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RESEARCH

Nigella sativa oil attenuates neuroinflammation and cognitive deficits in a rat model of Alzheimer's disease

Nigella sativa yağı, Alzheimer hastalığı rat modelinde nöroenflamasyonu ve bilişsel bozuklukları hafifletir

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Abstract

Purpose: The present study was conducted to investigate the neuroprotective effects of Nigella sativa oil in a rat model of Alzheimer's disease (AD) induced by bilateral intrahippocampal Aβ1-42 injection.

Materials and Methods: *Nigella sativa* oil was administered orally via gavage at doses of 0.5 ml/kg/day (NS-A), 1 ml/kg/day (NS-B), and 2 ml/kg/day (NS-C), starting seven days before and continuing for ten days after Aβ1–42 injection. Following the completion of NS administration, passive avoidance and Morris Water Maze experiments were conducted. After the behavioural experiments, hippocampal tissues were collected for immunohistochemical and biochemical analyses.

Results: On the second day of the passive avoidance test, escape latency were shorter in the AD group (47.5±11.5) compared to the control group (292.2±5.2) and longer in the NS-B (228.3±34.7) and NS-C (248.2±28.5) groups compared to the AD group. In the Morris Water Maze probe test, the time spent in the target quadrant was significantly reduced in the AD group (20.3±1) than in the control group (33.4±2.9). However, in the NS-B (29.3±1.3) and NS-C (34.8±4.1) groups, it was significantly higher compared to the AD group. Hippocampal TNF-α levels in the AD group (191.3±22) were higher compared to the control group (51.5±4.8), but this increase was suppressed in the NS-B (91.9±16.1) and NS-C (76.3±11.7) groups compared to the AD group. Levels of IL-1β in the hippocampus were elevated in the AD group (118.3±9.6) compared to the control group (34.6±4.3), whereas this increase was suppressed in both the NS-B (67.9±5.8) and NS-C (54±4.6) groups. Hippocampal immunohistochemical GFAP staining scores were increased in the AD group compared to the control, whereas NS-B and NS-C staining scores were lower compared to the AD group. Similarly, Iba1 staining levels

Öz

Amaç: Bu çalışmanın amacı, bilateral intrahipokampal Aβ1-42 enjeksiyonu ile indüklenen sıçan Alzheimer hastalığı (AH) modelinde Nigella sativa yağının nöroprotektif etkilerini araştırmaktır.

Gereç ve Yöntem: Nigella sativa yağı, Aβ1-42 enjeksiyonundan yedi gün önce başlayıp on gün boyunca devam edecek şekilde 0,5 ml/kg/gün (NS-A), 1 ml/kg/gün (NS-B) ve 2 ml/kg/gün (NS-C) dozlarında gavaj yoluyla oral olarak uygulandı. NS uygulamalarının tamamlanmasını takiben Pasif Sakınma ve Morris Su Labirenti deneyleri gerçekleştirildi. Davranış deneylerinden sonra, immünohistokimyasal ve biyokimyasal analizler için hipokampal dokular toplandı.

Bulgular: Pasif sakınma testinin ikinci günü AH grubunda (47.5±11.5) kontrol grubuna göre (292.2±5.2) kısa olan karanlık kompartmana geçiş süreleri NS-B (228.3±34.7) ve NS-C (248.2±28.5) gruplarında AH grubuna göre uzundu. Morris Su Labirenti prob testinde, hedef kadranda geçirilen süre AH grubunda (20.3±1) kontrol grubuna (33.4±2.9) göre anlamlı derecede kısaydı. Ancak, NS-B (29.3±1.3) ve NS-C (34.8±4.1) gruplarında bu süre AH grubuna kıyasla arttı. AH grubu hipokampal TNF-α düzeyi (191.3±22) kontrol grubuna (51.5±4.8) kıyasla artmışken, AH'ye göre NS-B (91.9±16.1) ve NS-C (76.3±11.7) gruplarında artış baskılandı. Hipokampal IL-1β düzeyleri AH grubunda (118.3±9.6) kontrole göre (34.6±4.3) artmışken, bu artış NS-B (67.9±5.8) ve NS-C (54±4.6) gruplarında baskılandı. Hipokampüs İmmünohistokimyasal GFAP boyanma skoru AH grubunda kontrole göre artmışken, AH grubuna göre NS-B ve NS-C boyanma skoru daha azdı. Benzer şekilde Iba1 boyaması AH grubunda kontrole göre artmışken, NS-B ve NS-C gruplarında bu artış baskılandı.

Sonuç: Deneysel bulgularımız, Nigella sativa yağının Alzheimer hastalık modelinde bilişsel işlev kaybını önleyici etkiler gösterebileceğini ortaya koymaktadır. Bu etkinin,

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were elevated in the AD group compared to the control, while this increase was suppressed in the NS-B and NS-C groups

Conclusion: Our experimental findings suggest that Nigella sativa oil may exert preventive effects on the loss of cognitive function in an Alzheimer's disease model. It is thought that this effect may be related to the suppression of excessive inflammatory response involved in the pathology of the disease.

Keywords:. Alzheimer's disease, amyloid beta, nigella sativa oil, neuroinflammation, rat.

hastalığın patolojisinde rol oynayan aşırı inflamatuar cevabın baskılanmasıyla ilişkili olabileceği düşünülmektedir.

Anahtar kelimeler: Alzheimer hastalığı, amiloid beta, nigella sativa yağı, nöroinflamasyon, sıçan

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia and poses a growing health concern worldwide due to the increasing life expectancy[1]. Despite extensive research, there is currently no definitive treatment that halts disease progression or reverses pathology. Current pharmacological interventions offer only symptomatic relief[2]. Numerous hypotheses have been postulated to elucidate the pathophysiology of the disease. The Amyloid β (Aβ) hypothesis, currently the most widely accepted hypothesis explaining AD pathology, suggests that amyloid beta peptides cluster and form plaques at the core of the disease, with other pathological changes occurring secondarily[3]. It has been demonstrated that pathophysiological processes, including Aβ accumulation, changes in intracellular ion concentrations and tau hyperphosphorylation, result in an increased inflammatory response. The inflammatory response, which is initially in the form of defence reactions, becomes detrimental when it is excessive and prolonged, leading to cognitive decline[4]. Several studies have been conducted to ascertain the potential of anti-inflammatory agents to mitigate the pathology of Alzheimer's disease[5,6].

Nigella sativa (black cumin) has been traditionally used for its medicinal properties, including its anti-inflammatory, antioxidant, and neuroprotective effects, especially in Eastern cultures. Nigella sativa and its active constituent, thymoquinone, have been evaluated in experimental disease models[7–9] , as well as in clinical trials. These investigations have indicated a potential therapeutic benefit, which has led to growing interest in recent years[10,11]. Moreover, recent studies suggest that it may have neuroprotective properties and enhance memory and cognitive function[11–13]. However, despite the increasing interest in Nigella sativa as a neuroprotective agent, its precise effects on Aβ-induced neuroinflammation and cognitive impairment have not been comprehensively elucidated.

This study aimed to investigate whether oral administration of *Nigella sativa* oil could ameliorate Aβ-induced neuroinflammation and cognitive impairment in rats. The novelty of this study lies in its focus on elucidating the mechanistic role of *Nigella sativa* oil in modulating neuroinflammatory pathways, particularly through the suppression of pro-inflammatory cytokines and the regulation of astrocyte and microglial activation. We hypothesize that *Nigella sativa* oil exerts neuroprotective effects by suppressing pro-inflammatory cytokines and reducing astrocyte and microglial activation, thereby alleviating Aβ-induced neuroinflammation and improving cognitive function.

MATERIALS AND METHODS

Chemicals

Amyloid Beta 1-42 peptide (Aβ1-42, California Peptide, 642-15-1mg), Nigella sativa oil (black cumin oil used in the study was obtained from fresh seeds by cold pressing every other day), Phosphate-buffered saline (PBS, Sigma-Aldrich, USA), Ketamine (Alfamine 10% solution for injection, Alfasan, Netherlands), and Xylazine (Alfazyne 2% solution for injection, Alfasan, Netherlands) were used. ELISA kits for TNF-α (Abcam ab100785) and IL-1β (Abcam ab100768) specific to rats were obtained from Abcam (Cambridge, UK).

Animals

In this study, 4-month-old Wistar-Albino male rats (250-350 g body weight) were used. Rats were kept in

groups of four per cage under controlled conditions (12-hour light/dark cycle, central ventilation) and acclimated for one week. They received standard chow and tap water ad libitum, with overnight fasting before oral gavage.

The study protocol was approved by the Bolu Abant İzzet Baysal University Animal Research Local Ethics Committee (Ethics approval dated 14.02.2018, decision number 2018/06), and conducted under standard ethical guidelines (NIH publication no. 85-23, revised 1985; European Communities Directive 2010/63/EU) at Bolu Abant İzzet Baysal University Experimental Animals Application and Research Centre.

Experimental groups

The rats were randomly divided into five groups, with seven rats in each group (Table 1). The study had three groups: NS-A, NS-B, and NS-C, each receiving *Nigella sativa* oil orally every morning. The control groups received corn oil in the same manner.

Table 1. Groups

Control (C)	Corn oil + intrahippocampal (i.h.) saline
Alzheimer's Disease (AD)	Corn oil + i.h. Aβ
Nigella sativa oil 0.5 (NS-A)	Oral 0.5 ml/kg/day NS + i.h. Aβ
Nigella sativa oil 1 (NS-B)	Oral 1 ml/kg/day NS + i.h. Aβ
Nigella sativa oil 2 (NS-C)	Oral 2 ml/kg/day NS + i.h. Aβ

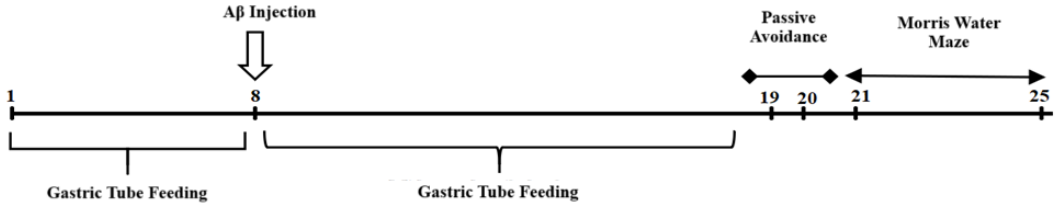

Figure 1. Experimental protocol

Experimental Alzheimer's disease model

Aβ1–42 was dissolved in 100 μL of distilled water and incubated at 37 °C for 7 days for fibril formation. All rats were anaesthetized with intraperitoneal Ketamine (100 mg/kg) and Xylazine (5 mg/kg). The coordinates of the injection site were determined according to the atlas of Paxinos & Watson[14]. The rat was placed on a stereotaxic apparatus and the Aβ1-42 peptide was injected into the hippocampus with a microsyringe (Hamilton Laboratory Products, Reno, USA) at the coordinates AP: -3.5 mm, ML: ± 2.0 mm, DV: -2.8 mm in an amount of 4μg/side bilaterally[15]. The control group was injected with the same amount of saline as the Aβ group.

Passive avoidance test

The Passive Avoidance test evaluates learning and short-term memory. The apparatus consists of two compartments, one illuminated and one dark, separated by a guillotine door with an electrified grid floor. On the first day, after 20 seconds in the light compartment, the guillotine door opened automatically, and the rat moved to the dark compartment. Upon entry, the door closed, and the animal received a 0.5 mA electric shock for three seconds. The rat was then returned to its home cage. On the second day, the animal was reintroduced to the light compartment, and the latency to enter the dark compartment was recorded (maximum 300 seconds). Rats that did not enter within 300 seconds were categorically considered to have learned the task [16].

Morris Water Maze (MWM) test

The Morris water maze (MWM) apparatus is the tool of choice for evaluating the spatial memory of rats. The MWM tank with dimensions of 160 cm in

diameter and 50 cm in height was filled with water to a height of approximately 30 cm. The temperature of the water was kept at 23±1°C. A video recording camera system was placed on the ceiling at the top of the tank, centred on the tank. A square platform with a side of 10 cm was placed in one quadrant, which was divided into 4 equal parts, 2 cm below the water. Visual cues were placed around the pool so that the animal could determine its direction. Over four days, rats were trained to find the platform from five different starting positions. If a rat failed to locate the platform within 90 seconds, it was guided to it and allowed to rest there for 20 seconds. On the fifth day, the probe test was conducted, removing the platform and allowing the rats to swim freely for 60 seconds. The time spent in the target quadrant was recorded[17].

Sample collection

Following behavioural tests, the rats were anaesthetized with intramuscular ketamine and xylazine. The right hippocampus was dissected for biochemical analysis and stored at -80°C. The left hippocampus was fixed in 10% formalin for histopathological analysis.

TNF-α and IL-1β measurements

All materials and reagents were brought to room temperature. Dilutions of the materials to be used in the experiment were made according to the ratios specified in the manufacturer's instructions. Briefly, hippocampus tissues were removed from -80ºC, thawed and weighed. After homogenizing the tissues, a Biotinylated TNF-α Detection Antibody was added. Then, HRP-Streptavidin solution was added. After incubation, the samples were read at 450 nm in the spectrometer.

Immunohistochemical analysis

Coronal brain sections (3-4 μm thick) were prepared and rehydrated. Iba1 antibody (1:8000 dilution) and GFAP antibody (1:100 dilution) were applied. After washing, DAB+ Chromogen was added, followed by counterstaining and cover-slipping. Staining intensity and distribution were scored as 0 (none) to +3 (severe).

Statistical analysis

The data obtained in the experimental studies were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows 25. For normally distributed data, a one-way ANOVA test followed by a post-hoc Bonferroni test was applied for intergroup comparisons. For non-normally distributed data, the Kruskal-Wallis test was used, followed by Dunn's post-hoc test when necessary. A p-value of <0.05 was considered statistically significant. Statistical significance levels were defined as follows: $p<0.05$: significant "*", $p<0.01$: highly significant "**", $p<0.001$: extremely significant "***". Results are given as mean ± standard error. GraphPad Prism 8.02 program was used for graph drawings.

Power analysis, based on Chen et al. (2015), determined that a minimum of seven rats per group was required to achieve a statistical power of 95% with an effect size of 1.96 and a Type I error probability of 0.05[18].

Data from behavioural tests (passive avoidance test and Morris Water Maze) and biochemical analyses (hippocampal TNF-α and IL-1β levels) were assessed for normality using the Shapiro-Wilk test. For normally distributed data, one-way ANOVA was performed followed by Bonferroni post-hoc tests to determine intergroup differences. For non-normally distributed data, the Kruskal-Wallis test was used, followed by Dunn's post-hoc test.

Histological scoring data (GFAP and Iba1 immunohistochemical staining) were analyzed using non-parametric tests (Kruskal-Wallis and Dunn's post-hoc), due to ordinal nature of the scores.

RESULTS

All groups exhibited an increase in average body weight. No statistically significant difference was found between the groups (Table 2).

The results of the passive avoidance experiment are given as the within-group average of the transition times of the animals to the dark chamber on the first day when electric shock was applied to induce learning and on the second day when learning was tested.

On the first day of the passive avoidance experiment, no statistically significant difference was found between the Control group (13.8±3.6 s), AD group (14.6±3.3 s), NS-A group (12.5±2.7 s), NS-B group (28.5±5.8 s) and NS-C group (19±5.4 s) for latency to enter the dark compartment (Fig. 2A). On the second day, the AD group (47.5±11.5 s) had significantly lower transition times than the Control group (292.2±5.2 s, $p<0.001$). In contrast, the NS-B

(228.3±34.7 s) and NS-C (248.2±28.5 s) groups exhibited significantly longer transition times than the AD group ($p<0.001$) (Fig. 2B). During the training days, the AD group required significantly more time to locate the platform than the Control group on days 2, 3, and 4 ($p<0.05$). NS-B and NS-C groups reached the platform significantly faster than the AD group ($p<0.05$) (Fig. 3A).

In the probe test, the AD group (20.3 ± 1 s) spent significantly less time in the target quadrant than the Control group (33.4 ± 2.9 s, $p<0.01$). In contrast, NS-B (29.3±1.3 s) and NS-C (34.8±4.1 s) groups spent significantly more time in the target quadrant than the AD group ($p<0.05$ and $p<0.001$, respectively). (Fig. 3B).

Table 2. The mean weight changes of the rats in the groups (Mean ± SEM)

	Weight at week 1 (g)	Weight at week 3 (g)
CONTROL	304.6±16.3	321.9±22.9
AD	296.6±23.3	303.9±19.6
NS-A	313.1±44.3	323.9±44.6
NS-B	295.5±37.3	304.8±37.0
NS-C	290.3±35.1	301.3±37.8

AD: Alzheimer's disease model, NS-A: 0.5 ml/kg, NS-B: 1 ml/kg, NS-C: 2 ml/kg Nigella sativa oil treatment groups.

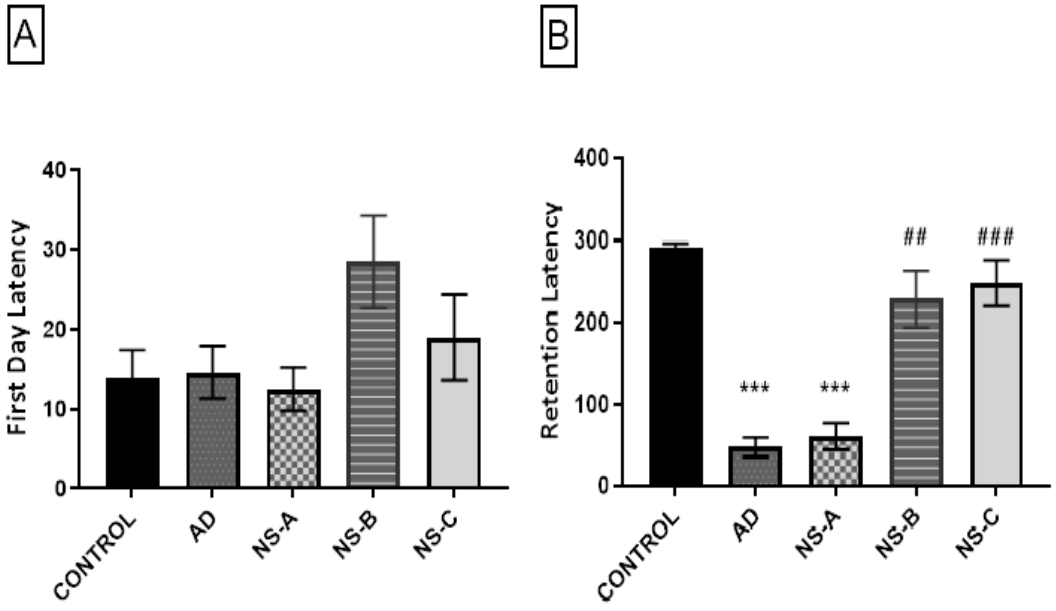

Figure 2. First-day latency times in the Passive Avoidance Test (A), Retention latency times in the Passive Avoidance Test (B) on the second day.

AD: Alzheimer's disease model, NS-A: 0.5 ml/kg, NS-B: 1 ml/kg, NS-C: 2 ml/kg Nigella sativa oil treatment groups. (*$p<0.05$, **$p<0.01$, ***$p<0.001$ indicate comparison to control group, #$p<0.05$, ##$p<0.01$, ###$p<0.001$ indicate comparison to AD group.)

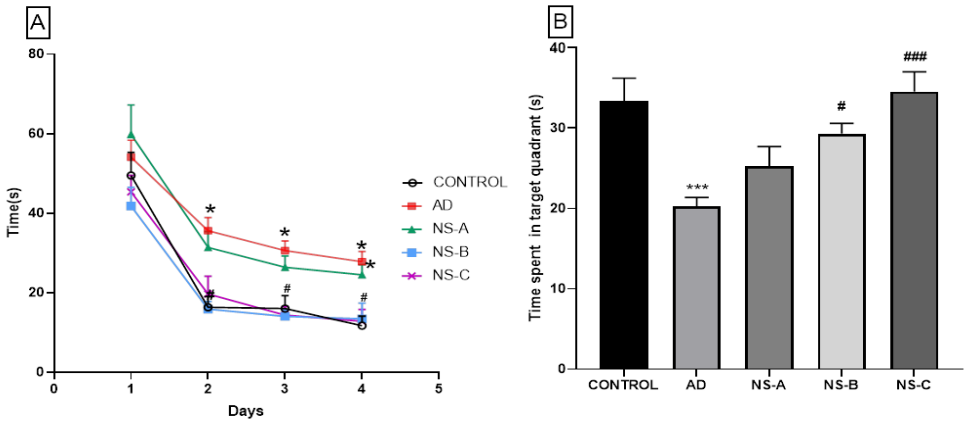

Figure 3. Time spent locating the platform during the acquisition days (A), and time spent in the target quadrant during the probe test (B) in the Morris Water Maze (MWM).

AD: Alzheimer's Disease model, NS-A: 0.5 ml/kg, NS-B: 1 ml/kg, NS-C: 2 ml/kg Nigella sativa oil treatment groups. (*p<0.05, **p<0.01, ***p<0.001 indicate comparison to control group, #p<0.05, ##p<0.01, ###p<0.001 indicate comparison to AD group.**)**

Swimming trace analyses confirmed that rats in the NS-B and NS-C groups stayed longer in the target quadrant during the probe test (Fig. 4).

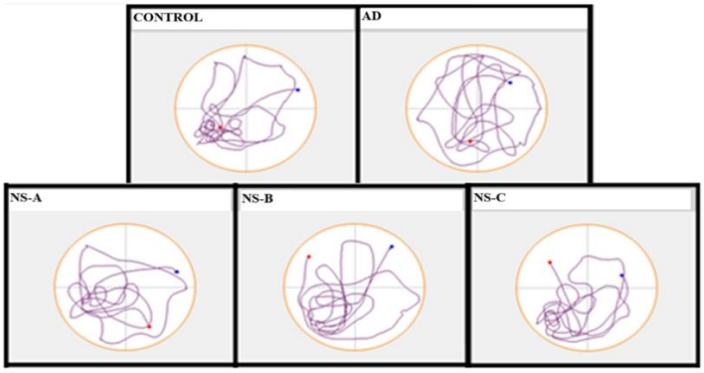

Figure 4. Swimming traces of rats during the probe test in the MWM.

AD: Alzheimer's Disease model, NS-A: 0.5 ml/kg, NS-B: 1 ml/kg, NS-C: 2 ml/kg Nigella sativa oil treatment groups.

Hippocampal TNF-α levels were significantly higher in the AD group (191.3±22) than in the Control group (51.5±4.8, p<0.001). However, TNF-α levels were significantly reduced in the NS-B (91.9±16.1) and NS-C (76.3±11.7) groups compared to the AD group (p<0.01, p<0.001, respectively) (Fig. 5A).

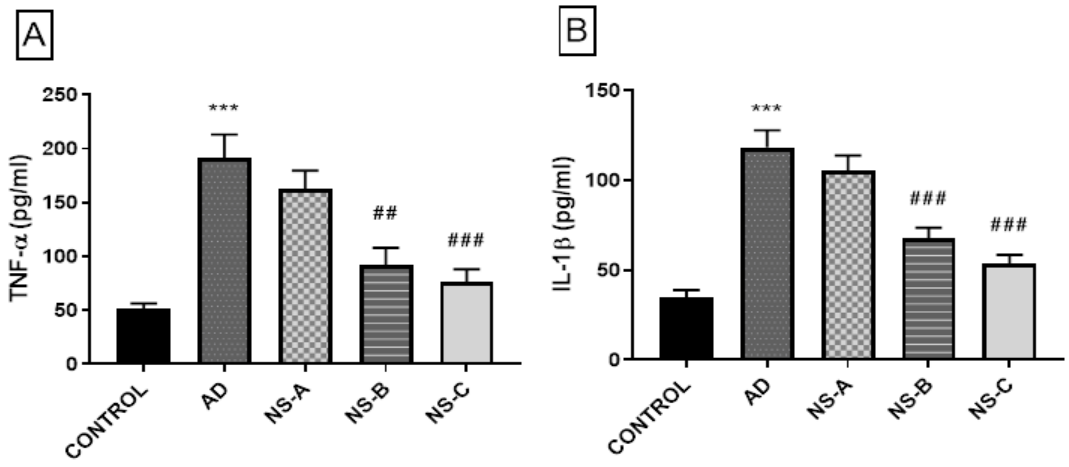

Figure 5. Hippocampal TNF-α levels (A). Hippocampal IL-1β levels (B).

AD: Alzheimer's Disease model, NS-A: 0.5 ml/kg, NS-B: 1 ml/kg, NS-C: 2 ml/kg Nigella sativa oil treatment groups. (*p<0.05, **p<0.01, ***p<0.001 indicate comparison to control group, #p<0.05, ##p<0.01, ###p<0.001 indicate comparison to AD group.)

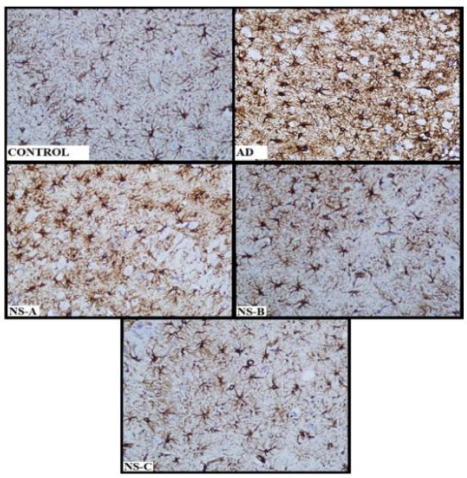

Figure 6. Immunohistochemical GFAP staining (40x magnification).

(AD: Alzheimer's Disease model, NS-A: 0.5 ml/kg, NS-B: 1 ml/kg, NS-C: 2 ml/kg Nigella sativa oil treatment groups.)

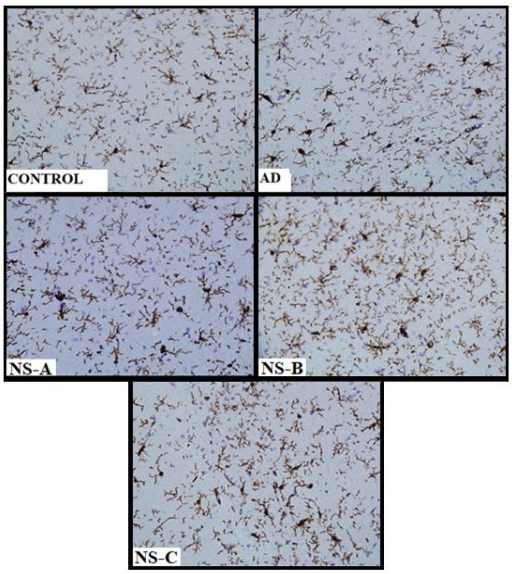

Figure 7. Immunohistochemical Iba1 staining (40x magnification).

(AD: Alzheimer's Disease model, NS-A: 0.5 ml/kg, NS-B: 1 ml/kg, NS-C: 2 ml/kg Nigella sativa oil treatment groups.)

Similarly, IL-1β levels were significantly elevated in the AD group (118.3±9.6) compared to the Control group (34.6±4.3, $p<0.001$). NS-B (67.9±5.8) and NS-C (54±4.6) groups had considerably lower IL-1β levels than the AD group ($p<0.001$) (Fig. 5B).

The AD group showed a significantly higher GFAP staining score compared to the Control group ($p<0.001$). The NS-B ($p<0.01$) and NS-C ($p<0.01$) treatment groups exhibited significantly lower GFAP staining scores than the AD group (Fig. 6).

Similarly, Iba1 immunohistochemical staining was significantly increased in the AD group compared to the Control group ($p<0.01$). The NS-B and NS-C groups showed significantly reduced Iba1 staining compared to the AD group ($p<0.05$ for both) (Fig. 7)

DISCUSSION

In our study, we investigated the effects of Nigella sativa oil on cognitive dysfunction and neuroinflammation in a rat model of Alzheimer's disease induced by Aβ1-42 injection into the hippocampus. NS oil was administered at three different doses (0.5, 1.0, and 2.0 ml/kg), and cognitive function was evaluated using behavioural tests, while neuroinflammatory responses were assessed via histological analysis. Our results demonstrate that NS oil significantly ameliorated cognitive impairment, as measured by the MWM and Passive Avoidance tests, particularly at doses of 1 and 2 ml/kg. These findings suggest that *Nigella sativa* oil may have therapeutic potential in reducing Aβ-induced neuroinflammation and cognitive impairment associated with Alzheimer's disease.

Among the various Aβ forms, Aβ1-42 is recognised as the most toxic and predominant form found in amyloid plaques[19]. In AD, hippocampal neurons and the cerebral cortex are the most affected brain regions [20]. In our study, we used Aβ1-42 injection into the hippocampus to model amyloid plaque formation and mimic AD pathology in rats. This model is widely used because it replicates key AD features, including neuroinflammation and cognitive impairment [16,17,21,22].

Neuroinflammation plays a central role in AD pathology by promoting Aβ deposition, neuronal loss, and cognitive impairment. Aggregation of Aβ peptides triggers microglial activation, leading to the release of pro-inflammatory cytokines such as IL-1, IL-6, IFN-γ, and TNF-α, as well as free radicals, which disrupt synaptic function and damage neurons[23,24].

While microglial activation initially aids in Aβ clearance, chronic inflammation exacerbates AD pathology by creating a vicious cycle of Aβ accumulation and neuronal injury[4,25,26]. Breaking this cycle has been suggested as a potential strategy for slowing or reversing AD progression[27]. Several studies indicate that anti-inflammatory compounds can reduce neuroinflammation, mitigate cognitive decline, and improve AD symptoms[16,21,27,28].

To investigate the neuroinflammatory response, we conducted immunohistochemical analyses of hippocampal sections using anti-Iba1 and anti-GFAP antibodies. Iba1 is a marker for activated microglia, while GFAP is used to assess astrogliosis. In our study, the AD group showed significantly increased microglial and astroglial activation compared to the control group. However, administration of NS oil at doses of 1 and 2 ml/kg markedly reduced this activation, indicating its potential anti-inflammatory effect.

Microglial activation in AD is known to contribute to increased secretion of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, thereby amplifying the neuroinflammatory response[29]. Activated microglia, in response to Aβ accumulation, adopt an ameboid morphology and release neurotoxic products that contribute to neuronal damage[30]. In our study, the anti-inflammatory effect of Nigella sativa (NS) oil was reflected by a reduction in microglial activation, as indicated by decreased Iba1 staining intensity. These findings are consistent with studies reporting the neuroprotective effects of other anti-inflammatory compounds, such as curcumin, which has been shown to reduce the microglial inflammatory response and pro-inflammatory cytokine levels[27,31].

Astrocytes, the most abundant glial cells in the central nervous system, play a critical role in maintaining synaptic function and neuronal homeostasis. In AD, reactive astrocytes are often observed surrounding amyloid plaques, exhibiting hypertrophy and increased GFAP [32,33]. While reactive astrocytes may initially have a protective role, prolonged astrogliosis can impair neuronal support and increase oxidative stress, contributing to disease progression[34]. In our study, administration of Nigella sativa (NS) oil led to

a reduction in astroglial activation, as indicated by decreased GFAP staining in hippocampal sections. This attenuation of astrogliosis may contribute to the observed improvements in cognitive performance, supporting previous findings that limiting astrocyte reactivity can enhance cognitive outcomes in AD models[30,35].

The levels of pro-inflammatory cytokines TNF-α and IL-1β in hippocampal tissues were significantly elevated following Aβ injection, confirming the presence of neuroinflammation. NS oil administration reduced these cytokine levels, indicating an anti-inflammatory effect. Similar findings have been reported in studies using other anti-inflammatory agents, such as TGF-β1 and Angelica sinensis, which have been shown to attenuate neuroinflammation in animal models of AD[29,36].

The behavioural test results that we conducted, including the passive avoidance test and MWM, are consistent with earlier studies [37]. The AD group exhibited significantly shorter latency times in the passive avoidance test compared to the control group, indicating impaired learning and memory. In contrast, rats treated with NS oil at doses of 1 and 2 ml/kg showed marked improvements in cognitive performance. Similarly, MWM testing revealed cognitive deficits in the AD group, as evidenced by prolonged escape latencies during training sessions and reduced time spent in the target quadrant during the probe test. Treatment with 1 and 2 ml/kg NS oil ameliorated these impairments, suggesting a protective effect of NS oil against Aβ-induced cognitive dysfunction.

Despite these promising findings, several limitations should be acknowledged. First, although the Aβ1-42-induced rat model replicates key aspects of Alzheimer's disease pathology, it does not fully capture the complexity of human AD, which involves multifactorial etiologies and a slowly progressive course. Second, the study duration was relatively short, and the long-term effects of Nigella sativa oil on disease progression and safety were not assessed. Lastly, while behavioural, biochemical, and histological analyses were performed, the underlying molecular mechanisms responsible for the observed neuroprotective effects remain to be fully elucidated.

In summary, our findings demonstrate that Nigella sativa oil has therapeutic potential to counteract Aβ-induced neuroinflammation and cognitive deficits in an experimental AD model. The improvements observed in learning and memory, along with reduced activation of microglia and astrocytes, indicate that its anti-inflammatory properties may underlie these protective effects. Although thymoquinone is considered the primary active constituent, the complex phytochemical profile of *N. sativa* oil suggests that other bioactive compounds may also contribute. Further studies are warranted to identify active compounds and elucidate their molecular targets in AD pathology. Additionally, exploring the potential of NS oil in clinical settings is essential to determine its feasibility as a treatment option for AD.

Author Contributions: Concept/Design : SAÇÖ, SOA; Data acquisition: SAÇÖ, FU, AK; Data analysis and interpretation: SAÇÖ, FU, AK; Drafting manuscript: SAÇÖ, FU; Critical revision of manuscript: SAÇÖ, FU, AK; Final approval and accountability: SAÇÖ, FU, AK, SOA; Technical or material support: SAÇÖ; Supervision: SAÇÖ, FU; Securing funding (if available): n/a.
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