**BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY** ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 739-746 | DOI: 10.14421/biomedich.2025.142.739-746 ISSN 2540-9328 (online)

# *Nigella Sativa* Oil Protects Against Aluminium Chloride-Induced Cognitive Impairment Via Modulation of Cholinergic Activity, Brain Neurotransmitter, and Oxidative Stress

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Manuscript received: 19 May, 2025. Revision accepted: 18 September, 2025. Published: 01 October, 2025.

**Abstract**

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and memory impairment, with no known cure. This study investigated the potential protective effects of *Nigella sativa* oil (NSO) on aluminium chloride ($AlCl_3$)-induced cognitive impairment in Wistar rats. Twenty-four rats were divided into four groups. Group I received 1 ml/kg of distilled water. Groups II-IV were administered $AlCl_3$ (100 mg/kg). Groups III and IV were co-treated with NSO at 1 ml/kg and 2 ml/kg, respectively. Neurobehavioral assessments (Morris water maze and Y-maze) were performed, followed by biochemical analysis of brain tissues. Aluminium chloride significantly ($p < 0.05$) impaired spatial learning and memory and decreased the percentage of alternation. It also significantly ($p < 0.05$) increased acetylcholinesterase level, glutamate concentration, and malondialdehyde level, and decreased antioxidant markers. Meanwhile, *Nigella sativa* oil (1 ml/kg and 2 ml/kg) significantly ($p < 0.05$) improved learning ability and spatial memory, and increased percentage alternation in the Y-maze test. *Nigella sativa* oil also significantly ($p < 0.05$) decreases acetylcholinesterase, glutamate, and malondialdehyde, and increases antioxidant biomarkers. This study showed that *Nigella sativa* oil can improve cognitive and spatial learning functions via modulation of cholinergic activity, brain neurotransmitters, and oxidative stress.

**Keywords:** Aluminium chloride; Alzheimer's disease; Cognitive impairment; *Nigella sativa* oil; Oxidative stress.

**Abbreviations**: Aβ: Amyloid-β; ACh: Acetylcholine; AChE: Acetylcholinesterase; AD: Alzheimer's disease; $AlCl_3$: Aluminium chloride; ANOVA: Analysis of variance; BBB: Blood-brain barrier; CAT: Catalase; GLU: Glutamate; GSH: Glutathione; MDA: Malondialdehyde; MWM: Morris water maze; NSO: *Nigella sativa* oil; SEM: Standard Error of Mean; SOD: Superoxide dismutase.

## INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative condition marked by a progressive degeneration of the hippocampal and cortical neurons that impairs memory and cognitive function. Alzheimer's disease is a multifactorial disease with no known etiology, and numerous risk factors are linked to its development and progression. The risk of developing AD increases proportionately with age, approximately doubling every 5 years after age 65 (Ojetunde, 2024).

Aluminium (Al) is found in antacids, deodorants, and food additives, which are easily absorbed by the body. Its neurotoxicity in animals has been well demonstrated and linked to the etiology of neurodegenerative diseases like AD (Niu, 2018). It facilitates the formation of amyloid-β (Aβ) protein plaques via the aggregation of tau proteins in the brain (Elreedy et al., 2023). Al has also been linked to aging-related alterations and neurodegeneration. Aluminium chloride ($AlCl_3$) administration mainly accumulates in the hippocampus, which is particularly susceptible to AD and plays a significant role in learning and memory functions (Ojetunde, 2024).

Alzheimer's disease is becoming a global burden due to its high prevalence yet poor treatment (Boland et al., 2018). Oxidative stress and inflammation are the major contributors to this neurodegenerative disease. It is reported that Al toxicity is due to potentiating the activity of $Fe^{2+}$ and $Fe^{3+}$ ions in the Fenton reaction to cause oxidative damage (Ojetunde, 2024).

There is currently no known cure for AD, and drug therapy for the disease is still in its early stages. Drugs approved for the treatment of possible AD help to regulate the symptoms of the disease, but do not reduce or reverse its progression, and are followed by with accompanying adverse side effects (Vaz et al., 2022). At the moment, medications targeting neurotransmitter systems in the brain form the basis of AD therapy (Singh et al., 2024).

Different studies (Ojetunde, 2021; Ojetunde et al., 2021; Tongshuwar et al., 2020) have summarized the use

of medicinal plants and herbs and their phytochemical components for the treatment of different diseases. *Nigella sativa* has garnered special attention in both traditional and modern medicinal research. According to compelling evidence, *Nigella sativa* oil has high antioxidant and anti-inflammatory properties (Sahak et al., 2016). This suggests its use as a potential remedy for cognitive impairments in AD. Therefore, this study investigated the modulatory role of *Nigella sativa* oil on cognitive impairments in aluminium chloride-induced cognitive impairment in Wistar rats.

## MATERIALS AND METHODS

### Drugs and Chemicals

Methylated spirit, aluminum chloride-hydrated ($AlCl_3.6H_2O$) was purchased from Sigma Chemical Co. (St. Louis, USA). The *Nigella sativa* Oil [black seed oil (100% pure natural oil)] was obtained from Masra Warda, Kingdom of Saudi Arabia.

### Animals

Twenty-four (24) apparently healthy Wistar rats weighing 200 ± 20 g were kept at the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria. They were randomized into experimental and control groups and were housed in plastic cages. Standard animal feed made of pellets from growers' mash was provided to the animals. The rats were allowed access to drinking water *ad libitum* throughout the study. They were allowed to acclimatize for 2 weeks before the commencement of induction and treatment. Rats were kept under constant conditions (temperature 25 ± 3 °C and humidity 50%) with 12/12 h light/dark cycles. Ethical approval was obtained from the Ahmadu Bello University ethical committee on animal use and care with approval number ABUCAUC/2023/102.

Animals were divided into four groups (6/group) and treated daily for six weeks as follows:

Group 1: received 1 ml/kg of distilled water orally (normal control).
Group 2: received 100 mg/kg of $AlCl_3$ orally (negative control) (Baburaj et al., 2023)
Group 3: received 100 mg/kg of $AlCl_3$ + *Nigella sativa* oil (1 ml/kg) orally (Imam et al., 2016)
Group 4: received 100 mg/kg of $AlCl_3$ + *Nigella sativa* oil (2 ml/kg) orally

At the end of the six-week administration, 24 hours after the last administration, the following tests were carried out:

### Morris water maze (MWM) test

The spatial learning and memory were assessed by the Morris water maze as described by Mahboubi et al. (2016). The MWM tank is 100 cm in width, and 62.5 cm in height. The MWM tank was filled to a depth of 40 cm with water and maintained at a temperature of 20 ± 1°C. Around the room, multiple visual cues were present and kept constant throughout the experiment. The maze was categorized geographically into four quadrants; north-east (NE), north-west (NW), south-east (SE) and south-west (SW) and starting positions, north (N), south (S), east (E), west (W) were equally spaced around the perimeter of the pool and a hidden circular platform (diameter: 13 cm) was placed at the centre of the NW quadrant, 1 cm below the surface of the water.

During four consecutive daily sessions (each session consisted of four trials), the rats were trained to find the submerged escape platform located in a fixed position. Each trial had a maximum duration of 60 seconds before removing the rats from the MWM. Two hours after the last training trial, the rats were subjected to a memory probe trial during which they were allowed to swim for 60 seconds in the absence of the training platform. All the rats started from the same position, opposite to the target quadrant (the quadrant where the escape platform was positioned).

### Y-maze test

Short-term working memory was assessed as a measure of spontaneous alternations using the Y-maze as described by Zaher et al. (2019). The Y-maze is composed of three equally spaced arms. Each of the rats was placed in one of the arm compartments and allowed to move freely until its tail completely entered another arm. The number of maximum spontaneous alternations is the total number of arms entered minus two, and the percentage alternation is calculated as {(total alternations /spontaneous alternations) x 100}. For each animal, the Y-maze testing was carried out for 5 minutes. The apparatus was cleaned with methylated spirit (10% methanol and 90% ethanol) alcohol and allowed to dry between sessions.

### Brain homogenate preparation

The animals were then sacrificed by decapitation under ketamine and diazepam (75 and 25 mg/kg). The rats' brain tissues were collected and prepared according to the method described by Zatta et al. (2002), and Habila et al. (2012). Treated and control animals were sacrificed, and brain tissue was immediately removed and placed on an inverted Petri dish on ice. The whole brain was harvested, weighed, and homogenized in 3 ml of a medium containing phosphate buffer solution, pH 7.5. The total homogenate was centrifuged at 5000 x g for 5 minutes. The supernatants were used for biochemical parameter assays.

### Biochemical assay

#### *Brain marker of oxidative stress*

The malondialdehyde (MDA) assay was carried out by using Esterbauer and Cheeseman (1990).

***Brain antioxidant markers***

The brain superoxide dismutase (SOD) was determined according to the method described by Fridovich (1989). Catalase (CAT) was measured using the method of Sinha (1972). Glutathione (GSH) was measured using Ellman's method (1959).

**Neurochemical Assessment**

***Measurement of brain acetylcholinesterase (AChE) level***

The concentration of brain tissue homogenate, AChE level was assessed using ELISA Kits (Ray Biotech, Inc., USA) according to the manufacturer's instructions.

***Measurement of brain glutamate (GLU) concentration***

The brain tissue homogenate GLU concentration was assessed using ELISA Kits (Ray Biotech, Inc., USA) according to the manufacturer's instructions.

**Data analysis**

The data obtained were expressed as Mean ± Standard Error of Mean (SEM) and were analyzed using one-way and mixed analysis of variance (ANOVA) with *Tukey's* post hoc test to compare the level of significance between control and experimental groups. SPSS version 20 software was used for the analysis, and values of $p < 0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

Table 1 shows the effect of *Nigella sativa* oil on escape (Es.) latency in $AlCl_3$-induced cognitive impairment in Wistar rats. The escape latency of the Morris water maze indicated an impairment of memory, as the $AlCl_3$ exposed group showed a significant increase ($p < 0.05$) in time (s) to reach the escape latency in all 4 days when compared to the normal control group. *Nigella sativa* oil administration groups (1ml/kg and 2ml/kg) showed a significant decrease ($p < 0.05$) in the time to reach the escape latency of the Morris water maze when compared with the $AlCl_3$ exposed group.

**Table 1.** Effect of *Nigella sativa* oil on escape latency in $AlCl_3$-induced cognitive impairment in rats using MWM.

| Groups | Es. Latency (s) (Day 1) Mean ± SEM | Es. Latency (s) (Day 2) Mean ± SEM | Es. Latency (s) (Day 3) Mean ± SEM | Es. Latency (s) (Day 4) Mean ± SEM |
|---|---|---|---|---|
| **DW (1ml/kg)** | 12.36 ± 0.54 | 5.47 ± 0.59 | 3.74 ± 0.09 | 2.92 ± 0.20 |
| **$AlCl_3$** | 22.73 ± 0.37$^{a}$ | 19.64 ± 1.02$^{a}$ | 13.63 ± 0.95$^{a}$ | 12.45 ± 0.54$^{a}$ |
| **$AlCl_3$ + NSO (1ml/kg)** | 15.97 ± 0.52$^{ab}$ | 9.38 ± 0.82$^{ab}$ | 7.72 ± 0.72$^{ab}$ | 5.68 ± 0.26$^{ab}$ |
| **$AlCl_3$ + NSO (2ml/kg)** | 14.92 ± 0.24$^{ab}$ | 7.61 ± 0.15$^{b}$ | 5.62 ± 0.16$^{b}$ | 3.41 ± 0.09$^{bc}$ |

Values along the same column with superscripts $^{a, b,}$ and $^{c}$ are significantly different ($p < 0.05$) when compared to the normal control, $AlCl_3$, and $AlCl_3$+NSO (1ml/kg) group, respectively. $AlCl_3$: Aluminium chloride, DW: Distilled water, NSO: *Nigella sativa* oil

Figure 1 shows the effect of *Nigella sativa* oil on the Memory Probe Trial of $AlCl_3$-induced cognitive impairment in rats. The Memory probe trial of the Morris water maze indicated an impairment of memory, as the $AlCl_3$-exposed group showed a significant decrease in time (s) on the Memory probe trial when compared to the normal control group. However, *Nigella sativa* oil administration groups (1ml/kg and 2ml/kg) showed a significant increase ($p < 0.05$) in the time of memory probe trial of the Morris water maze when compared with the $AlCl_3$ exposed group.

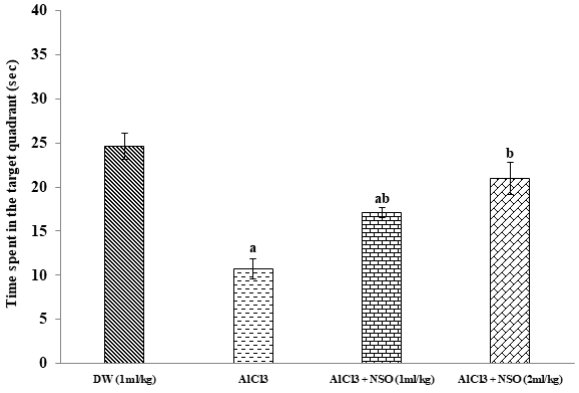


**Figure 1.** Memory probe trial. Values with superscripts $^{a}$ and $^{b}$ are significantly different ($p < 0.05$) when compared to normal control, and $AlCl_3$ group, respectively. $AlCl_3$: Aluminium chloride, DW: Distilled water, NSO: *Nigella sativa* oil.

The result of the mean values of percentage alternation was estimated as shown in Figure 2. The $AlCl_3$-treated group percentage alternation decreased significantly ($p < 0.05$) when compared to the normal group. Groups co-treated with *Nigella sativa* oil (1ml/kg and 2ml/kg) had a significantly ($p < 0.05$) higher percentage of alternation when compared with the $AlCl_3$-treated group.

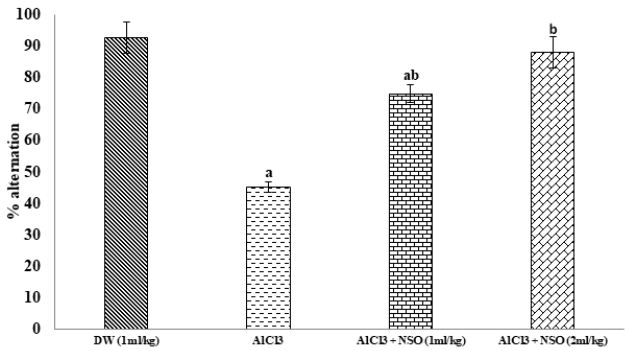


**Figure 2.** Effect of *Nigella sativa* oil on spontaneous alternation in rats with $AlCl_3$-induced cognitive impairment. Values with superscripts [a] and [b] are significantly different ($p < 0.05$) when compared to normal control, and $AlCl_3$ group, respectively. $AlCl_3$: Aluminium chloride, DW: Distilled water, NSO: *Nigella sativa* oil.

The acetylcholinesterase level in the $AlCl_3$-treated group was significantly ($p < 0.05$) increased when compared with the normal control group, as shown in Figure 3. However, treatment with *Nigella sativa* oil (1ml/kg and 2ml/kg) showed a significantly ($p < 0.05$) decreased acetylcholinesterase level.

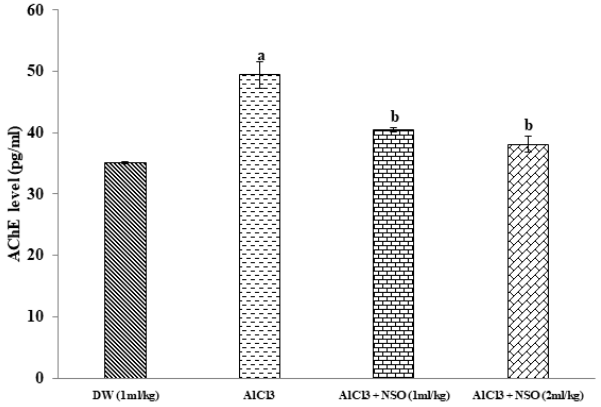


**Figure 3.** Effect of *Nigella sativa* oil on brain homogenate level of acetylcholinesterase in $AlCl_3$-induced cognitive impairment in rats. Values with superscripts [a] and [b] are significantly different ($p < 0.05$) when compared to normal control, and $AlCl_3$ group respectively. $AlCl_3$: Aluminium chloride, DW: Distilled water, NSO: *Nigella sativa* oil.

The results of the mean values of glutamate are shown in Figure 4. The $AlCl_3$-treated group glutamate level significantly ($p < 0.05$) increased when compared with the normal control group. Groups treated with *Nigella sativa* oil (1ml/kg and 2ml/kg) had a significant ($p < 0.05$) decrease in the level of glutamate compared with the $AlCl_3$-treated group.

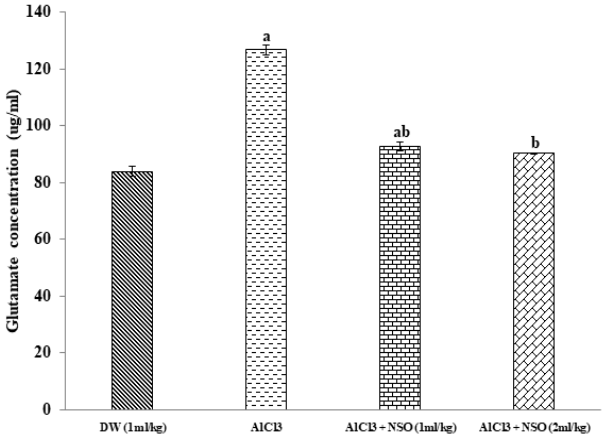


**Figure 4.** Effect of *Nigella sativa* oil on brain homogenate level of glutamate in $AlCl_3$-induced cognitive impairment in rats. Values with superscripts [a] and [b] are significantly different ($p < 0.05$) when compared to normal control, and $AlCl_3$ group respectively. $AlCl_3$: Aluminium chloride, DW: Distilled water, NSO: *Nigella sativa* oil.

As shown in table 2, there was a significant decrease ($p < 0.05$) in the mean values of all the anti-oxidant biomarkers SOD, CAT, and GSH in the $AlCl_3$ treated group compared with the normal control group, while there was a significant increase ($p < 0.05$) in the MDA of $AlCl_3$-treated group when compared with the normal control group. A significant increase ($p < 0.05$) was observed in the anti-oxidant biomarkers SOD, CAT, and GSH of groups treated with *Nigella sativa* oil (1ml/kg and 2ml/kg) when compared with the $AlCl_3$-treated group, while for the MDA a significant decrease ($p < 0.05$) was observed in the groups treated with *Nigella sativa* oil (1 ml/kg and 2 ml/kg) when compared with the $AlCl_3$ treated group. However, the increase in GSH level of the group receiving 1 ml/kg of *Nigella sativa* oil was not statistically significant when compared to the $AlCl_3$-treated group.

**Table 2.** Effect of *Nigella sativa* oil on oxidative stress and antioxidant parameters in $AlCl_3$-induced cognitive impairment in Wistar rats.

| Groups | MDA (nmol/mg protein) Mean ± SEM | SOD (U/mg protein) Mean ± SEM | CAT (U/mg protein) Mean ± SEM | GSH (ug/mg protein) Mean ± SEM |
|---|---|---|---|---|
| **DW (1ml/kg)** | 40.40 ± 2.31 | 44.17 ± 0.94 | 28.07 ± 1.30 | 25.57 ± 0.57 |
| **$AlCl_3$** | 56.87 ± 1.62$^{a}$ | 23.00 ± 1.65$^{a}$ | 9.2 ± 0.40$^{a}$ | 19.13 ± 1.41$^{a}$ |
| **$AlCl_3$ + NSO (1ml/kg)** | 47.87 ± 1.27$^{b}$ | 31.23 ± 1.36$^{ab}$ | 19.23 ± 0.88$^{ab}$ | 21.50 ± 1.03 |
| **$AlCl_3$ + NSO (2ml/kg)** | 42.13 ± 1.73$^{b}$ | 39.50 ± 2.25$^{bc}$ | 26.97 ±1.47$^{bc}$ | 27.60 ± 1.45$^{bc}$ |

Values along the same column with superscripts $^{a, b,}$ and $^{c}$ are significantly different ($p < 0.05$) when compared to normal control, $AlCl_3$, and $AlCl_3$+NSO (1 ml/kg) groups, respectively. $AlCl_3$**:** Aluminium chloride, DW: Distilled water, NSO: *Nigella sativa* oil

## Discussion

The present study results revealed that the administration of $AlCl_3$ impairs spatial learning and memory as assessed via the Morris Water Maze task, as it took a significant time to escape. Aluminium can cross the blood-brain barrier (BBB) and accumulate in various regions of the brain tissues, which promotes the impairment of learning and memory (Hamdan et al., 2022). Aluminium can also interfere with the downstream effector molecules (cyclic GMP) necessary for long-term potentiation. This interruption could explain the observed memory loss and neurobehavioral changes (Colizzi, 2018).

Rats treated with *Nigella sativa* oil at all dosages used showed improved learning ability and spatial memory with a significant decrease in time to escape latency during assessment and an increase in the time spent in the target quadrant in the probe task in the Morris Water Maze task. In models of neurotoxicity and neurodegenerative disorders, *Nigella sativa* has been linked to be effective in enhancing neurocognitive and psycho-cognitive functions (Norouzi et al., 2019). Thymoquinone, present in *Nigella sativa* oil, is known to improve learning, memory, and cognitive functions and may be responsible for the cognitive modulation of *Nigella sativa* oil (Bargi et al., 2017). Thymoquinone has also been reported to improve memory by increasing acetylcholine immunoreactivity and decreasing AChE activity (Abulfadl et al., 2018).

The current study revealed that the rats treated with only $AlCl_3$ exhibited a significant decrease in spontaneous alternation percentage when compared to control rats in the Y-maze test. This diminished percentage of alternation as observed in the present study indicated an impaired spatial working memory (Rout et al., 2012), which is mostly attributed to the neurological cell damage and synaptic dysfunction encountered in rats' brains with AD (Mohamed et al., 2020). However, co-administration of *Nigella sativa* oil improves the rats' cognitive ability by significantly modulating these memory deficits via a significant increase in percentage alternation. This report supports the findings of several studies (Imam et al., 2021), which reported that *Nigella sativa* oil has the potential to protect against or improve spatial working memory deficits. This effect is supported by the findings of Khan et al. 2014, where thymoquinone (the active component of *Nigella sativa* oil) increased percentage alternation in an animal model of neurological disorder. It has also been shown that thymol present in *Nigella sativa* oil enhances cognitive functions in a model of dementia (Asadbegi et al., 2017).

Acetylcholinesterase activity is a well-known indicator of damage to cholinergic neurons in the brain. It is the primary enzyme inactivating acetylcholine in the synaptic cleft (Anwar et al., 2021). Dementia and the severity of neuropathological alterations associated with AD are strongly linked to the disruption of cholinergic neurotransmission in the cortex and hippocampus (Lao et al., 2019). In this study, the administration of $AlCl_3$ to the experimental rats showed a significant increase in the level of AChE, which is in agreement with the work done by Ekundayo et al. (2022) and Hejaziyan et al. (2023). Aluminium ion interacts with the peripheral site of AChE, which modifies its secondary structure and eventually increases its activity (Auti & Kulkarni, 2019). The therapeutic administration of *Nigella sativa* oil to $AlCl_3$-induced cognitively impaired rats showed the possible modulatory role of *Nigella sativa* oil by reducing AChE levels. A study with *Nigella sativa* demonstrated almost identical levels of AChE activity compared to donepezil (Sudha et al., 2021).

Thymoquinone, the active ingredient of *Nigella sativa* oil, was reported to possess anti-cholinesterase activity (Jukic et al., 2007). The principal constituents of its essential oil, thymol and carvacrol, and their derivatives (e.g., thymohydroquinone) had inhibitory effects on AChE (Jukic et al., 2007). So, these compounds can be identified as prospective therapeutic agents for the treatment of AD and/or cognitive disorders. Cholinergic depletion has been noted to increase Aβ deposition (Ramos-Rodriguez et al., 2013), tau phosphorylation, & pro-inflammatory cytokines formation (Field et al., 2012). Therefore, the pathophysiological characteristics and the clinical presentation of AD may be improved by restoring cholinergic functions (Zaher et al., 2019).

The main post-excitatory neurotransmitter, glutamate, is involved in almost all central nervous system functions, particularly in the hippocampal region and cortical area of the brain (Kim et al., 2011). Glutamate is neurotoxic when present in excessive amounts and increases neuronal excitability by activating proteolytic enzymes (Weil et al., 2008). In this study, there was a significant elevation of this glutamate neurotransmitter in

the $AlCl_3$-treated group. This elevation of glutamate concentrations has been linked to increased sensitivity and/or activity of the glutamatergic system, leading to neuronal dysfunction and cell death in AD (Gasparini & Dityatev, 2008). Aluminium activates glutamate-mediated excitotoxicity, which results in severe neuronal damage and loss (Baburaj et al., 2023). Due to overstimulation of N-methyl-D-aspartate receptors, glutamate hinders learning and memory by causing cognitive decline and neuronal degeneration (Alghamdi, 2018).

In contrast, the result of the present study indicates that *Nigella sativa* oil modulates $AlCl_3$-induced cognitive impairment, by inhibiting the excessive elevation of glutamate, as shown in *Nigella sativa* oil-treated groups, thereby decreasing the level of $Ca^{2+}$ influxes to hippocampal neurons by blocking the L-type calcium channel, which may reduce excitotoxicity and neuronal death. In another study, *Nigella sativa* diminished glutamate secretion, leading to decreased neuronal excitatory activity (El-Naggar et al., 2010). Also, *Nigella sativa* oil pre-treatment lowered glutamate levels in mice models of essential tremor (Folarin et al., 2020). The possible cause of the improving effects of *Nigella sativa* oil on the level of glutamate in treated rats is the antioxidant activities of its components.

In previous research, aluminium neurotoxicity effects produced an imbalance between the generation of reactive oxygen species and antioxidants, leading to oxidative stress in neurons (Abbas et al., 2022). The administration of $AlCl_3$ in the present study resulted in marked oxidative stress in the brain tissues as indicated by reduced levels of SOD, CAT, and GSH, then significantly increased levels of MDA compared to the control group. However, treatment with *Nigella sativa* oil showed significant SOD, CAT, and GSH increase and significant MDA decrease compared to the $AlCl_3$-treated group supporting the antioxidant effect of *Nigella sativa* oil which may be linked to the presence of the phytochemicals present in *Nigella sativa* oil.

*Nigella sativa* oil contains quinine, carvacrol, and 4-terpineol, which are effective in connecting free radicals (Umar et al., 2012). It also contains aglycones and flavonol glycosides, which have higher anti-oxidant and anti-radical effects, so they function as a revealer of superoxide radicals in the blood to eliminate free radicals and inhibit the oxidation process in cells (Kooti et al., 2016).

It has also been demonstrated that thymoquinone (obtained from *Nigella sativa* seed oil) can cross the BBB and scavenge free radicals generated by various pro-oxidant stimuli, including heavy metals, thereby preventing neurodegeneration (Hosseinzadeh et al., 2012; Elmaci & Altinoz, 2016). Thymoquinone was previously found to increase antioxidant enzyme activities in a rat model of chlorpromazine toxicity (Safhi, 2016). As a result, the antioxidant property of thymoquinone found in *Nigella sativa* seed oil could be considered as one protective mechanism against learning and memory impairment in AD (Lotfi et al., 2022).

## CONCLUSIONS

It is concluded that *Nigella sativa* oil significantly protected neurobehavioral alterations and improved cognitive, and spatial learning functions in rats. *Nigella sativa* oil can prevent oxidative damage by increasing neuronal antioxidant enzyme activities (SOD, CAT & GSH) and decreasing biomarker of lipid peroxidation (MDA). Moreover, *Nigella sativa* oil can modulate the cholinergic function (AChE), and abnormal levels of neurotransmitters (Glutamate).

***Authors' Contributions:*** AOO, AA, IS, and ASI designed the study. AOO carried out the laboratory work. AOO, AA, IS, and ASI analyzed the data. AOO wrote the manuscript. All authors read and approved the final version of the manuscript.

***Competing Interests:*** The authors declare that there are no competing interests.

***Funding:*** The author declares no funding.

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