasing recognition of the special benefits of training, patient participation in the rehabilitation program is still low (12). Today, regular training and herbs are used instead of medicine to improve the physical condition of patients with MS. Herbal medicines have less toxicity and fewer side effects than chemical medicines and are more widely used (13). In Iran, medicinal plants are frequently used in traditional medicine to treat diseases. One of these plants is Nigella sativa, which is native to Southwest Asia and belongs to the Ranunculaceae family (14). Nigella sativa is a rich source of unsaturated fatty acids. Various studies indicate its anti-inflammatory, antioxidant, and neuroprotective properties. In addition, it has been widely used in the treatment of neurodegenerative diseases such as Parkinson's and Alzheimer's due to their potential antioxidant effects (15). Therefore, this study was aimed to investigate the effect of Nigella sativa plant extract and aerobic training and their possible synergistic effect can provide a suitable solution for the treatment of MS.

Materials and methods

Experimental Design

The statistical population of this study consisted of 64 adult male mice (8 weeks) of C57BL / 6 weights in the weight range of 20-26g. At the time of the study, these animals had unrestricted access to drinking water and standard food, and for the same period of time (12 hours) in the dark (night) and light (day) and at a temperature of 21 to 23 °C. To avoid any stress and accustom the animals to the new environment, the study started after 10 days. The animals were randomly divided into 8 groups of 8: groups 1-healthy (sham) 2- Multiple sclerosis (MS) model 3- Healthy with aerobic training (EX) 4- Multiple sclerosis model with aerobic training (MS+EX) 5- Healthy recipient of Nigella sativa (NS) oral extract 6- Multiple Sclerosis Model Receiving Oral Extract of Nigella sativa (MS+NS) 7- Healthy Receiving Oral Extract of Nigella sativa with Aerobic Training (EX+NS) 8- Model Multiple Sclerosis Receiving Oral Extract of Nigella sativa along with aerobic training (MS+NS+EX). Four groups of samples (MS+NS, MS+EX, MS+NS+EX, and MS) were fed with 0.2% cuprizone-impregnated feed for 7 weeks. To ensure demyelination, a tissue sample from the brain was taken three weeks after the induction of multiple sclerosis, and the corpus callosum was examined. All experiments were conducted in adherence to the ethical standards set by the Lorestan University Animal Ethics Committee, under the code LU.ACRA.2021058, and in accordance with the guidelines outlined by the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals.

Administration of Nigella sativa and Treadmill Training Protocol

In previous studies, one-tenth of the lethal dose of Nigella sativa in rats (870.9) was used for oral gavage (16). Therefore, in this study, the amount for oral gavage was 87 mg/kg body weight. Alcoholic extract was prepared from Nigella sativa powder by the Soxhlet method. At the end of the seventh week, three specimens were examined in the preclinical laboratory by MRI imaging to ensure demyelination. Seven days after the induction of the Cuprizone model, oral gavage was performed every morning in the groups treated with Nigella sativa until the end of the study. In the untreated groups, normal saline was gavaged. Corpus callosum tissue sections were used to evaluate the disease development process or its recovery. BDNF was measured by

immunohistochemistry to examine brain sections of the hippocampus.

After assigning the animals to 8 groups, the training groups were trained on a treadmill for a week. The training program included forced running on a treadmill at a speed of 15 to 20 meters per minute for 8 weeks, 6 days a week and twice a day, each session lasting 15 minutes with a minimum interval of 2 hours. It started at 15 meters per minute in the first week and gradually increased to 20 meters per minute in the fifth week and continued unchanged until the end of the eighth week. To maintain the speed of the mice and at the same time minimize external stressors, the sponge was used as a tactile stimulus. This training program is modeled on a study by Landers et al., Which has been modified according to the study conditions (17).

Statistical Analysis

Data were analyzed using SPSS software. The results were reported as Mean±SEM. In order to evaluate the percentage of myelination between sham and MS groups in the third week, independent t-test, statistical difference between the studied groups, one-way ANOVA test, and Tukey test were used to determine the groups that differed from each other.

Results

The results of this study showed that in the sham group and the three groups receiving Nigella sativa (NS), exercise (EX), and NS+EX that were not injured, the clinical test score was zero, and no movement disorders and weakness were observed in the front and rear limbs. In contrast, in the negative control group or cuprizone, there was an increase in score from the first to the eighth week. Additionally, the three treatment groups (Cup+NS, Cup+EX, and Cup+NS+EX) exhibited a decrease in score from the first to the eighth week, and this decrease was significant compared to the first week. In all four groups, this decrease was also significantly different from the Cup group (P<.05) (Figure 1).

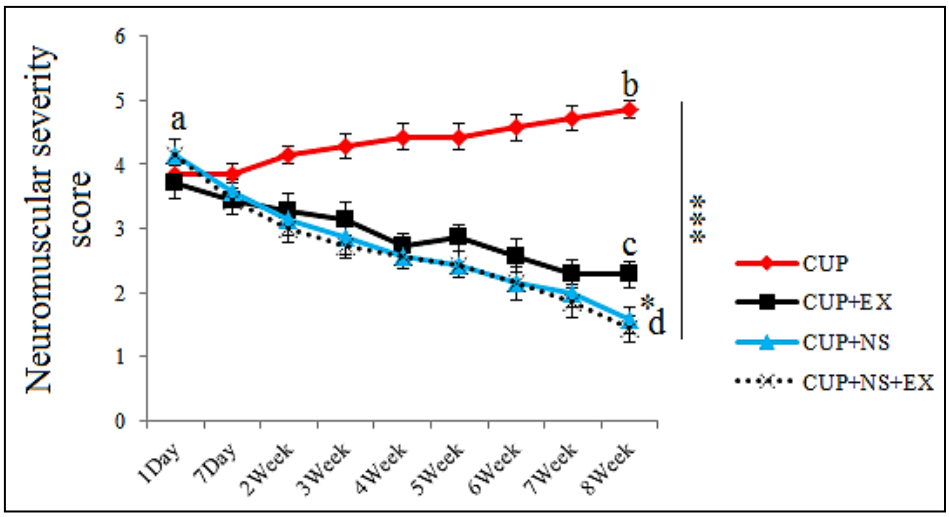

Figure 1. Trends in movement and muscle tone, assessed through the Clinical Test (Neuromuscular Severity Score) line chart, among the tested groups—Cup, Cup+NS, Cup+EX, and Cup+NS+EX. Data are presented as mean±SEM, and significance levels are denoted by *P<0.05 and ***P<0.001.

The results also revealed a significant difference between the studied groups, as determined by one-way ANOVA analysis, in the impact of the training test and Nigella sativa (NS) extract separately and in combination on the amount of corpus callosum brain-derived neurotrophic factor (BDNF) (P<0.001). In Tukey's post hoc analysis, a notable difference was observed between the treatment groups and the Cup group (P<0.001). After 8 weeks, BDNF levels increased in the experimental test group (37.69±2.04), the NS group (34.96±1.84), and the combined group (43.17±3.2). When comparing the treated groups, a significant difference was noted between the NS group and the other two groups (P<0.05) (Figure 2).

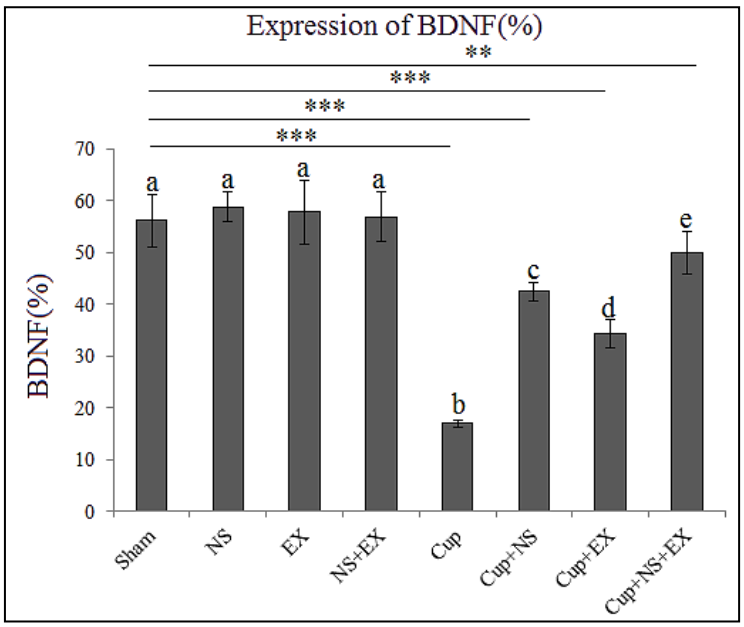

Figure 2. Significant difference between the three intervention groups and the Cuprizone group. Significance denoted by *P<0.01 and ***P<0.001.

In the study of demyelination rate, the cuprizone group exhibited the highest rate of damage, with affected areas extending beyond the entire tissue, and this difference was significant compared to other groups (P<0.05). In the four groups, sham, Nigella sativa (NS), exercise (EX), and NS+EX, the least amount of damage was observed, but there was no significant difference among these four groups. However, a significant difference was noted between these groups and the Cuprizone group. Within the study groups, Cup+EX showed the highest rate of injury, and this difference was significant compared to the two treatment groups Cup+NS and Cup+NS+EX. Additionally, this damage was significantly less compared to cuprizone. Among the treatment groups, the lowest rate of injury was observed in the Cup+NS+EX group, and this difference was significant compared to both the other two treatment groups and cuprizone (Figure 3).

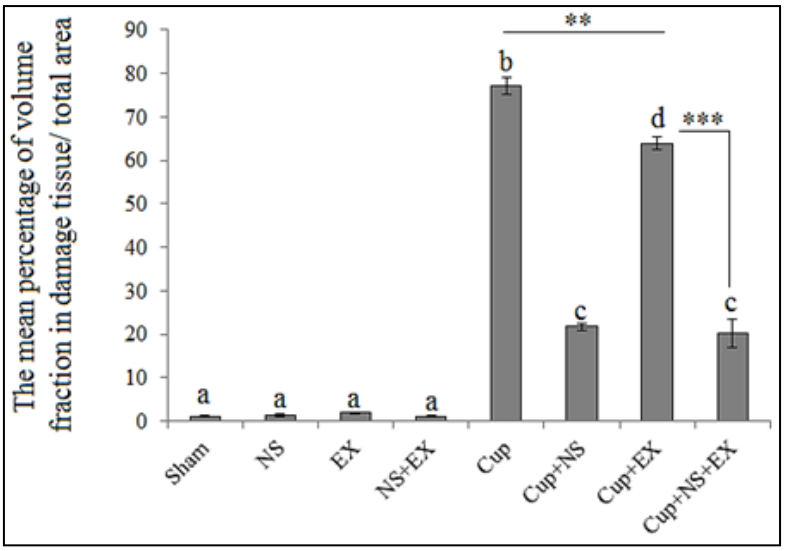

Figure 3. Histogram of Average Percentage of Volume Fraction Reflecting Tissue Damage. Data Averaged ± SEM. Significance denoted by *P<0.01 and ***P<0.001 for groups a, b, c, and d.

Discussion

This study aimed to investigate the impact of various interventions on clinical test scores, BDNF levels in the hippocampus, and demyelination rates in the context of Multiple Sclerosis (MS). The results revealed distinct patterns within the study groups, providing valuable insights into the potential effectiveness of Nigella sativa extract and aerobic training in mitigating inflammation and severity of symptoms associated with MS.

This study showed that in the groups with zero clinical test score, no movement disorders and weakness were observed in the front and back limbs. However, in the negative control group or Cuprizone, an increase in clinical test scores was observed from the first to the eighth week. Additionally, three treatment groups (Cup+NS, Cup+EX, and Cup+NS+EX) showed a significant decrease in clinical test scores from the first to the eighth week compared to the first week. The difference in all four groups compared to the Cup group was significant.

In the study of the effect of training and Nigella sativa test on Brain-Derived Neurotrophic Factor (BDNF) level in the hippocampus, a significant difference was observed between the studied groups with one-way ANOVA analysis. In Tukey's post hoc, a significant difference was observed between the treatment groups and the Cup group. After 8 weeks, BDNF levels increased in the training test group, Nigella sativa group, and the combination group, aligning with the results of Bansi et al., 2013 (1); Bricken et al., 2016 (18); Castellano & White, 2008 (5); and Gold et al., 2003 (7). Compared to the treated groups, a significant difference was observed between the Nigella sativa group and the two groups.

The results of this study indicate a significant difference between the treatment groups and the Cup group. After 8 weeks, BDNF levels increased in the training test group, the Nigella sativa group, and the combination group. Compared to the treated groups, there is a significant difference between the Nigella sativa group and the other two groups, consistent with the results of Mohammadi Rad et al., 2019 (19), and

Sean Bricken et al., 2016 (18). Mohammadi Rad et al. reported in a study that there are interactions between neurotrophic and inflammatory factors in the brain that play an important role in the course of MS. Also, the cause of inflammatory symptoms in MS can be the inverse relationship between TNF-α and BDNF levels in the brain tissue and serum of Cuprizone mice, which is consistent with the results of the present study (19). Bricken et al., in a study entitled "Effect of Training on Serum BDNF Levels in Patients with Progressive MS," showed that BDNF is strongly induced during acute training even in patients with progressive MS and advanced physical disability (20). Noor et al. in a 2014 study entitled "The effect of Nigella sativa on the severity of symptoms in animals with MS" found that consumption of Nigella sativa extract 2 weeks before induction of the EAE model increases the motor activity of animals and reduces their anxiety (21). Considering the confirmation of the anti-inflammatory effects of Nigella sativa extract in previous studies, it can be said that the reason for improving the motor symptoms of animals with MS is the reduction of inflammation in their brain tissue. The results of another study showed that hydroalcoholic extract of Nigella sativa has a significant ability to prevent nerve damage to the hippocampus and to prevent learning and memory disorders in mice. The results suggest that oral Nigella sativa extract and aerobic training may be effective in reducing inflammation and the severity of symptoms of MS (14). In the study of demyelination, the highest amount of damage was observed in the Cup group, which was significantly different from the other groups. Also, the lowest amount of damage was observed in four groups: sham, NS, EX, and NS+EX, but there was no significant difference between these four groups. In the study of treatment groups, Cup+EX showed the highest amount of damage, and Cup+NS+EX showed the least amount of damage. This finding confirms that Nigella sativa and training testing repair damaged areas. Consistent with the results of the present study, Jensen et al., in studying the effect of training on oligodendrogenesis in central myelination, showed an increase in the number of oligodendrocytes with training and stated that oligodendrocyte regeneration as a result of training leads to increased remyelination (22). Also, Mendelsohn et al. in 2019, in a study investigating the effect of training in the Cuprizone model, concluded that training regenerates oligodendrocytes in the Cuprizone demyelinating model (23), which is consistent with the results of the present study. Regarding the role of immune system attacks against myelin axons of the central nervous system (CNS) and as a result of demyelination and degeneration caused by it, it can be briefly said that following the attack of the immune system, the infiltration of inflammatory cells occurs in the CNS, which results in the death of oligodendrocytes, the activation of microglia and astrocytes, and finally, axonal degeneration will occur. Therefore, it is considered that the damage of oligodendrocytes and demyelination occurs as a result of inflammation of the CNS (24). From the obtained results, it can be concluded that Nigella sativa extract and aerobic training can be effective in reducing inflammation and severity of symptoms of MS.

Conclusion

The induction of Multiple Sclerosis (MS) causes tissue changes in the hippocampus and corpus callosum. These changes are accompanied by cell death, destruction of axons and myelin, and the induction of gliosis. Behavioral findings indicate that tissue and morphometric disorders lead to cognitive

and memory impairments, as well as movement and balance disorders. Aerobic training and Nigella sativa extract alone and in combination can be effective in treating and preventing the exacerbation of symptoms in individuals with MS. Probably due to the reduction of inflammation and the improvement of the myelination status, the balance, and the level of motor activity of the animals have also improved. These structural changes in the anatomy of the mouse brain, which indicate histological changes and align with its behavioral function, can pave the way for a new path for faster diagnosis and prevention of debilitating symptoms.

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Conflict of interest

The authors declare no affiliation or involvement with any organization or institution that has any financial or non-financial interest in the topic or content discussed in this manuscript.

Authors' contributions

A ZL and RM designed this study; A ZL, RM, MR and MD performed the research and wrote the paper. A ZL, RM and MR, MD analyzed the data. All authors have given their final approval for this paper to be published.

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