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Thymoquinone ameliorate oxidative stress, GABAergic neuronal depletion and memory impairment through Nrf2/ARE signaling pathway in the dentate gyrus following cypermethrin administration

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Abstract

Background Exposure to chemical toxins, including insecticides, harms bodily organs like the brain. This study examined the neuroprotective of thymoquinone on the cypermethrin's harmful effects on the histoarchitecture of the dentate gyrus and motor deficit in the dentate gyrus.

Methods Forty adult male rats (180–200 g) were randomly divided into 5 groups ($n=8$ per group). Groups I, II, III, IV, and V received oral administration of 0.5 ml of phosphate-buffered saline, cypermethrin (20 mg/kg), thymoquinone (10 mg/kg), cypermethrin (20 mg/kg) + thymoquinone (5 mg/kg), and cypermethrin (20 mg/kg) + thymoquinone (10 mg/kg) for 14 days respectively. The novel object recognition test that assesses intermediate-term memory was done on days 14 and 21 of the experiment. At the end of these treatments, the animals were euthanized and taken for cytoarchitectural (hematoxylin and eosin; Cresyl violet) and immunohistochemical studies (Nuclear factor erythroid 2-related factor 2 (Nrf2), Parvalbumin, and B-cell lymphoma 2 (Bcl2).

Result The study shows that thymoquinone at 5 and 10 mg/kg improved Novelty preference and discrimination index. Thymoquinone enhanced Nissl body integrity, increased GABBAergic interneuron expression, nuclear factor erythroid 2-derived factor 2, and enhanced Bcl-2 expression in the dentate gyrus. It also improved the concentration of nuclear factor erythroid 2-derived factor 2, increased the activities of superoxide dismutase and glutathione, and decreased the concentration of malondialdehyde level against cypermethrin-induced neurotoxicity.

Conclusion thymoquinone could be a therapeutic agent against cypermethrin poisoning.

Keywords Cypermethrin, Thymoquinone, Cresyl fast violet, GABBAergic interneuron, Dentate gyrus

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Background

Oxidative stress arises from an imbalance between reactive oxygen species (ROS) and the antioxidant defense system. ROS can lead to covalent oxidative modifications, such as ribonucleic acid (RNA) oxidation, and induce mutations in mitochondrial DNA (mtDNA), thereby destabilizing nucleic acids [1, 2]. These modifications may result in cellular dysfunction and apoptosis. The mitochondrial-dependent caspase pathway is crucial in apoptosis [3]. Stimulation of this cascade releases pro-apoptotic factors, including cytochrome c (Cyc), activating caspase-9 and caspase-3, ultimately triggering cellular apoptosis [4, 5]. Hence, antioxidant pathways that mitigate oxidative damage may exhibit neuroprotective effects [6].

Persistent pesticide exposure, such as pyrethroids, adversely affects various physiological functions [7]. For example; long-term exposure can disrupt the functioning of different organs, posing serious health risks [8, 9]. Nigeria has witnessed numerous cases of food poisoning due to pesticides, resulting in significant fatalities and economic losses [10]. Pyrethroids, widely used in agriculture and household insect control, were detected in a large portion of the population [11]. Despite their broad application, pyrethroids exhibit adverse effects, including neurobehavioral and molecular target disruption in the nervous system [12, 13]. Cypermethrin, a common pyrethroid, crosses the blood-brain barrier, inducing oxidative stress and apoptotic cell death [14, 15].

Medicinal plants offer a sustainable therapeutic approach against chemical toxins; for example, thymoquinone (TQ), derived from Nigella sativa L., possesses antioxidant and anti-inflammatory properties [16, 17]. TQ has shown neuroprotective effects in various models of brain injury and neurodegenerative diseases by inhibiting lipid peroxidation and apoptosis [18, 19]. Its antioxidant effects are mediated through the nuclear factor erythroid 2-related factor 2 (NRF2) pathway, which regulates cellular defense mechanisms against oxidative stress [20, 21]. Activation of NRF2 induces the expression of antioxidative and detoxifying enzymes, crucial for cellular function [22]. Whereas, the Dysregulation of NRF2/ARE signaling has been implicated in neurodegenerative disorders [23].

Given the increasing incidence of pesticide-induced food poisoning, there is a need for effective antidotes with shared mechanisms of action. This study aims to evaluate the efficacy of thymoquinone against cypermethrin-induced neurotoxicity, focusing on GABAergic interneuron disruption, dentate gyrus cytoarchitectural disorganization, and oxidative stress-induced cell damage.

Methods

Experimental design

The experimental design involved 40 adult male Wistar rats (180–200 g). Thymoquinone was obtained from MedChemEpress (MCE) USA (Cat No: HY-d0803) Cypermethrin 10% EC product was sourced from Yubaili Agrotec (ACEC20L068) and NAFDAC No: A5-0108 was obtained from Ibukun Oluwa Agrochemical Distop. Ilorin, Nigeria. The rats were housed in the a Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin's animal holding facility under natural day-night cycles, with a standard chow diet and water ad libitum. Informed consent was obtained from the owner(s) of the animals involved in this study. All procedures were conducted with the owners' approval and were reviewed and approved by the University of Ilorin Ethical Review Committee (Approval No. UERC\ASN\2021\2137).

The rats were randomly divided into 5 groups ($n=8$ per group). Groups I, II, III, and IV received oral administration of 0.5 ml of phosphate buffered saline, 20 mg/kg of cypermethrin, 10 mg/kg of thymoquinone, 20 mg/kg cypermethrin plus 5 mg/kg of thymoquinone, and 20 mg/kg of cypermethrin plus 10 mg/kg of thymoquinone for 14 days respectively. Memory behavior was assessed on the 14th day of the experiment.

Behavioral evaluation

Intermediate memory recognition of the experimental rats following exposure to cypermethrin and thymoquinone was assessed using the novel object recognition (NOR) paradigm. The test apparatus is made from plywood measuring 100 cm by 100 cm with walls that are 50 cm high. Novelty preference and Discriminatory index were evaluated [24]. Twenty-four hours after the behavioral study, the animals were euthanized using 20 mg/kg bw ketamine intramuscularly, and brain tissue was excised and processed for histological, immunohistochemistry, and biochemical analysis.

Tissue collection

After the completion of perfusion (brain tissues for histology and immunohistochemistry), the whole brain tissues were excised and were post-fixed in 4% paraformaldehyde overnight. The whole hippocampal CA regions were excised and equilibrated in 30% sucrose solution, before histological and immunohistochemical analyses. The sections were taken at 2 µm on paraffin wax-embedded tissue blocks and mounted on a glass slide [25].

Histological analysis and immunohistochemistry

The hematoxylin and eosin (H and E) staining technique was used to demonstrate the general histo-architecture of the cells; and to show the location of the normal or

abnormal nucleus of the hippocampal cells [26]. Cresyl violet: This technique was used to demonstrate Nissl bodies (endoplasmic reticulum and ribosomes) in the cells; and to show normal or abnormal protein synthesis in the cytoplasm of the hippocampus [27].

For immunochemistry: Nuclear factor erythroid 2-related factor 2 (Nrf2), Parvalbumin, and B-cell lymphoma 2 (Bcl2) (human monoclonal; Elisa and microarray) were used to understand their roles in oxidative stress response, neuronal survival, and apoptosis regulation within dentate gyrus. The avidin-biotin complex method was used. The antibody dilution factor used was 1:100 for all the antibody markers. The processed tissues were sectioned at two microns on the rotary microtome and placed on a hotplate at 90 °C for at least 40 min. Image J software cell counter was used for counting the immunopositive cells for Nrf2, Parvalbumin, and Bcl2 in the dentate gyrus [28].

Biochemical investigation

Following the end of the various treatments, some hippocampal tissues were homogenized. The homogenates were collected in a 5 ml plain bottle and centrifuged for 10 min at 5000 rpm using a centrifuge. The supernatant was carefully decanted and stored at -4 °C for enzymatic assays of superoxide dismutase (SOD) activity [29], glutathione (GSH) concentration [30], malondialdehyde (MDA) [29] concentration and nuclear factor erythroid 2-derived factor 2 (NrF2) a product of Elabscience Biotechnology Inc.USA (E-EL-R0673) method and absorbance was read using microplate reader. A four-parameter logistic curve (4PL-curve) was plotted and values obtained from the samples were extrapolated using GraphPad Prism 8.0.

Statistical analysis

Data from the behavioral investigation, biochemical assays, and immunopositive cell count were analyzed using one-way analysis of variance (ANOVA) and subjected to post hoc Bonferroni's multiple comparison test. The results were expressed as mean ± SEM. Statistical analyses were performed using GraphPad Prism software (version 8.0.2). Values of $p \leq 0.05$ were considered statistically significant.

Results

Thymoquinone restore inter-mediate related behaviors following cypermethrin exposure

The novelty preference of Wistar rats in the CYM group for new objects in the novel object recognition test was significantly low (32.70 ± 3.93) compared to the PBS control group at $p < 0.05$, compared to the CYM group, the TQ group, and the CYM-LHQ group showed higher preference for new object which was significant at $p < 0.05$. However, the CYM-10mgTHQ group showed a higher preference than the CYM group with no significance at $p < 0.05$ Fig. 1B. The discrimination index was significantly reduced in the CYM group (-0.34 ± 0.08) compared to the PBS group (0.47 ± 0.10) and THQ group (0.81 ± 0.11) at $p < 0.05$. Groups CYM-LTHQ and CYM-HHQ showed a higher discrimination index when compared to the CYM group but were not significant at $p < 0.05$ (Table 1).

Oxidative stress biomarker

The concentration of Nrf2 in the CYM group was significantly reduced $p < 0.05$ when compared to both the PBS control and the other experimental groups (Fig. 1A). Moreover, a significant reduction $p < 0.05$ in the SOD activity was observed in the CYM-exposed group, relative to the PBS control group, while all the other experimental groups showed a significant increase in the SOD activities $p < 0.05$ when compared to the CYM group (Fig. 1B). However, SOD activities in the TQ, CYM-LTQ and CYM-HTQ groups were not significantly lower compared to the PBS control (Fig. 1B). In addition, the GSH concentration was significantly reduced ($p < 0.05$) in the CYM group relative to the PBS group (Fig. 1C). Subsequent treatment with LTQ and HTQ led to a significant increase in the GSH levels ($p < 0.05$) when compared to the CYM-only group (Fig. 1C). Regarding the lipid peroxidation marker MDA, its level was notably higher ($p < 0.05$) in the CYM group compared to the PBS group (Fig. 1D). While MDA levels were lower in other experimental groups, significance ($p < 0.05$) was only observed in the CYM-LTQ group compared to the CYM group (Fig. 1D).

PBS = phosphate-buffered saline, CYM = cypermethrin, TQ = thymoquinone, CYM-LTQ = cypermethrin followed by low dose thymoquinone and CYM-HTQ = cypermethrin followed by High dose thymoquinone.

Histochemical examination of the dentate gyrus revealed chromatolytic-like alterations in rats exposed to CYM. This was characterized by the disrupted integrity of Nissl granules and distortions in the shape and organization of granule cells. Additionally, numerous pyknotic and vacuolated cells were observed due to CYM exposure. Thymoquinone demonstrated a mitigating effect against CYM-induced toxicity, this was observed as a result of a reduction in the extent of neurodegenerative-like changes. Specifically, there was an improvement in Nissl body integrity and better enhancements were observed in the cellular shape and arrangement in the rats exposed to thymoquinone following CYM neurotoxicity (Fig. 2).

The immunohistochemical assessment of the dentate gyrus utilizing anti-Nrf2, anti-Parvalbumin (Parv), and anti-Bcl2 antibodies revealed diminished expression of

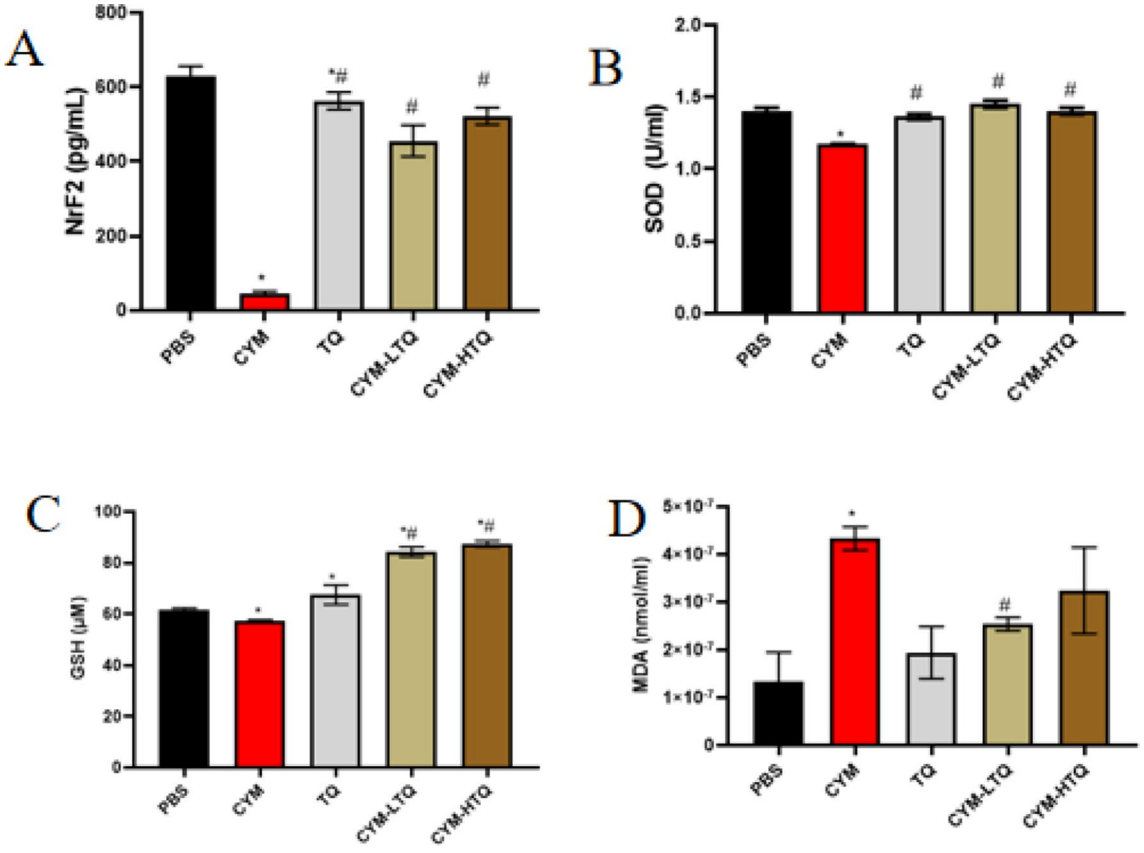

Fig. 1 Effects of thymoquinone post treatments on the cpermethrin-induced dentate gyrus oxidative stress. (**A**) Nrf2 (**B**) SOD (**C**) GSH (**D**) MDA. One-way ANOVA followed by Bonferroni's post hoc test multiple comparisons was used for analysis. Data is presented as mean ± SEM ($n = 5$). *$p < 0.05$ compared to PBS group; #$p < 0.05$ compared to CYM group

Table 1 Novelty preference and discrimination index of rats following cypermethrin and thymoquinone exposure

Groups *N* = 5	Novelty preference (%)	Discrimination index
PBS	77.60 ± 3.82	0.28 ± 0.24
CYM	39.30 ± 7.00*	-0.20 ± 0.15
TQ	90.30 ± 5.41#	0.81 ± 0.11#
CYM-LTQ	65.00 ± 11.20	0.44 ± 0.09
CYM-HTQ	46.00 ± 12.00	0.23 ± 0.06

PBS = phosphate-buffered saline, CYM = cypermethrin, TQ = thymoquinone, CYM-LTQ = cypermethrin followed by low dose thymoquinone and CYM-HTQ = cypermethrin followed by High dose thymoquinone. Single asterisk (*) indicates significant ($p < 0.05$) compared to PBS, Hash (#) indicates significant ($p < 0.05$) compared to CYM

Nrf2, Parv, and Bcl2 positive cells in the dentate gyrus of rats exposed to CYM. Conversely, post-treatment with thymoquinone exhibited a notable enhancement in the expression of Nrf2, Parv, and Bcl2 immunopositive cells in the dentate gyrus (Figs. 3, 4 and 5).

Quantification of immuno-positive cell counts using ImageJ software demonstrated a significantly higher ($p < 0.05$) Nrf2 cell count in the dentate gyrus of PBS control rats compared to those exposed to CYM (Fig. 3A & B). Thymoquinone administration led to a significant increase ($p < 0.05$) in Nrf2 immunopositive cells in the CYM-LTQ and CYM-HTQ groups compared to CYM-exposed rats (Fig. 3A & B). Similarly, there was a significantly higher ($p < 0.05$) count of Parvalbumin-positive cells in the dentate gyrus of PBS control rats compared to CYM-exposed rats (Fig. 4A & B). Thymoquinone significantly ($p < 0.05$) increased the number of Parvalbumin cells in the CYM-LTQ and CYM-HTQ groups compared to CYM-exposed rats (Fig. 4A & B).

Moreover, the cell count of the anti-apoptotic protein Bcl2 was higher in the PBS control group than in CYM-exposed rats (Fig. 5A & B). Administration of thymoquinone resulted in a higher Bcl2 count in the CYM-LTQ and CYM-HTQ groups compared to CYM-exposed rats (Fig. 5A & B).

Discussion

Continuous application of pesticides and other agrochemicals, driven by the need to increase food production and prevent pest and insect-induced crop damages, has led to increased exposure to the harmful effects of these chemicals, including pyrethroid insecticides, due to their residual accumulation in crops, fruits, and

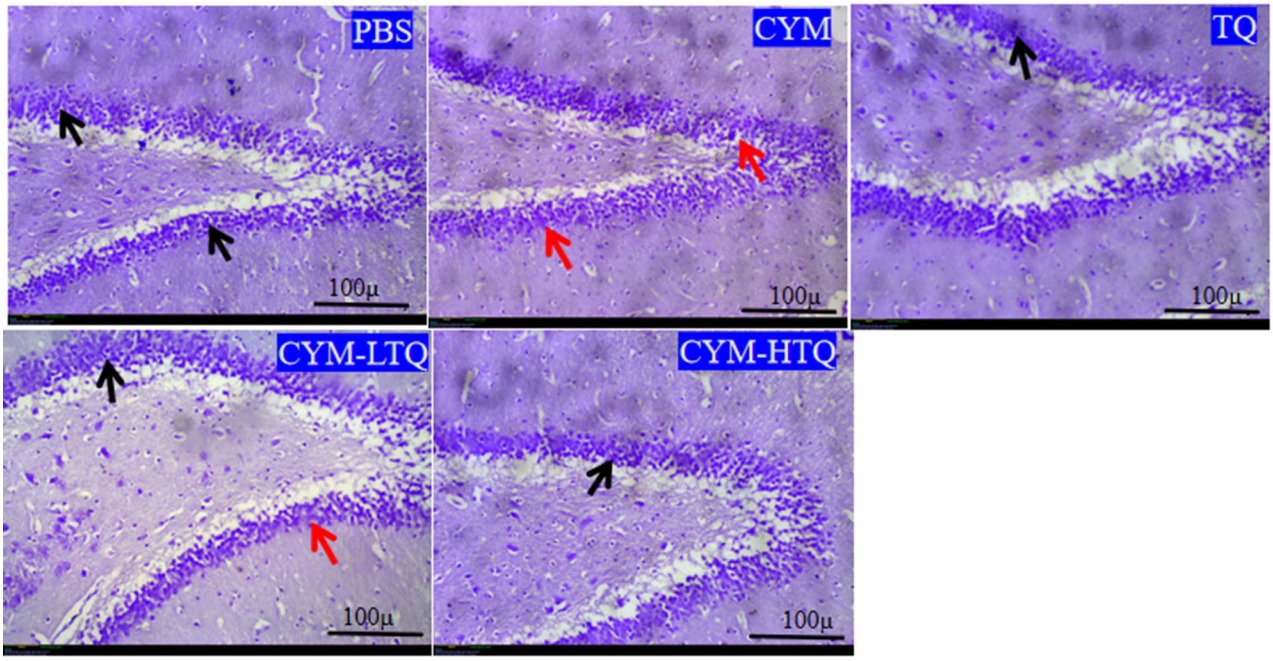

Fig. 2 Nissl substance of Dentate gyrus stained with cresl fast violet (CFV). PBS group showed neurons with intact Nissl bodies (black arrows). the CYM group presented chromatolytic-like cytoplasm, indicating Nissl body loss with vacoulations (red arrow): TQ group displayed neurons with intact Nissl bodies (black arrows); CYM-LTQ group showed numerous neurons with well-stained cytoplasm indicating intact Nissl bodies (black arrows) and few pale neurons (red arrows): Also, CYM-HTQ group showed more neurons with well-stained cytoplasm indicating intact Nissl bodies (black arrows) and few pale neurons (red arrows). TQ = thymoquinone, CYM-LTQ = cypermethrin followed by low dose thymoquinone and CYM-HTQ = cypermethrin followed by High dose thymoquinone

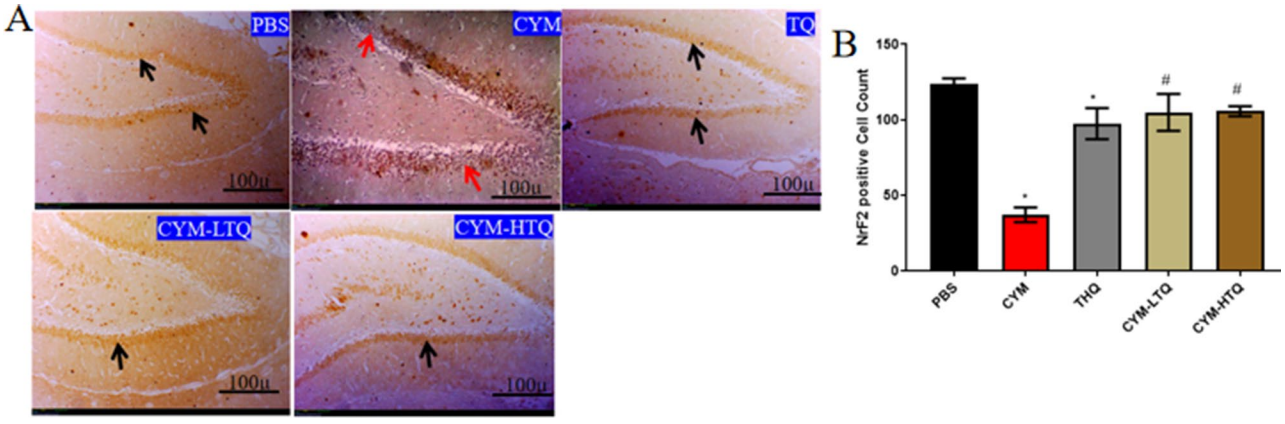

Fig. 3 (**A**) Immunostaining for Nrf2 in the dentate gyrus (scale bar = 100 µm), PBS group showed numerous Nrf2 expression (black arrows). CYM group displayed low Nrf2 expression indicating high Nrf2 inactivation (red arrows): TQ group showed high Nrf2 positive cells (black arrows): CYM-LTQ presented high Nrf2 positive cells (black arrows) compared to CYM group: CYM-HTQ group also displayed numerous Nrf2 positive cells (black arrow) compared to CYM group. (**B**). Nrf2 positive cell count. PBS group had significantly high Nrf2 positive cells (*$p < 0.05$) compared to CYM group. CYM group showed significant difference (#$p < 0.05$) compared to CYM-LTQ and CYM-HTQ groups $N = 6$. PBS = phosphate-buffered saline, CYM = cypermethrin, TQ = thymoquinone, CYM-LTQ = cypermethrin followed by low dose (5 mg/kg) thymoquinone and CYM-HTQ = cypermethrin followed by High dose (10 mg/kg) thymoquinone

vegetables. When humans and other animals are exposed to these chemicals, they can induce toxicity through mechanisms involving mitochondrial dysfunction, oxidative stress, and inflammation. This toxicity can manifest as movement disorders, loss of cognition, or a combination of both.

This study demonstrates that thymoquinone increases the activities of antioxidant enzymes, akin to its parent molecule Nigella sativa oil [31, 32], thereby preventing lipid peroxidation and preserving dentate gyrus architecture, ultimately enhancing memory function against CYM toxicity.

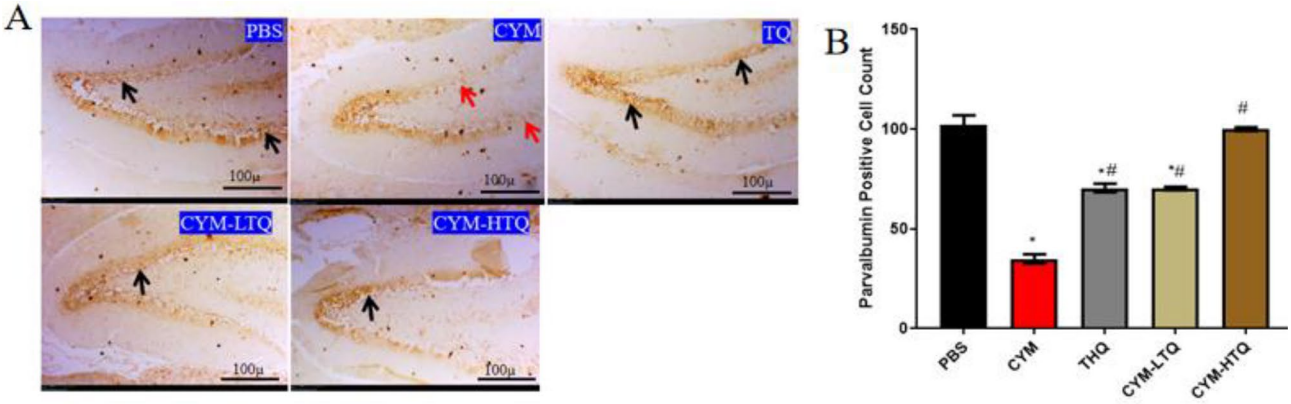

Fig. 4 GABAergic interneuron of the dentate gyrus. (**A**) immunostaining for Parvalbumin in the dentate gyrus (scale bar = 100 µm), PBS group showed numeruos Parvalbumin positive cells (black arrows). CYM group displayed low parvalbumin positive cells indicating high inactivation of GABAergic interneuron (red arrows): TQ group showed high pavalbumin positive cells (black arrows): CYM-LTQ presented high parvalbumin positive cells (black arrows) compared to CYM group: CYM-HTQ group also displayed numerous parvalbumin positive cells (black arrow) compared to CYM group. (**B**). Parvalbumin positive cell count. PBS group had significantly high parvalbumin positive cells (*$p < 0.05$) compared to CYM group. CYM group showed significant difference (#$p < 0.05$) compared to TQ, CYM-LTQ and CYM-HTQ groups ($N = 6$). PBS = phosphate-buffered saline, CYM = cypermethrin, TQ = thymoquinone, CYM-LTQ = cypermethrin followed by low dose (5 mg/kg) thymoquinone and CYM-HTQ = cypermethrin followed by High dose (10 mg/kg) thymoquinone

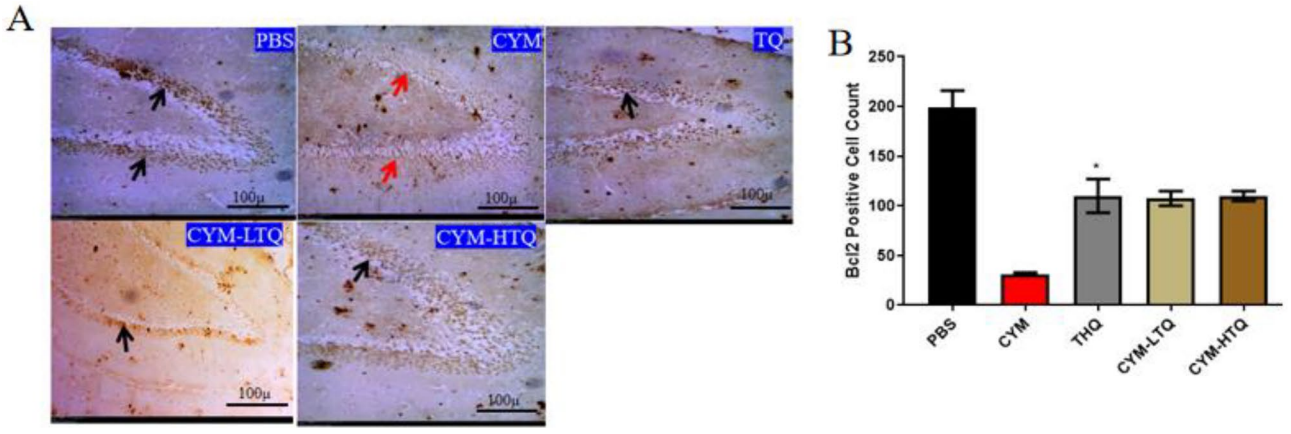

Fig. 5 Bcl2 expression in the dentate gyrus. (**A**) immunostaining for Bcl2 in the dentate gyrus (scale bar = 100 µm), PBS group showed high Bcl2 expression (black arrows). CYM group displayed low Bcl2 expression (red arrows): TQ group showed high Bcl2 expression (black arrows): CYM-LTQ presented high Bcl2 (black arrows) compared to CYM group: CYM-HTQ group also displayed numerous Bcl2 expression (black arrow) compared to CYM group. (**B**).Bcl2 positive cell count. PBS group had significantly high Bcl2 positive cells (*$p < 0.05$) compared to CYM group. CYM group showed no significant difference (#$p < 0.05$) compared to TQ, CYM-LTQ and CYM-HTQ groups ($N = 6$). PBS = phosphate-buffered saline, CYM = cypermethrin, TQ = thymoquinone, CYM-LTQ = cypermethrin followed by low dose (5 mg/kg) thymoquinone and CYM-HTQ = cypermethrin followed by High dose (10 mg/kg) thymoquinone

In this study, cypermethrin caused a reduction in the expression of the Nuclear factor erythroid 2-related factor 2 (Nrf2), a regulatory protein responsible for initiating and expressing the antioxidant system. The reduced concentration and low expression of Nrf2 cells in the dentate gyrus of CYM-exposed rats are undoubtedly responsible for the reduction in the activities of the antioxidant enzymes SOD and GSH, leading to oxidative stress as indicated by the high level of MDA in CYM-exposed rats. Due to high unsaturated fatty acids, the brain is especially susceptible to oxidative stress, which causes membrane lipid peroxidation and disrupts the normal organizational structure of brain cells, as observed in the dentate gyrus of CYM-exposed rats. Cypermethrin exposure caused neuronal damage, impaired Nissl body integrity, and induced chromatolytic-like changes in the dentate gyrus due to oxidative stress. It was observed that continuous CYM exposure not only disrupted neuronal shapes in the dentate gyrus but also induced Nrf2 expression. Since appropriate activation of Nrf2 and its nuclear translocation establishes the Nrf2/ARE complex and subsequently boosts the expression and synthesis of antioxidant enzymes, the decreased level of Nrf2 observed contributes to the lower activity of antioxidant enzymes SOD and GSH, which encourages further oxidative stress damage and raises the level of MDA. Cypermethrin

decreased the level and activity of antioxidant enzymes like SOD, GSH, and catalase (CAT) in cypermethrin-induced toxicity in the Wistar rat model of Parkinson's disease and peripheral blood. The findings of this study are indeed strengthened by earlier studies by [33–35], which reported an excessive increase in the level of MDA and reduced antioxidant capacity of SOD, CAT, GSH, and GPx, leading to increased lipid peroxidation in the peripheral blood and in the nigrostriatum of cypermethrin-exposed rats.

Intervention with thymoquinone was observed to reactivate Nrf2, as shown by the high expression of Nrf2 immunopositive cells. This increased nuclear availability of Nrf2 leads to the Nrf2/ARE complex, thereby stimulating the production and expression of antioxidant enzymes and resulting in high SOD and GSH activity, as reported in this study, and reduced MDA levels, indicating a low level of lipid peroxidation. This finding is consistent with an earlier study by [31], who found that the parent plant of thymoquinone, black seed oil, increased total antioxidant capacity and GSH while decreasing total ROS levels in rats exposed to Dichlorvos. The findings of this study are also strengthened by the study of Kanter, who reported enhancements in hepatic and pancreatic antioxidant capacities of catalase and GSH following Nigella sativa against STZ-induced diabetes in rats [36].

Apoptosis is characterized by morphological changes in cells such as nuclear pyknosis, DNA fragmentation, chromatin condensation, cytoskeleton destruction, membrane blebbing, formation of membrane apoptotic bodies phagocytosed by macrophages and and so on [37]. Continuous exposure to environmental toxins frequently causes apoptosis in cells [38] Anti-Bcl-2-stained dentate gyrus had a low expression of Bcl-2-positive cells due to cypermethrin exposure. Cypermethrin caused apoptosis in the rat brain by producing ROS and cytotoxins. Cypermethrin also induced apoptosis via mitochondrial damage, cytochrome c release, and activation of caspases 3 and 9, which are involved in both extrinsic and intrinsic apoptosis pathways [39–41]. When Bcl-2 and other anti-apoptotic proteins are cleaved by caspases following the initiation of apoptosis, their anti-apoptotic action is frequently converted to pro-apoptotic action [37]. The findings of this study are similar to the report of the previous study where a type 2 pyrethroid, deltamethrin, following its exposure in rats, induced apoptosis by increasing the level of Bax, caspase-3, cytochrome c, and decreasing the expression of Bcl-2 pro-survival proteins [42, 43]. Thymoquinone exhibits anti-apoptotic effects, as administration of thymoquinone brings about a marked increase in the expression of Bcl-2 immunopositive cells in the hippocampal dentate gyrus of the experimental rats. Bcl-2, as a pro-survival protein, has a hydrophilic carboxyl-terminal domain linked to mitochondria outer membrane and helps preserve mitochondrial integrity, preventing unnecessary cytochrome c release and caspase activation [37]. Bcl-2 prevents Bax and other pro-apoptotic genes from oligomerizing, which stimulates the release of apoptogenic molecules from the mitochondria. Apart from inhibiting Bax oligomerization, Bcl-2 directly binds and inactivates B blocks cytochrome c release, and thus inhibits adaptor molecule APAF-1 and caspase-9 activation, thereby preventing caspase cascade activation [37, 44]. In accordance with the findings of this study [45], showed that thymoquinone, in concentrations of 10 M and 20 M, prevented arsenic-induced neurotoxicity, apoptosis, and cytotoxicity by either decreasing the levels of Bax or increasing the level of Bcl-2. Also, in agreement with the data of this study, a previous study revealed that thymoquinone administration decreased p53 and Bcl-2 gene expression but increased BAD gene expression in MCF-7 cells; however, it increased the expression of Bcl-2 gene and p53 gene but decreased Bax/BAD gene expression in non-cancer HEK293 cells [46].

In the dentate gyrus, basket cells constitute the GABAergic neurons in the granule layer with the receptors localized in the molecular layer. Reduced levels of parvalbumin-positive cells in the dentate gyrus of cypermethrin-exposed rats indicate that CYM inhibits GABAergic interneurons. GABAergic interneurons constitute the inhibitory neurons in the CNS that are vital for modulating various physiological activities [47]. Reduced GABAergic interneuron expression due to CYM exposure interferes with the activity of GABAergic interneurons and disrupts excitatory and inhibitory balance in the brain. Previous studies have shown that CYM hinders the opening of the voltage-gated chloride channels and inhibits the GABA-dependent uptake of chloride ions, resulting in hyper-excitation of neuronal cells and leading to changes in the delayed rectifier voltage-dependent potassium channel, which regulates neuronal excitability [15, 48, 49].

Thymoquinone enhances parvalbumin-positive cell expression against cypermethrin toxicity. The improvement in motor functions observed in this study, which is one of the crucial functions controlled by GABAergic interneurons, complements the increased expression of the Parvalbumin-positive cells [15] As a result of thymoquinone's activation of GABA receptors, which results in hyperpolarization and inhibits neuronal activity, the N-methyl-D-aspartate NMDA receptor's enhanced glutamate functions produce prolonged neuronal stimulation [50]. According to earlier research by [51, 52], TQ increased GABA receptor activation after prilocaine-induced cardiotoxicity, epileptiform activity, and seizures in rats as well as seizures brought on by pentylenetetrazole.

Conclusion

Thymoquinone improves motor function by activating Nrf2, reducing the level of NF-κB, increasing the activities of SOD and GSH, and decreasing the concentration of MDA against cypermethrin neurotoxicity. It also enhances the expression of parvalbumin-positive cells as well as Bcl-2-positive cells. Therefore, thymoquinone can be employed to manage pyrethroid and other insecticide poisoning.

Abbreviations

ROS	Reactive oxygen species
RNA	Ribonucleic acid
mtDNA	Mutations in mitochondrial DNA
Cyc	Cytochrome c
TQ	Thymoquinone
NRF2	Nuclear factor erythroid 2-related factor 2
NOR	Novel object recognition
Nrf2	Nuclear factor erythroid 2-related factor 2
Bcl2	B-cell lymphoma 2
MCE	MedChemEpress
NAFDAC	National authority for food and drugs administration control
SOD	Superoxide dismutase
GSH	Glutathione
MDA	Malondialdehyde
E	EL-Elabscience Biotechnology
4PL	Curve-four-parameter logistic curve
ANOVA	Analysis of variance
SEM	Standard error of mean
CYM	Cypermethrin
PBS	Phosphate-buffered saline
CYM	LTQ-cypermethrin followed by low-dose thymoquinone
CYM	HTQ-cypermethrin followed by High dose thymoquinone
H and E	Hematoxylin and eosin
CYM	LTQ-cypermethrin followed by low-dose thymoquinone
CYM	HTQ-cypermethrin followed by High dose thymoquinone
NMDA	N-methyl-D-aspartate *-singleasterisk
#	Hash

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12868-024-00896-7.

Supplementary Material 1

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Author contributions

ALI: Conception, design, data collection data analysis, and interpretation, animal breeding, animal treatments, and writing of the manuscriptAAO: Conception, design, histochemical analysis and interpretation, critical revision and writing of the manuscriptFAS: Conception, design, histochemical analysis and interpretation, and critical revisionAI: Conception, design, histochemical analysis and interpretation, and critical revisionRYI: Conception, design, histochemical analysis and interpretation, and critical revision LAO: Conception, design, data collection, biochemical analysis and interpretation SAB: Conception, design, data collection, biochemical analysis and interpretationSM: Conception, design, data collection, biochemical analysis and interpretationAOA: Conception, design, histochemical analysis and interpretation, and critical revision OOO: Critical revision and referencing SMA: Conception, design, and project supervision.

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Data availability

Data submitted as supplementary file.

Declarations

Ethics approval and consent to participate

This study was approved by the University of Ilorin ethical review committee (UERC\ASN\2021\2137).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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