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ORIGINAL ARTICLE

Prospective Cognitive-Enhancing Benefits of Black Cumin in Epileptic Conditions

[1] Mohd. Shoeb Abdul Mukhtar*, [2] Ravikant Gupta
[1-2*]Department of Pharmaceutical Science, Oriental University, Indore, Madhya Pradesh, India
*Correspondence author email: mshoeb839@gmail.com[1]

ABSTRACT
This study investigates the potential neuroprotective effects of Nigella sativa, sometimes known as black seed, on rats' kindling-induced memory impairment. The kindling model, a tried-and-true method of inducing epileptic convulsions, has been used to mimic the neurological effects of epilepsy and related memory problems. The neuroprotective potential of Nigella sativa was assessed using a battery of behavioral tests, biochemical analyses, and histological evaluations. Our findings indicate that Nigella sativa extract may be utilized as a therapy for epilepsy-induced memory impairment as it significantly lowers the cognitive loss produced by kindling and the neuronal damage caused by seizures.
Keywords: *Epilepsy, Black Cumin, Memory dysfunction, seizures, anti-inflammatory, memory impairment.*

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INTRODUCTION

This study examines the possible neuroprotective benefits of *Nigella sativa*, commonly referred to as black seed, on rats' memory impairment caused by kindling. The neurological consequences of epilepsy and associated memory issues have been replicated using the kindling model, a well-proven technique for causing epileptic convulsions. Biochemical assays, histological assessments, and a series of behavioral tests were used to evaluate *Nigella sativa*'s neuroprotective potential. According to our research, *Nigella sativa* extract considerably reduces the cognitive loss brought on by kindling and the neuronal damage brought on by seizures, suggesting that it might be employed as a treatment for epilepsy-induced memory impairment.

Similarly, Parkinson's disease (PD) is marked by the loss of dopaminergic neurons in the substantia nigra, resulting in motor impairments such as bradykinesia, tremors, and rigidity [3]. Beyond motor symptoms, PD is also associated with cognitive decline, mood disorders, and autonomic dysfunction, making its management particularly challenging. Existing treatments, such as levodopa and dopamine agonists, provide symptomatic relief but do not halt disease progression.

Movement issues, cognitive decline, and psychiatric symptoms are all consequences of Huntington's disease (HD), a hereditary neurodegenerative illness that damages the basal ganglia [4]. It is brought on by a huntingtin gene mutation that causes neurons to gradually degenerate. HD presently has no known cure, and available treatments concentrate on symptom management rather than treating the underlying illness.

Motor neurons are the main target of the deadly neurodegenerative disease amyotrophic lateral sclerosis (ALS), which causes progressive muscular weakening, paralysis, and respiratory failure [5]. While some ALS cases are related to genetic abnormalities, the majority are spontaneous. There are still few therapeutic options available despite a lot of research; the only FDA-approved medications that provide any help are riluzole and edaravone.

Oxidative stress, which arises from an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense systems, is one of the main pathogenic processes in neurodegenerative diseases. This oxidative damage accelerates the course of the illness by causing neuronal death [6].

Neuronal degeneration and cognitive decline are further exacerbated by excitotoxicity, mitochondrial dysfunction, and chronic neuroinflammation [7]. There is an urgent need for therapeutic agents that can target multiple pathways at once because neurodegeneration is complex and multifactorial.

The pharmacological characteristics of Nigella sativa, sometimes referred to as black seed or black cumin, have been extensively researched, especially its neuroprotective benefits. Thymoquinone (TQ), the main bioactive component of Nigella sativa, has potent anti-inflammatory, antioxidant, and neuroprotective qualities [8]. Nigella sativa has long been used in traditional medicine to treat neurological conditions, and new research indicates that it may also help reduce oxidative stress and neuroinflammation in neurodegenerative illnesses [9].

According to preclinical research, TQ alters a number of signaling pathways that are important for neuronal survival, such as nuclear factor erythroid 2-related factor 2 (Nrf2), which is essential for the cellular antioxidant response [10]. In animal models of AD, TQ has also been demonstrated to enhance cognitive performance, suppress acetylcholinesterase activity, and decrease amyloid-beta aggregation [11]. These results imply that Nigella sativa could provide a fresh strategy for reducing the rate of AD development and maintaining cognitive function.

By improving dopaminergic cell survival and lowering oxidative damage, Nigella sativa has shown neuroprotective benefits in animal models of Parkinson's disease [12]. It is believed that TQ's preventive benefits in Parkinson's disease (PD) are mediated by its capacity to improve mitochondrial function, lower neuroinflammation, and alter dopamine metabolism. Furthermore, research has shown that Nigella sativa extract lessens motor impairments in models of Parkinson's disease, suggesting that it might be used as an adjuvant treatment to help manage the condition [13].

Additionally, research on models of HD and ALS shows that TQ reduces neuronal death and mitochondrial dysfunction, indicating that it may be a broad-spectrum neuroprotective drug [14]. TQ's promise as a viable therapeutic candidate for the treatment of neurodegenerative illnesses is highlighted by its capacity to alter several cellular pathways implicated in neurodegeneration.

Current treatment approaches for neurodegenerative diseases are mostly symptomatic and do not stop the disease's development, despite breakthroughs in our knowledge of these conditions. The necessity for alternative and supplemental therapeutic techniques is highlighted by the limited effectiveness and side effects of traditional therapies. Nigella sativa and other natural substances with neuroprotective qualities present a possible path for the creation of innovative treatments to combat neurodegeneration [15].

In a kindling model of memory failure, this study intends to investigate the neuroprotective properties of Nigella sativa by assessing its influence on oxidative stress indicators, inflammatory responses, and cognitive function. Through clarifying the molecular processes that underlie its neuroprotective qualities, this study might aid in the creation of supplementary approaches to the treatment of neurodegenerative illnesses.

MATERIAL AND METHODS

Animals:

The study involved male Wistar rats each weighing between 250 to 300 grams. These rats were sourced from the Central Animal Research Facility (CARF). The animals were housed under standard laboratory conditions, following a natural light and dark cycle (12 hours light and 12 hours dark). They were provided with standard laboratory feed and water ad libitum. The protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (No. IAEC/ REC/04/2024-25).

Collection of Plant Material:

Nigella sativa was collected in April from various sources in and around the Bhopal District, Madhya Pradesh, India. The plant was authenticated by Dr. Kaushaki Gutam, former Associate Professor of Botany at JNKVV University, Jabalpur, Madhya Pradesh. The plant was cleaned thoroughly with tap water and then with distilled water. The leaves were shade-dried and ground into a coarse powder.

Experimental planning

TQ to Detect Antiepileptic and Neuroprotective Activity:

The bioassay to evaluate the antiepileptic and neuroprotective effects of TQ was conducted across four experimental sets: **Experiment-IV (Electrophysiological Study), Experiment-V (Behavioral Study), Experiment-VI (Histological Study), and Experiment-VII (Biochemical Study)**.

Sample Size: A total of **180 male Wistar rats** were used for the bioassay, with 60 rats allocated for the electrophysiological study, and the remaining 120 rats divided between two behavioural studies (n=60 per group). After behavioural assessments, the rats' brains were used for histological and biochemical analysis. Each experiment involved six groups, with 10 animals per group.

Random Allocation:
Rats were randomly allocated to the experimental groups using the lottery method, ensuring unbiased distribution across the groups.
Experimental Groups:
In each experiment, a total of **60 rats** were divided into six groups, each containing 10 animals:

- **Group I (Cage Control)**: Animals remained undisturbed in their cages throughout the experimental period, except for handling by the investigator.
- **Group II (Vehicle Control)**: Animals received **0.9% NaCl** intraperitoneally (i.p.) and an equal volume of distilled water orally for 30 days (as received by Groups IV, V, and VI).
- **Group III (Distilled Water + PTZ)**: Animals received **30 mg/kg body weight (b.w.) of PTZ i.p.** every 48 hours, along with an equal volume of distilled water orally for 30 days (to induce seizures).
- **Group IV (Sodium Valproate 200 mg/kg + PTZ)**: Animals received **200 mg/kg of Sodium Valproate** orally, 30 minutes before receiving **30 mg/kg b.w. of PTZ i.p.** every 48 hours for 30 days.
- **Group V (TQ 40 mg/kg + PTZ)**: Animals received **40 mg/kg of TQ** (Nigella) orally, 30 minutes before **35 mg/kg b.w. of PTZ i.p.** every 48 hours for 30 days.
- **Group VI (TQ 80 mg/kg + PTZ)**: Animals received **80 mg/kg of TQ** (Nigella) orally, 30 minutes before **35 mg/kg b.w. of PTZ i.p.** every 48 hours for 30 days.

PTZ-Induced Kindling Model for Chronic Generalized Epilepsy:
The **PTZ-induced kindling model** is a well-established method for inducing chronic epilepsy in experimental animals. Kindling refers to a progressive increase in the susceptibility to seizures, which is often used to simulate epilepsy. **PTZ (pentylenetetrazole)**, a chloride channel inhibitor associated with the GABAA receptor, is commonly used to induce kindling.
In this study, rats were injected with **35 mg/kg b.w. of PTZ i.p.** every 48 hours for 30 days to induce chronic epilepsy, as per the method described by [17-20].
Animal Body Weight Changes:
Body weight changes were monitored every two days, prior to scheduled dosing. This was an important parameter used to assess the overall health and efficacy of the pharmacological treatments.
Detection of Seizure Stages during Kindling Progression:
After each PTZ injection, the convulsive behavior of the rats was observed for 30 minutes. The intensity of seizures was classified into the following stages:

- **Stage 0**: No response
- **Stage 1**: Ear and facial twitching
- **Stage 2**: Convulsive waves throughout the body
- **Stage 3**: Myoclonic jerks, rearing
- **Stage 4**: Turning over onto one side
- **Stage 5**: Generalized tonic-clonic seizures (turning onto the back)

Seizure intensity was observed after each PTZ injection on the **1st, 3rd, 5th, 7th,** and subsequent days. Seizure intensities were recorded following the injection to evaluate the effects of TQ.
Latency for Onset of Seizures During Kindling Progression:
The **latency** for the onset of seizures was recorded for each group during the one-month kindling period. This provided an important measure of how the treatment with TQ affected the time it took for seizures to occur as the kindling progressed.
Induction of Kindling
Racine's 1972 explanation of the igniting procedure was adhered to [16]. Simply stated, ketamine Intraperitoneal (i.p.) doses of xylazine (10 mg per kilogram of body weight) and (75 mg per kilogram of body weight) were used to induce sleep in the experimental subjects. A bipolar electrode (-3.5 mm for AP, ±2.5 mm for ML, and -3.0 mm for DV) was implanted in the right hippocampus area. Unless the mice developed stage 5 convulsions (Racine's scale), electric stimulation of the hippocampus (0.5 mA, 1-second duration, 20 Hz) was used to induce seizures once per 48 hours. The kindling procedure was continued until stage 5 convulsions were consistently seen for a minimum of three consecutive sessions.
Preparation of *Nigella sativa* extract
Through a local herbal store, we purchased *Nigella sativa* seeds. To make a powdered extract, the seeds were crushed and macerated with 70% ethanol for 48 hours. The ethanol was then evaporated under reduced pressure to produce a concentrated extract. The extract was administered orally for 14 days at a dose of 100 mg per kilogram after being diluted in ordinary saline the day after the first kindling session.

Assay of Acetylcholinesterase (AChE) Activity

Acetylcholinesterase (AChE) activity was measured using the method [18]. The substrate, acetylthiocholine (ATC), was used, and AChE catalyzed the hydrolysis of ATC to release thiocholine. The released thiocholine reacted with the reagent 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), producing a yellow-colored anion, thionitrobenzoic acid, which had an absorption maximum at 412 nm. The concentration of thionitrobenzoic acid was measured spectrophotometrically and was used as a direct indicator of AChE activity. The extinction coefficient for thionitrobenzoic acid was 1.36×10^{-4}/mole/cm.

RESULT

Table 1: Detection of Antiepileptic and Neuroprotective Activity of TQ:

Parameter	Group I (Cage Control)	Group II (Vehicle Control)	Group III (PTZ-induced Epilepsy)	Group IV (Sodium Valproate + PTZ)	Group V (TQ 40 mg/kg + PTZ)	Group VI (TQ 80 mg/kg + PTZ)
EEG Amplitudes	Normal	Normal	Increased (spike-wave discharges)	Decreased	Reduced	Most significantly reduced
Latency to Seizure Onset	-	-	Significantly reduced	Prolonged	Prolonged	Most significantly prolonged
Seizure Severity	-	-	High	Reduced	Reduced	Most significantly reduced
Retention Latency (24h, sec)	151.50 ± 18.41	131.00 ± 31.02	48.00 ± 9.71	126.33 ± 11.08	82.67 ± 16.15	136.83 ± 18.62
Retention Latency (48h, sec)	99.33 ± 20.20	102.83 ± 24.59	51.33 ± 3.06	104.83 ± 17.21	89.17 ± 11.65	113.67 ± 8.39
(AChE) Activity	Normal	Normal	Reduced (2-fold decrease)	Restored to near-normal levels	Restored	Most significantly restored
Body Weight Changes	Stable	Stable	Weight loss	Mild recovery	Moderate recovery	Most significant recovery

Death rate, myoclonic jerk delay, and generalized seizures in the PTZ model under nigella sativa treatment:

Death rate:

The mortality rate was found to be 50% in the control group that received 1% CMC. Conversely, every other experimental group that was treated had protective effects, leading to a 0% death rate.

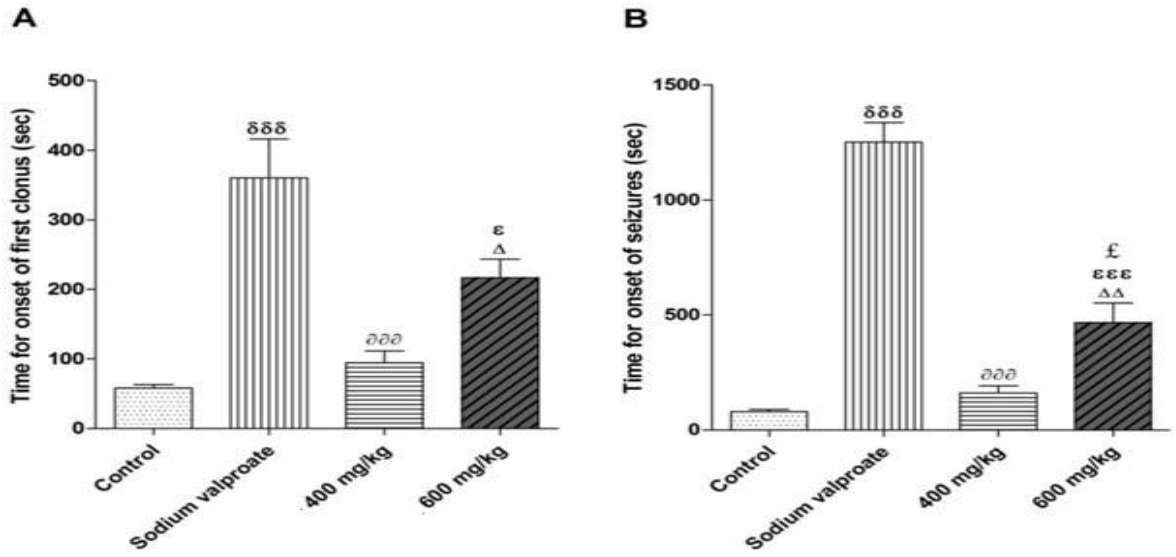
myoclonic jerk delay:

Nigella sativa and sodium valproate's impact on myoclonic jerk latency in a control group. While Nigella sativa at 400 mg/kg demonstrated a delay that was not statistically significant, sodium valproate considerably prolonged this latency. However, Nigella sativa showed protective benefits against PTZ-induced seizures by considerably delaying the start of the first clonus at 600 mg/kg. Overall, sodium valproate continued to be more effective in spite of this.

The time it takes for generalized seizures to start:

An investigation on the anti-epileptic properties of Nigella sativa with sodium valproate. The delay for seizure onset was about 15 times greater in the sodium valproate group than in the control group. Although it was still less efficacious than sodium valproate, a greater dose of Nigella sativa (600 mg/kg) greatly delayed the start of seizures, whereas a lesser dose (400 mg/kg) had no discernible impact. The promise of Nigella sativa in the treatment of epilepsy was highlighted by statistical analysis, which validated the protective effect of the higher dosage against PTZ-induced seizures.

Effect of TQ extract on the latency for the onset of first clonus (A) and seizures (B) in rats



Graph 1 : Effect of TQ extract on the latency for the onset of first clonus (A) and seizures (B) in rats. Both doses of TQ extract was effective in increasing the latency forthe onset of clonus and seizures, but the high dose of TQ extract had a significantly better effect comparing to the low dose. δδδ, *∂∂∂*, εεε $p<0.001$, ΔΔp <0.01, £$p<0.05$

Table 2: Mortality and Seizure Onset Under Nigella Sativa Treatment

Parameter	Control (1% CMC)	Sodium Valproate	Nigella sativa (400 mg/kg)	Nigella sativa (600 mg/kg)
Death Rate (%)	50%	0%	0%	0%
Myoclonic Jerk Delay	No delay	Significantly prolonged	No significant delay	Considerably delayed
Generalized Seizure Onset	Baseline (fast onset)	15× delayed	No significant delay	Significantly delayed

DISCUSSION

Black cumin (*Nigella sativa*), particularly its active compound thymoquinone, has shown promising potential in improving cognitive function in individuals with epilepsy. Epileptic patients often suffer from memory loss and learning difficulties due to recurrent seizures and the side effects of antiepileptic drugs.

Studies suggest that black cumin may help protect brain cells by reducing oxidative stress and inflammation, both of which are linked to seizure-induced cognitive decline. Its anticonvulsant properties, possibly through enhancement of GABAergic activity, also contribute to fewer seizures, indirectly supporting better cognitive performance.

In animal models, black cumin has been associated with improved memory and learning, especially through its effects on the hippocampus, a key brain region for cognition. These findings support its potential as a natural adjunct therapy to enhance brain health and reduce cognitive side effects in epileptic patients.

However, more clinical research is needed to confirm these benefits in humans and establish safe, effective dosing.

CONCLUSION

This study highlights the neuroprotective and anticonvulsant effects of Nigella sativa, particularly its active component thymoquinone (TQ), in epilepsy. Epilepsy often leads to cognitive impairment and oxidative stress, which current antiepileptic drugs (AEDs) fail to fully address due to their side effects. The research demonstrates that in the pentylenetetrazol (PTZ) kindling model, TQ effectively reduced seizure severity, enhanced GABAergic transmission, and modulated excitatory pathways. Additionally, TQ's antioxidant properties lowered oxidative stress markers, protecting neurons in the frontal cortex and hippocampus.

Behavioral assessments confirmed that TQ improved spatial learning and memory retention, supporting its role as a cognitive enhancer in epilepsy-related impairments. Given its ability to reduce seizures while preventing neuronal damage, TQ emerges as a potential alternative or adjunct therapy to AEDs. However, further clinical studies are needed to optimize dosage, formulations, and long-term safety in humans.
In conclusion, Nigella sativa and TQ offer a promising natural therapeutic approach for epilepsy, improving seizure control, neuroprotection, and cognitive function. Their incorporation into epilepsy treatment could enhance patient outcomes and quality of life while minimizing AED-related side effects.

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