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Research article

Nigella sativa extract abrogates traumatic brain injury-induced memory impairment in adult mice

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ABSTRACT

Background: Traumatic brain injury (TBI) is an increasing widespread cause of disability and mortality, typically leading to dementia and memory impairment.
Objective: This study aims to investigate the neuroprotective potential of *Nigella sativa* extract against TBI induced memory impairment in adult albino mice.
Methods: Adult male mice were divided into four groups randomly: Control, *Nigella sativa* extract alone, TBI alone and TBI plus *Nigella sativa* extract. TBI induction was carried out in mice using a weight dropping method then *Nigella sativa* extract (10 mg/kg) was administered intraperitoneally for two weeks. Morris water maze and Y-maze tests were used to measure memory improvement ability and Western blot technique was used to analyse the neuroinflammatory and synaptic protein markers.
Results: Nigella sativa extract significantly decreased phosphorylated c-Jun N-terminal kinase (p-JNK), Tumor necrosis factor-alpha (TNF-α), and nuclear factor kappa B (NF-κB) proteins to reduce TBI-induced neuroinflammation accompanied by the restoration of both pre- and post-synaptic protein expression in adult mice model. Furthermore, *Nigella sativa* extract enhanced both short and long-term spatial memory against TBI in adult mice model.
Conclusion: Nigella sativa extract abrogated neuroinflammation mediated memory impairment in TBI mice model. Further research is needed to determine *Nigella sativa* extract ingredients detail completely and to understand its mechanisms of neuroprotection in reducing memory impairments associated with traumatic brain injury and other neurodegenerative diseases.

1. Introduction

The neurons cells in central nervous system (CNS) undergoes progressive fatal injuries due to certain neurological disorders like Alzheimer disease (AD), Parkinson disease (PD) and traumatic brain injury (TBI) [1,2]. The quality of life of these neurological diseases

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affected people suffers and produces a wide range of psychological and motor disorders [1]. As a result of TBI the key symptom is neuritis which is the main point for the generation of pathophysiology of neurodegenerative diseases [3]. Moreover, the pathophysiology of the neuronal degenerative disease is exacerbates by oxidative stress and mitochondrial dysfunction [4].

The traumatic brain injury is also known as "silent epidemic" and is a significant public health issue worldwide affecting millions of people per annum [5]. Due to severe head trauma or some external forces may lead to traumatic brain injuries (TBIs) which results in multiple neurological abnormalities, such as motor dysfunction and cognitive impairments [5]. The key components of TBI pathophysiology are oxidative stress, neuritis and excitotoxicity which lead to synaptic malfunctioning and loss of neurons [6]. TBI is considered as a most common cause of mortality and disability within all trauma associated injuries worldwide [7], and is also recognised as a persistent public health issue [8,9]. TBI survivors are more likely to experience neurodegenerative diseases in their later years, highlighting the interaction between immediate brain damage and long-term neurological disorders [10]. Effective methods of therapy are still desperately required for reducing the long-term effects of traumatic brain injuries, despite the context of medical care improvements [11].

Nigella sativa belongs to the Ranunculaceae family of medicinal plants. The common name of *Nigella sativa* is black seed or black cumin, has a rich religious as well as historical background and is known as a miracle herb due to its wide range of pharmacological potential, which is being revealed by multiple studies [12–17]. Although *Nigella sativa* is native to North Africa, Southwest Asia, and Southern Europe, but it is also grown around the world, including Pakistan, Iran, Syria, Turkey, Saudi Arabia, and the Middle East Mediterranean region [12]. A wide range of illnesses, including rheumatism, diarrhea, asthma, bronchitis, and skin conditions, are treated with *Nigella sativa* seeds [18]. In addition, it is used as liver tonic, digestive assistance, antidiarrheal, appetite booster, and to strengthen the immune system and foster the production of milk in nursing mothers so as to prevent parasite infections [19]. These black seeds have gain importance due to presence of large number of bioactive components in *Nigella sativa*. Thymoquinone is its one of the most important compound having antimicrobial, anti-inflammatory, and antioxidant properties presenting that *Nigella sativa* seeds may be promoted as a therapeutic component of the herb to treat TBIs and other neurological diseases [20–22]. Thymoquinone has demonstrated potent antioxidant as well as anti-inflammatory qualities, which may reduce neurodegenerative processes and neuropathology linked with traumatic brain injury [23].

This study evaluated the neuroprotective benefits of *Nigella sativa* extract against TBI-induced neuroinflammation mediated memory impairment by using Western blotting technique and behavioural tests in adult mice.

2. Materials and methods

2.1. Chemicals

Glycine, Ethanol, HCl, Tris-base, Skim milk powder, Acrylamide, Bisacrylamide, Sodium dodecyl sulfate (SDS), Tween 20, Ammonium Per sulfate (APS), Tetramethyl ethylene diamine (TEMED) were purchased from Sigma Aldrich, USA. All the chemicals commercially purchased were of purely analytical grade.

2.2. Plant Collection and extraction

Nigella sativa high-grade seeds were purchased from the local market. The seeds were washed with normal tap water and dried under shadow in lab. The dry seeds were pounded into a powder by using a mortar and pestle and then the undesired particles were sieved out. The extracts of *Nigella sativa* were obtained by using solvents, such as methanol and chloroform separately [24,25]. The extraction was carried out by the use of apparatus which was filled with *Nigella sativa* powdered seeds, and later on the solvent was run through it for the necessary number of cycles needed for extraction [26]. The resultant extract was further processed into filtration and evaporation to produce a pure powder and made a solution for administering to adult mice.

2.3. Experimental protocols

The male adult albino (BALB/c) mice weighted (28–30 g) were utilized in the experiments. These mice were provided by the Veterinary Research Institute (VRI) located in Peshawar. They were kept in separate cages at the Neuro Molecular Medicines Research Centre (NMMRC, Peshawar). Prior to the study, the mice were allowed to acclimate for one week in a temperature-controlled setting including a regular cycle of light and dark. They had unrestricted availability of water and food. Ethical approval of the experimental protocol has been taken from the NMMRC (Ref. No. NMMRC/11/2023) was submitted to the departmental ethics committee of Qurtuba university of Science and information Technology, D.I. Khan/Peshawar. Mice were randomly divided into four ($n = 6$) groups, as below.

1. Control Mice (treated with normal saline)
2. TBI-Induced Mice
3. TBI-Induced Mice + *Nigella sativa* Extract-treated Mice (10 mg/kg)
4. *Nigella sativa* Extract-treated Mice (10 mg/kg)

2.4. Induction of TBI

Traumatic brain injury (TBI) was induced in mice using a weight dropping method as previously described [27,28]. The animals were anesthetized with ketamine and positioned under a weight-drop platform. The mice were subsequently permitted to recover from anaesthesia under sunlight. An incision was made on the top of the skull after the hair was removed to expose the cranium. A weight of around 50 g was dropped onto the exposed skull from a height of 10 cm to induce brain injury. This technique was performed two times in order to ensure uniformity in the injury. Then the scalp was closed by applying sutures and later on the mice were exposed to sunlight for recovery from anaesthesia. All these mice were handled with great care.

2.5. Drug treatment

Mice in the control group were treated with 0.9 % saline intraperitoneally (i.p.), while *Nigella sativa* extract administered for two weeks (10 mg/kg) intraperitoneally with great care.

2.6. Behavioral tests

Behavioural tests were performed in order to examine the neuroprotective effect of *Nigella sativa* extract on the memory impairment and learning deficits induced by TBI in mice.

2.7. Y- maze test

Y-maze spontaneous alternation is also a behavioural test used to measure the mice willingness to explore new environment. This test was performed for short to long term spatial memory function of mice. Y- Maze apparatus was built from wood having three arms fixed at an angle of 120°. Each arm of the Y-maze was 20 cm high, 50 cm long and 10 cm wide. The mice were first trained for two days for 10 min so that they can get used to the new environment. Then the mice were allowed for 4 8 min sessions to explore the new environment by keeping each mouse at the centre of the Y-maze assembly and allowed to freely move. The number of arm entries of the mice were observed visually and noted. Spontaneous alternation is the successive entries of the mice into the 3 arms in overlapping sets of triplet. The alternation behaviour was calculated from the following formula as reported earlier [29];

$$\%\ Spontaneous\ Alternation = \frac{\text{Succesive Triplet Sets}}{Total\ Number\ of\ Arm\ Entries\ -2} \times 100$$

2.8. Morris Water Maze test

The Morris water maze (MWM) test was executed to examine the spatial learning of experimental models. For this test the apparatus consists of a circular water tank of about 100 cm in diameter and about 40 cm height. The MWM mechanical assembly was filled with water (26 cm deepness, 23 ± 1 °C). Firstly the mice were practiced twice a day for three consecutive days and then to discover the hidden platform (placed in one quadrant 1 cm below the water surface) the escape latency of the animals were noted for 1 min. If the mice failed to find the platform the animals were manually guided to the platform and leave there for 10 s. This training was continued for five days, and each group has its own data recorded in seconds to search the platform. After giving rest to the mice for two days, the mice were allowed for performing the probe test (when platform is absent) in which mice have to find the submerged platform and the time was noted they spent in the target quadrant [29].

2.9. Western blotting analysis

After the therapy, all of the animals were killed [29]. The brain tissue from the hippocampus was carefully taken from the beheaded mice and immediately transferred to the RNA later solution and PBS (1:1) on ice. T-PER solution (Thermo Scientific) was used to homogenize the hippo campus brain tissue after which the supernatant was collected and stored at −20 °C for future investigation. The protein concentration was determined using the Bio-Rad protein estimation assay, and the absorbance measurement was made at 595 nm. All protein samples were standardized to 30g per group and separated using SDS-PAGE on gels with a 12–15 % gel concentration. For the first 20–30min of the run, running conditions were maintained at 50 mA, and then for the last hour to an hour and a half, running conditions were changed to 120V. Proteins were moved from the gel to a PVDF membrane (Santa Cruz Biotechnology, USA) using the semi-dry transblot technique (Bio Rad). Anti-SYP, anti-PSD95, anti-p-JNK, anti-actin, anti-TNF-α, and anti-NF-kB monoclonal antibodies (Santa Cruz, CA, USA) were used as primary antibodies, followed by anti-mice HRP conjugated (Santa Cruz, CA, USA) secondary antibodies. For the development of the results, X-ray films were employed [29].

2.10. Statistical analysis

After that, all of the X-ray films were scanned. The blots were cropped and the behaviour results were compiled and then statistical analyses were done by using computer-based software. Among the programs Adobe Photoshop, Image J and Prism 5 Graph Pad are included. To represent the data, mean values ± standard error of the mean (SEM) were computed. The one-way analysis of variance

(ANOVA) and student t-test were used in the statistical analysis, which was carried out using the Graphpad Prism 5 program (San Diego, CA). Significance was considered as $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

3. Results

3.1. *Nigella sativa* extract inhibited phosphorylated-JNK to reduce TBI induced neuroinflammation in mice brain

TBI caused the activation of phosphorylated-JNK proteins to induce neuroinflammation in mice brain. Additionally, TBI also caused the activation of NF-κB and TNF-α protein respectively. On the other hand treatment with *Nigella sativa* extract significantly inhibited TBI-induced activation of phosphorylated JNK (p-JNK) protein as shown in Fig. 1a and b. Similarly, *Nigella saliva*'s extract inhibited crucial modulators of inflammatory responses such as NF-κB and TNF-α protein in the brain induced by TBI. *Nigella sativa* extract administration significantly reduced NF-κB and TNF-α activity, indicating a strong anti-inflammatory effect as depicted in Fig. 1A–D.

3.2. *Nigella sativa* extract improved pre- and post-synapse against TBI in mice brain

The administration of *Nigella sativa* extract exhibited significant neuroprotective effects against TBI induced synaptic dysfunction and memory impairment in experimental animals. By using Western blotting analysis, changes in the expression of important synaptic

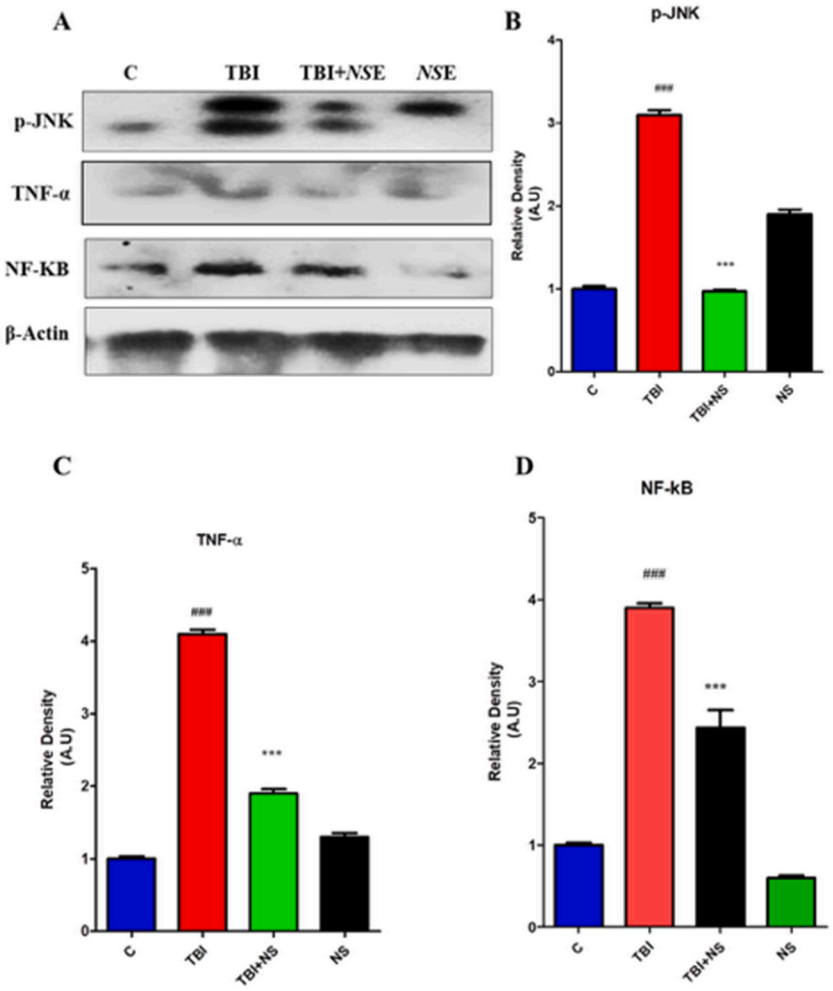

Fig. 1. *Nigella sativa* inhibited p-JNK to reduce neuroinflammation against TBI in mice. **(A)** Shown are the immunoblot of p-JNK, NF-κB, TNF-α, and β-actin of all experimental mice including brain homogenates including control, TBI either alone or treated with *Nigella sativa* extract and alone *Nigella sativa* extract treated respectively. **(B–D)** The histograms of respective relative densities of p-JNK, NF-κB and TNF-α are shown. Image J software was used to know the densities and to make graphs. The results were expressed in arbitrary unit (A.U) and were determined using Graphpad Prism 5 program and histogram indicates mean in A.U ± SEM. Significance of control vs TBI is expressed as #, while * denotes TBI vs TBI + *Nigella sativa* extract. $^{**,\#\#}p \leq 0.01$ and $^{***,\ \#\#\#}p \leq 0.001$.

proteins, such as synaptophysin, a marker of presynaptic terminals and, PSD95, a postsynaptic density protein, were evaluated. TBI led to a significant dysregulation of both pre- and post-synaptic proteins, indicating synaptic damage. Nevertheless, treatment with *Nigella sativa* extract remarkably restored the expression levels of both SYP and PSD95, nearly reinstating synaptic integrity to baseline levels. These results highlight the potential of *Nigella sativa* extract in mitigating synaptic dysfunction associated with TBI in adult mice brain as given in Fig. 2A–C.

3.3. *Nigella sativa* extract improved memory dysfunction against TBI in mice

Memory impairment was evaluated after TBI using the Morris water maze (MWM) paradigm. Compared to control animals, mice that had suffered TBI showed longer mean escape latencies from day 1 to day 5 respectively, which is a sign of poor spatial learning and memory. Remarkably, *Nigella sativa* extract treatment significantly lowered the mean escape latencies, starting from day 1 to day 5 indicating a potential restoration of spatial memory function. This enhancement demonstrates *Nigella sativa* extracts therapeutic effectiveness in reducing TBI-induced memory impairment as shown in Fig. 3A.

Similarly, in the probe trial (where the submerged platform was removed) TBI mice again showed signs of impaired spatial

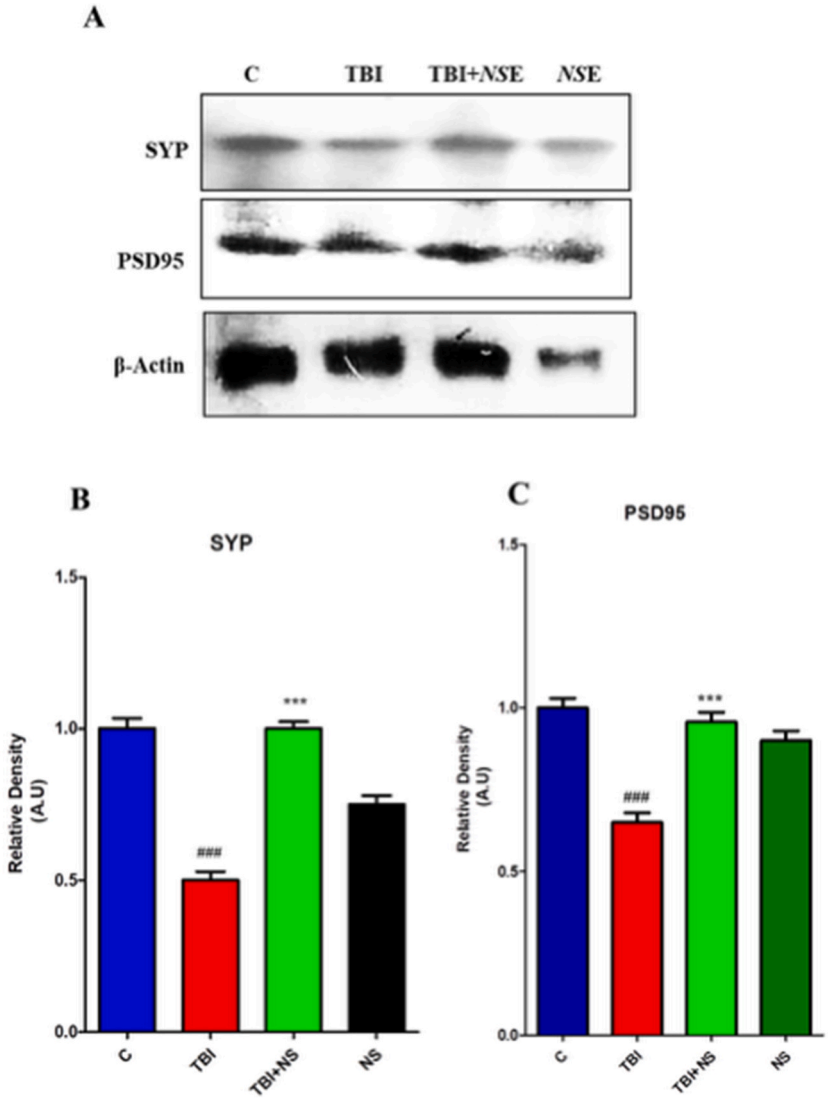

Fig. 2. *Nigella sativa* extract enhanced both pre- and post-synapse proteins expression against TBI in mice brain **(A)** Shown are the Western blot results of both pre- and post-synapse proteins expression of Synaptophysin and PSD95 in experimental mice brain homogenates including control, TBI either alone or treated with *Nigella sativa* extract and alone *Nigella sativa* extract treated respectively. **(B, C)** The histograms of respective relative densities of both SYP and PSD95 are shown. Image J software was used to know the densities and to make graphs. The results were expressed in arbitrary unit (A.U) and were determined using Graphpad Prism 5 program and histogram indicates mean in A.U ± SEM. Significance of control vs TBI is expressed as #, while * denotes TBI vs TBI + *Nigella sativa* extract. $^{**,\#\#}p \leq 0.01$ and $^{***,\ \#\#\#}p \leq 0.001$.

memory, such as disoriented exploration and spending more time in non-target quadrants. On the other hand, *Nigella sativa* extract treated mice showed increased spatial memory retention as seen by their concentrated exploration and longer stays in the target quadrant. These findings highlight the advantages of *Nigella sativa* extract in helping to maintain spatial memory function after TBI as shown in Fig. 3B.

Using the Y-maze for evaluating short-term memory, TBI mice's working memory function appeared to be impaired, as evidenced by a reduced percentage of spontaneous changes. On the contrary, compared to TBI animals, *Nigella sativa* extract supplementation raised the percentage of spontaneous modifications, suggesting that working memory had improved as given in Fig. 3C. *Nigella sativa* extract has shown neuroprotective effects against TBI. This action has been depicted in Fig. 3D as the proposed mechanism of *Nigella sativa* extract in TBI mice model.

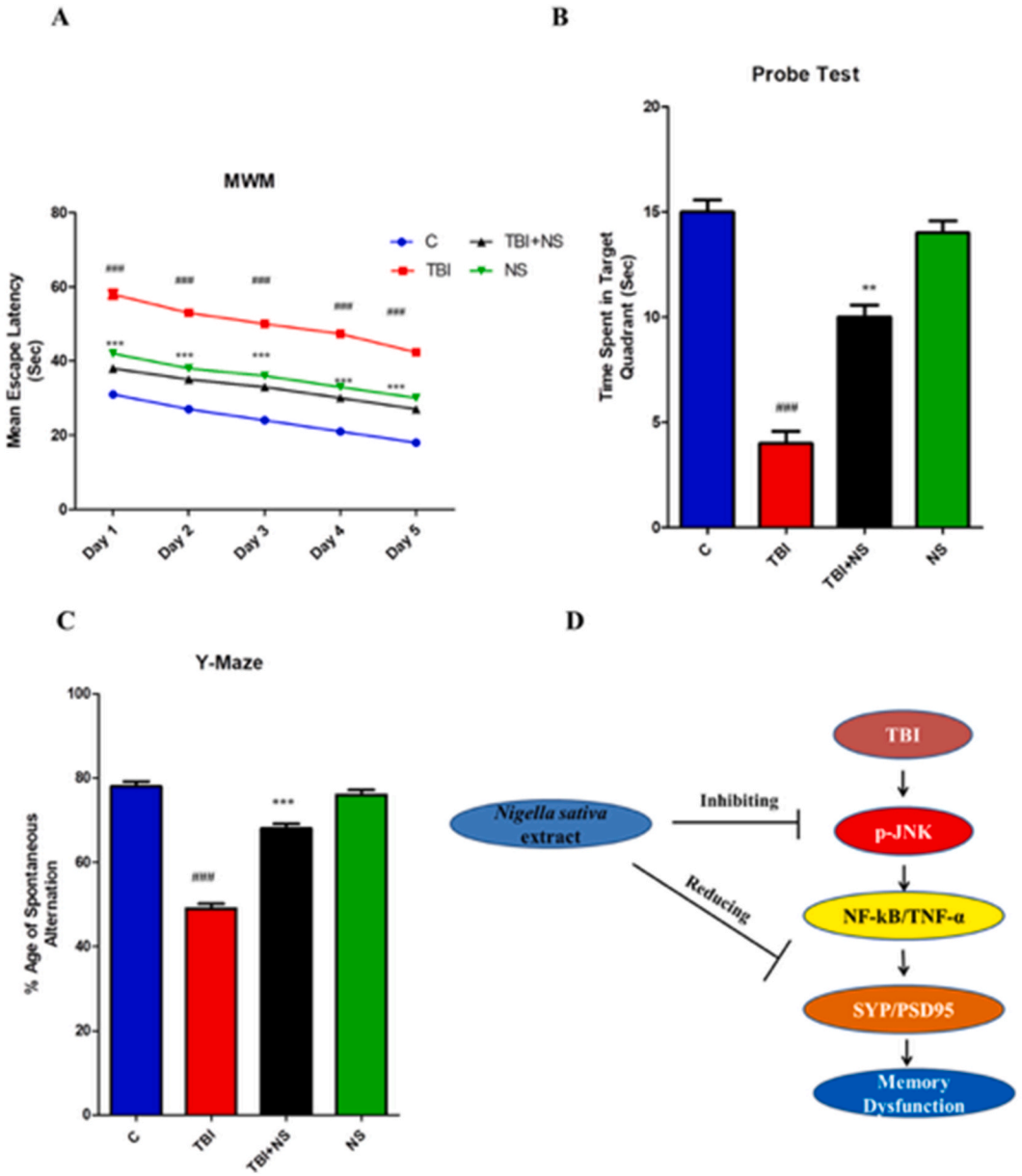

Fig. 3. *Nigella sativa* extract improved the memory impairment against TBI in adult mice brain. The result of behavioural tests is given as **(A)** mean escape latency in Morris Water Maze test from day 1 to day 5 **(B)** Probe test and **(C)** %age of spontaneous alteration in Y-Maze test. **(D)** Shown is the proposed mechanism of *Nigella sativa* extract in TBI mice model. All the data given is as a mean ± S.E.M. Significance of control vs TBI is expressed as #, while * denotes TBI vs TBI + *Nigella sativa* extract. $^{**,\#\#}p \leq 0.01$ and $^{***,\#\#\#}p \leq 0.001$.

4. Discussion

This study examined for the neurotherapeutic abilities of *Nigella sativa* extract against TBI-induced synaptotoxicity and memory impairment in adult albino mice. These male adult mice were administered *Nigella sativa* extract (10 mg/treated kg of body weight) after induction of TBI. Morris water maze and Y-maze tests were performed to assess their memory and western blotting technique was used to evaluate the anti-neuroinflammatory and synapse proteins restoration efficacy of *Nigella sativa* extract against TBI in male adult mice. The results of our current findings reveal that TBI significantly induced neuroinflammation leading to the neuronal synapse and cognitive decline in adult male albino mice. However, *Nigella sativa* extract significantly inhibited phosphorylated JNK, TNF-α and NF-kB proteins to reduce neuroinflammation to rescue adult mice. Additionally, *Nigella sativa* extract restored synapse proteins and improved the memory loss induced by TBI in adult mice.

In our study, the Y-Maze Test results showed that administration of *Nigella sativa* extract improved the behaviour against TBI by enhancing spatial recognition and enhanced working memory. Similar findings have been reported by another study indicating the broad-spectrum role of *Nigella sativa* as a potent neuroprotective compound across different cognitive impair models. These results are quite consistence to the previous findings evidencing our hypothesis of role of *Nigella sativa* in improving cognitive functions in lowering neuroinflammation and involve in synaptic proteins repair [30]. In this study, mice administrated with *Nigella sativa* extract demonstrated noticeably decreased escape latencies and increased time spent in the target quadrant during the probe trial in the Morris water maze test compared to TBI mice lacking treatment represents improved spatial learning and memory. Previous study reported similar findings focusing on a prominent bioactive compound of *Nigella sativa* i.e. Thymoquinone, decreased escape latency and path length in streptozotocin-induced cognitive impairment model. The consistency of these results among various investigations and experimental setups illustrated the prominent neuroprotective properties of *Nigella sativa* [24].

Similarly, while conducting the probe trial of the Morris water maze test, it was recorded that the time duration spent in the target quadrant by *Nigella sativa* extract treated group was more as compared to the TBI group suggesting improved memory recall. This finding aligns with another study investigation which demonstrated that *Nigella sativa* therapy improved neuropathic condition and reduced post-traumatic degenerative neurons and neurological dysfunctions in a murine mouse model [31]. These results are also supported by our research, suggesting that spatial learning, consolidation and retention of memory has been improved by the use of *Nigella sativa* extract in TBI induced mice models.

Studies conducted highlighted the dual involvement of cytokines in synaptic plasticity and damage [32,33]. Our research expands upon this finding by demonstrating that cytokines levels has been regulated by *Nigella sativa* extract perhaps upholding their functions in neural development and reducing adverse impacts. This dual performance of *Nigella sativa* extract may results in increase in its ability in enhancing both short-term and long-term memory as evidenced in our results from the Morris water maze and Y-maze tests. Similarly, while undergoing the comparative analysis of our findings with those of other researchers a consistent pattern may be noticed in effectiveness of *Nigella sativa* in decreasing the cognitive deficiencies and synaptic disorder resulting from traumatic brain injury (TBI) and other neurodegenerative impairments. The present study's investigation aligns with previous research findings that highlighted decrease in phosphorylated JNK, TNF-α, and NF-kB proteins indicating the anti-inflammatory and antioxidative properties of *Nigella sativa* [34]. These proteins are important indicators of neuro-inflammation and their decline implies a possible mechanism through which *Nigella sativa* extract confers its neuroprotective benefits.

To check more details about the mechanisms working as *Nigella sativa* extract neuroprotective effects, we focused on Western blot tests to evaluate significance of inflammatory and synaptic proteins expression [35,36]. Our main concern was on synaptophysin and PSD95 a pre-synaptic and post-synaptic protein respectively due to their important roles in both synaptic function as well as plasticity. At the same time, we also examined the levels of phosphorylated JNK, TNF-α, and NF-kB proteins which serve as the markers of neuronal inflammation. The Western blot test performed shows *Nigella sativa* extract increased the expression of PSD95 and synaptophysin, which enhances synaptic regeneration and repair. Moreover, *Nigella sativa* extract also decreased phosphorylated JNK, TNF-α, and NF-kB protein levels in nerve cells, which indicates that *Nigella sativa* has potent anti-neuroinflammatory properties. The recovery of synaptic proteins suggested that *Nigella sativa* extract have neuroprotective effects against TBI-induced cognitive impairment [37], also supports our findings. Furthermore, *Nigella sativa* has strong anti-inflammatory abilities therefore; it can be affective against several neurodegenerative disorders other than TBI [38].

5. Conclusion

To summarize, *Nigella sativa* extract exhibit potent anti-inflammatory properties, this might make it a potentially effective treatment agent for TBI and associated complications. Neuroinflammation is reduced by lowering important pro-inflammatory proteins, which provide protection against synapse loss and neuronal cells dysfunction. Therefore, *Nigella sativa* extract may have broad spectrum in the treatment of a number of neurodegenerative and neuroinflammatory diseases. It is required to conduct additional research in pure clinical settings in order to fully comprehend its full functioning and optimal utilization in therapeutic. Further studies are required for additional knowledge about the underlying mechanism and treatment methods of *Nigella sativa* in neuro-diseases associated with TBI.

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Ethics approval

Ethical approval letter has been taken from Research Ethical Committee of Neuro Molecular Medicine Research Center (NMMRC), Ring Road, Peshawar, KP-Pakistan.

Consent to participate

Written informed Consent was taken from all the participants prior to enrolment into this research.

Consent for publication

All authors are mutually agreed for publication.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Rifat Ullah Khan: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Sahibzada Muhammad Jawad:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Mubin Mustafa Kiyani:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Shahid Ali Shah:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Shahid Bashir:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Hamid Khan:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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