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Research Article

Kolaviron Attenuates Bifenthrin-INDUCED Cerebellar Neurotoxicity Through Multi-Target Modulation of NF-κB/Nrf2/p38 MAPK Signaling

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Abstract

Bifenthrin is a widely used type I pyrethroid insecticide whose exposure has been associated with neurotoxicity mediated by oxidative stress, inflammation, and apoptotic pathways. However, effective protective strategies against bifenthrin-induced cerebellar injury remain inadequately explored. This study investigated the protective effects of kolaviron against bifenthrin-induced cerebellar toxicity in rats and examined the possible involvement of oxidative, inflammatory, and apoptotic signaling pathways. Forty-eight rats were randomly assigned to six groups (n = 8): control, bifenthrin (1 mg/kg), kolaviron (200 mg/kg), kolaviron pretreatment (200 mg/kg) followed by bifenthrin (1 mg/kg), bifenthrin exposure followed by kolaviron post-treatment (200 mg/kg), and kolaviron co-treatment (200 mg/kg) with bifenthrin (1 mg/kg). Treatments were administered for 28 days. Oxidative stress markers, antioxidant enzymes, inflammatory mediators, apoptotic proteins, and relevant signaling molecules were evaluated alongside histopathological changes in the cerebellum. Bifenthrin exposure significantly decreased superoxide dismutase, catalase, glutathione, glutathione peroxidase, glutathione S-transferase, interleukin-4, and nuclear factor erythroid 2-related factor 2 (Nrf2), while significantly increasing malondialdehyde, tumor necrosis factor-α, interleukin-1β, cyclooxygenase-2, inducible nitric oxide synthase, caspase-3, p38 mitogen-activated protein kinase, and nuclear factor-κB ($P < 0.05$). Histological examination further revealed Purkinje cell degeneration and loss, vacuolation, and inflammatory cell infiltration. Kolaviron significantly ameliorated these biochemical, inflammatory, apoptotic, and histopathological alterations, with pretreatment producing the most consistent protective effects. conclusively, kolaviron attenuates bifenthrin-induced cerebellar toxicity by restoring redox homeostasis, enhancing Nrf2-associated antioxidant defense, and suppressing inflammatory and apoptotic signaling. Its protective activity may involve modulation of the Nrf 2/p38 MAPK/NF-κB signaling axis, resulting in the preservation of cerebellar architecture. These findings highlight kolaviron as a potential protective agent against bifenthrin-induced neurotoxicity.

Keywords

Bifenthrin, Cerebellum, Kolaviron, Neurotoxicity, Nrf 2, NF-κB, Oxidative Stress, Neuroinflammation

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1. Introduction

The extensive use of synthetic pyrethroid insecticides has generated increasing concern regarding their potential effects on human and environmental health. Among these compounds, bifenthrin, a type I pyrethroid insecticide, is widely applied in agricultural production, household pest management, and public health programmes because of its high insecticidal potency, broad-spectrum activity, and environmental persistence [1-3]. Although pyrethroids were initially introduced as relatively safer alternatives to organophosphate pesticides [4], growing experimental evidence indicates that prolonged or repeated exposure to bifenthrin can induce neurotoxicity through mechanisms involving oxidative stress, inflammatory responses, mitochondrial dysfunction, and neuronal degeneration [5, 6]. Consequently, understanding the molecular events associated with bifenthrin-induced brain injury and identifying effective neuroprotective agents remain important areas of toxicological research.

The cerebellum is particularly vulnerable to toxic insults because of its high oxygen consumption, abundant polyunsaturated fatty acids, and intense metabolic activity. Beyond its established role in motor coordination and balance, the cerebellum contributes to cognitive processing and emotional regulation [7]. Exposure to environmental toxicants has been shown to disrupt cerebellar cytoarchitecture, impair Purkinje cell function, and compromise neuronal communication, leading to motor dysfunction and behavioural abnormalities [8, 9]. Several studies have attributed these pathological changes to excessive reactive oxygen species (ROS) generation, lipid peroxidation, depletion of endogenous antioxidant defences, and sustained inflammatory responses following pesticide exposure [10, 11]. These findings suggest that oxidative stress and neuroinflammation represent central mechanisms underlying bifenthrin-induced cerebellar injury.

Oxidative stress and inflammation are closely interconnected biological processes that reinforce one another during toxicant-induced neuronal damage [12]. A central regulator of inflammatory signalling is nuclear factor-kappa B (NF-κB), a transcription factor that is activated by oxidative stress, xenobiotics, and inflammatory stimuli [13]. Activation of NF-κB stimulates the production of several pro-inflammatory mediators, including tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS), thereby amplifying inflammatory responses and promoting neuronal injury [14, 15]. Conversely, nuclear factor erythroid 2-related factor 2 (Nrf2) serves as a major endogenous defence system against oxidative stress by regulating the expression of antioxidant and cytoprotective proteins, including heme oxygenase-1 (HO-1), NAD (P) H quinone oxidoreductase-1 (NQO1), glutamate-cysteine ligase catalytic subunit (GCLC), and related antioxidant enzymes [16-19]. The balance between NF-κB-mediated inflammatory signalling and Nrf2-mediated antioxidant defence is therefore critical for maintaining neuronal homeostasis during exposure to environmental toxicants.

Another important signalling molecule involved in cellular stress responses is p38 mitogen-activated protein kinase (p38 MAPK). Activation of p38 MAPK occurs following oxidative stress and inflammatory stimulation, where it regulates proteins involved in inflammation, apoptosis, and cellular adaptation [20]. Excessive activation of this pathway contributes to neuronal dysfunction by enhancing inflammatory signalling and facilitating programmed cell death [21]. Importantly, p38 MAPK interacts with both NF-κB and Nrf2 signalling pathways, forming an integrated network that influences cellular responses to toxic stress [22]. Because these pathways collectively regulate oxidative injury and inflammation, alterations in their protein expression provide valuable insight into the molecular mechanisms of pesticide-induced neurotoxicity.

Natural products possessing antioxidant and anti-inflammatory properties have emerged as promising candidates for mitigating neurotoxic damage. Kolaviron, a biflavonoid complex isolated from the seeds of *Garcinia kola* Heckel, exhibits potent antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective activities [23]. Experimental evidence indicates that kolaviron reduces oxidative damage, enhances endogenous antioxidant capacity, suppresses inflammatory mediator production, and protects tissues against xenobiotic-induced injury [23]. These biological activities suggest that kolaviron may preserve cerebellar integrity during bifenthrin exposure by modulating oxidative stress and inflammatory processes.

Despite substantial evidence demonstrating the neurotoxic effects of bifenthrin and the antioxidant potential of kolaviron, the molecular events underlying kolaviron-mediated protection against bifenthrin-induced cerebellar injury remain incompletely understood. In particular, limited information is available regarding the changes in key inflammatory, antioxidant, and stress-response proteins that collectively regulate oxidative injury and neuroinflammation following bifenthrin exposure. Since these proteins represent the functional mediators of cellular responses to toxic insults, evaluating their concentrations provides important insight into the biological processes governing neuronal injury and protection. A clearer understanding of how these biomarkers respond to kolaviron treatment may therefore elucidate the mechanisms through which this natural biflavonoid preserves cerebellar integrity under pesticide-induced oxidative stress.

Accordingly, the present study investigated the neuroprotective effects of kolaviron against bifenthrin-induced cerebellar toxicity by determining the concentrations of selected protein biomarkers involved in oxidative stress, inflammation, and the NF-κB/Nrf2/p38 MAPK signalling network.

2. Materials and Methods

2.1. Chemicals

Bifenthrin (97% TC) (with batch number 20211012) was procured from Nanjing Essence Fine-Chemical Co., Ltd. China. All other reagents were of analytical grade and obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) through ND-Harris & associates Onitsha, Nigeria.

2.2. Isolation of Kolaviron

Seeds of *Garcinia kola* were obtained from a local vendor in Boki, Cross River State, Nigeria (GPS: 6.2500° N, 9.0333° E). The seeds were authenticated by Dr. Emmanuel Ekpiken, a qualified plant taxonomist at the Herbarium of the University of Cross River State, Nigeria, and a voucher specimen (Voucher No. UCS/HB/2025/012) was deposited for future reference. Prior to extraction, the seeds were screened for common mycotoxins (aflatoxins B1, B2, G1, G2) and pesticide residues, and were confirmed to be free of detectable contaminants.

The seeds were air-dried, pulverized, and kolaviron was isolated following a previously validated protocol with minor modifications [24]. Briefly, 1.5 kg of the pulverized seeds was defatted using light petroleum ether (60–80 °C) in a Soxhlet extractor for 24 h. The defatted residue was air-dried and subsequently extracted with acetone using a Soxhlet apparatus for an additional 24 h. The acetone extract was concentrated under reduced pressure at 40 °C using a rotary evaporator.

The concentrated extract was diluted with distilled water in a 1:2 (v/v) ratio and successively partitioned with ethyl acetate (6 × 250 mL). The combined ethyl acetate fractions were concentrated under reduced pressure to obtain a golden-yellow solid identified as kolaviron. The extraction yield of kolaviron was 9.4% (w/w) relative to the initial dry weight of the seeds.

Purity and structural validation of the isolated kolaviron were carried out using ^{1}H-NMR, ^{13}C-NMR, and electron ionization mass spectrometry (EI–MS). The obtained spectral data were in agreement with previously reported reference spectra for kolaviron [24], confirming compound identity. Further purity assessment was performed using high-performance liquid chromatography (HPLC), which demonstrated a purity of ≥95%, and thin-layer chromatography (TLC), which revealed a single major spot, indicating minimal impurities. The purified kolaviron was stored in airtight amber-colored vials at −20 °C until use to prevent photodegradation and oxidative degradation.

2.3. Animal Protocol

All experimental procedures involving animals were conducted in accordance with the ARRIVE 2.0 guidelines and complied with institutional and national regulations for the care and use of laboratory animals. Ethical approval was obtained from the Faculty of Basic Medical Sciences Animal Ethics Committee, University of Cross River State, Okuku Campus (Approval No.: FBMS/UNICROSS/25/10; approved on 12 March 2025).

A total of forty-eight (48) young adult male Wistar rats (*Rattus norvegicus*), aged 10–12 weeks and weighing 110–180 g at the start of the experiment, were obtained from the Animal House, Faculty of Basic Medical Sciences, University of Cross River State. All animals were sourced from the same breeding colony to ensure uniformity in age, genetic background, and health status. Following acclimatization for 7 days, rats with comparable body weights were randomly assigned to experimental groups to minimize inter-group variability.

Rats were housed in standard polypropylene cages (60 cm × 40 cm) fitted with stainless steel mesh covers, with eight animals per cage. Environmental conditions were maintained at 26 ± 2°C, relative humidity of approximately 45%, and a 12-hour light/12-hour dark cycle. Animals were provided with standard commercial pelletized rat chow (Mazuri®) and clean drinking water *ad libitum*. Cages were cleaned regularly to maintain hygienic conditions throughout the study.

Following a 7-day acclimatization period, animals were randomly assigned to six experimental groups (n = 8 per group):

1) Group I (Control): Canola oil vehicle only for 28 days.
2) Group II (Bifenthrin): Bifenthrin (1 mg/kg body weight) for 28 days.
3) Group III (Kolaviron): Kolaviron (200 mg/kg body weight) for 28 days.
4) Group IV (Kolaviron Pre-treatment): Kolaviron (200 mg/kg) for 28 days, followed immediately by bifenthrin (1 mg/kg) for an additional 28 days (total 56 days).
5) Group V (Kolaviron Post-treatment): Bifenthrin (1 mg/kg) for 28 days, followed immediately by kolaviron (200 mg/kg) for 28 days (total 56 days).
6) Group VI (Co-treatment): Bifenthrin (1 mg/kg) and kolaviron (200 mg/kg) administered concurrently for 28 days.

All compounds were administered orally by gavage once daily. Bifenthrin, being highly lipophilic, was dissolved in canola oil to ensure complete solubilization and accurate dosing. Kolaviron, the isolated biflavonoid fraction, was dissolved in a minimal volume of 0.5% dimethyl sulfoxide (DMSO) and then diluted with canola oil to achieve the desired concentration. The standardized dosing volume for all groups was 0.2 mL/100 g body weight (2 mL/kg). Vehicle control animals received an equivalent volume of canola oil to account for any potential vehicle effects. The doses of bifenthrin (1 mg/kg) and kolaviron (200 mg/kg) were selected based on pilot investigations and previous reports demonstrating sub-chronic toxicity and protective efficacy, respectively [23, 25, 26].

At the end of the experimental period, rats were euthanized

under deep anesthesia induced by intraperitoneal administration of ketamine in accordance with institutional ethical guidelines. Adequate anesthesia was confirmed by the absence of pedal withdrawal and corneal reflexes. Death was further verified by cessation of respiration and cardiac activity before tissue collection. The cerebellum were then rapidly excised, carefully trimmed of adhering fat and connective tissue, and rinsed in ice-cold normal saline to remove blood contaminants prior to further processing.

2.4. Determination of Biomarkers of Cerebellar Oxidative Stress

Cerebellar tissues were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) using a Teflon homogenizer and centrifuged at 10,000 × g for 15 min at 4 °C. The resulting supernatant was collected and used for the biochemical analyses. Superoxide dismutase (SOD) activity was determined according to the method of Zhidkova et al. (2011) [27]. Catalase (CAT) activity was measured using hydrogen peroxide as the substrate following the method of Pine et al. (1984) [28]. Reduced glutathione (GSH) concentration was determined spectrophotometrically at 412 nm according to the method of Kand'ár et al. (2007) [29]. Glutathione peroxidase (GPx) activity was determined using the method described by Saydam et al. (1997) [30]. Glutathione S-transferase (GST) activity was measured according to the method of Culley et al. (2002) [31]. Lipid peroxidation was quantified by measuring malondialdehyde (MDA) levels following the method described by Valenzuela (1991) [32].

2.5. Determination of Cerebellar Inflammatory Proteins

Testis tissues were rinsed with 1× phosphate-buffered saline (PBS), homogenized in four volumes of ice-cold 0.1 M phosphate buffer (pH 7.4) using a Teflon homogenizer, and centrifuged at 10,000 × g for 15 min at 4 °C. The resulting supernatants were collected and stored at −20 °C until analysis. After two freeze–thaw cycles to ensure complete cell membrane disruption, the homogenates were further centrifuged at 5,000 × g for 5 min at 4 °C.

The concentrations of TNF-α, IL-1β, COX-2, iNOS, IL-4, caspase-3, p38 MAPK, NF-κB, and NrF2 proteins were quantified in the supernatants using commercially available ELISA kits according to the manufacturer's instructions (CUSABIO Life Science Inc., Wuhan, China). All samples were assayed in duplicate, and the mean values were used for statistical analysis.

2.6. Histological Analysis

To minimize observer bias, histological assessments were conducted under blinded conditions, such that the histopathologist was unaware of the treatment groups during tissue processing, slide labeling, and microscopic evaluation. Following euthanasia, cerebellar tissues were immediately excised, cleared of adherent fat and connective tissue, and rinsed in ice-cold normal saline. The tissues were fixed in 10% phosphate-buffered formalin for 48 h, dehydrated through ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax according to standard histological procedures [33].

Paraffin-embedded tissues were sectioned at 5 µm thickness using a rotary microtome (Leica RM2235, Leica Microsystems, Germany). The sections were mounted on glass slides, deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E) following established protocols [34]. Stained sections were examined under a light microscope (Olympus CX31, Olympus Corporation, Tokyo, Japan), and representative photomicrographs were captured at ×400 magnifications using an attached digital camera (Olympus DP12).

2.7. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to compare the various experimental groups followed by Duncan post hoc to identify significantly different groups (GraphPad Prism statistics, version 8.0). Results are presented as mean ± standard deviation (SD). Values of $P < 0.05$ were considered statistically significant.

3. Kolaviron Suppresses Oxidative Stress Parameters in Bifenthrin-induced Cerebellar Toxicity

Bifenthrin exposure significantly disrupted cerebellar redox homeostasis compared with the normal control group. Activities of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and glutathione S-transferase (GST) were significantly reduced ($P < 0.05$), whereas malondialdehyde (MDA) concentration was significantly increased ($P < 0.05$), (Figure 1a-f). Kolaviron administration ameliorated these alterations, with pretreatment producing the most consistent restoration of antioxidant indices and reduction in MDA.

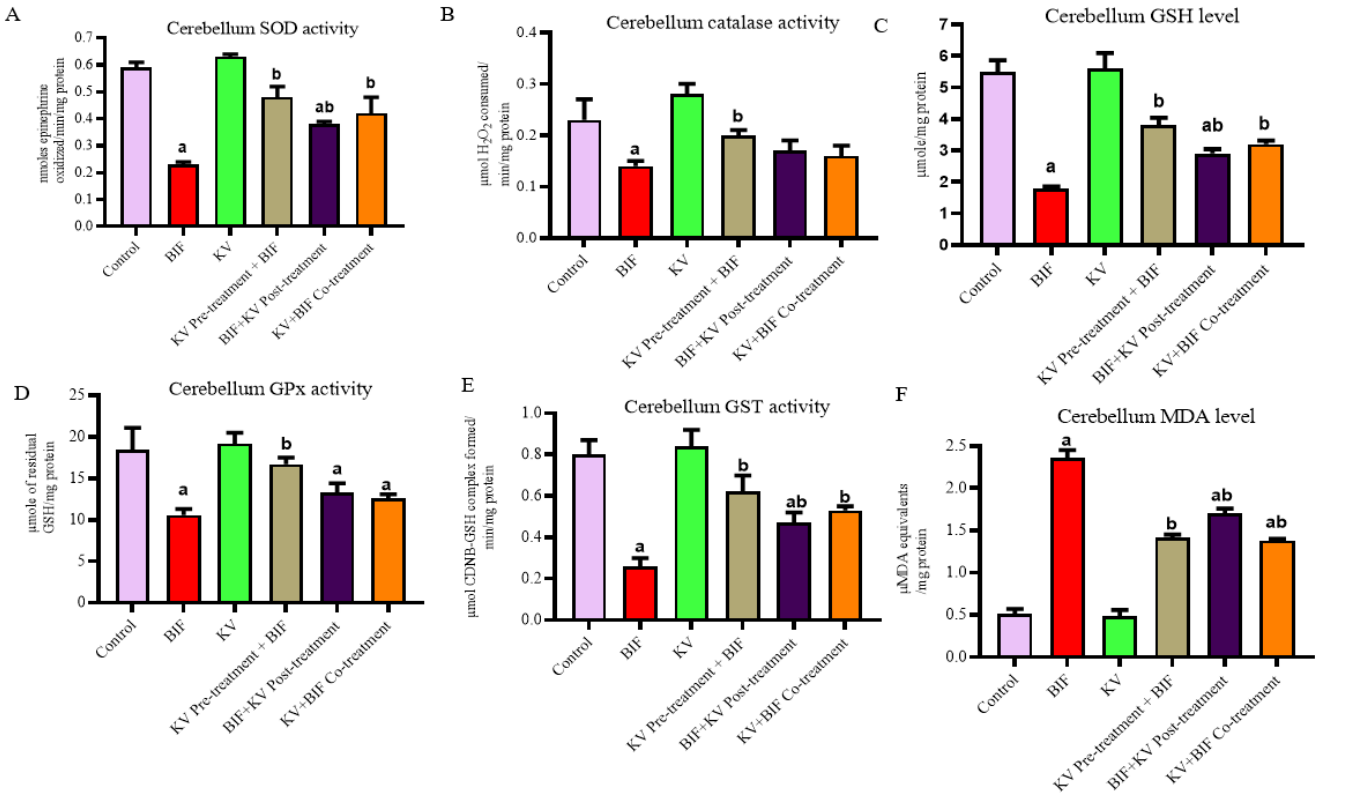
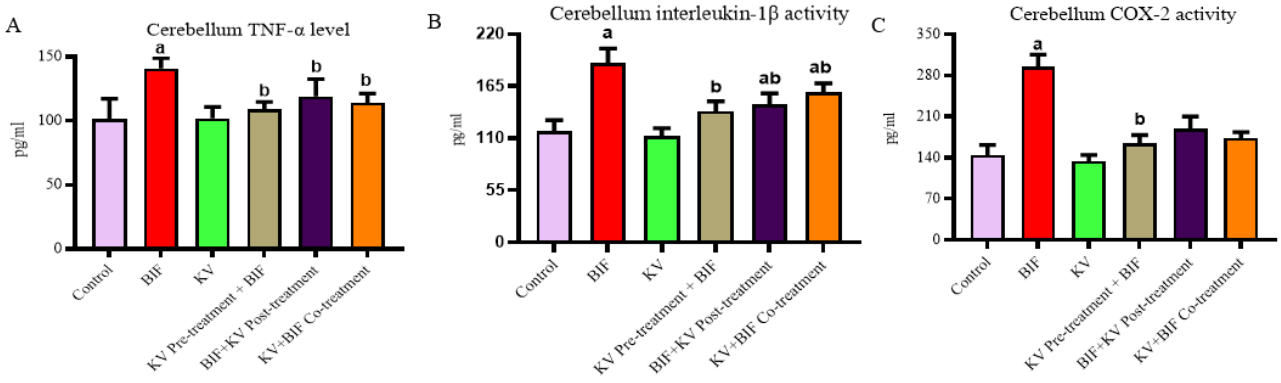


Figure 1. *Effect of kolaviron (KV) on oxidative stress biomarkers in bifenthrin-induced cerebellar neurotoxicity. (A) Superoxide dismutase (SOD), (B) catalase (CAT), (C) glutathione (GSH), (D) glutathione peroxidase (GPx), (E) glutathione S-transferase (GST), and (F) malondialdehyde (MDA). Values are expressed as mean ± SD.* a$P < 0.05$ *versus the control group;* b$P < 0.05$ *versus the bifenthrin group;* ab$P < 0.05$ *versus both control and bifenthrin groups.*

3.1. Kolaviron Attenuates Inflammatory Markers in Bifenthrin-induced Cerebellar Toxicity

Bifenthrin also elicited a significant cerebellar inflammatory response, characterized by increased tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS), together with a significant reduction in interleukin-4 (IL-4) compared with the control group ($P < 0.05$; Figure 2a–e). Kolaviron treatment attenuated these inflammatory alterations. In particular, kolaviron pretreatment significantly reduced the elevated pro-inflammatory markers and restored IL-4 relative to the bifenthrin-only group. Post-treatment and co-treatment similarly reduced several inflammatory indices, although significant differences were not observed for all markers across all treatment regimens.



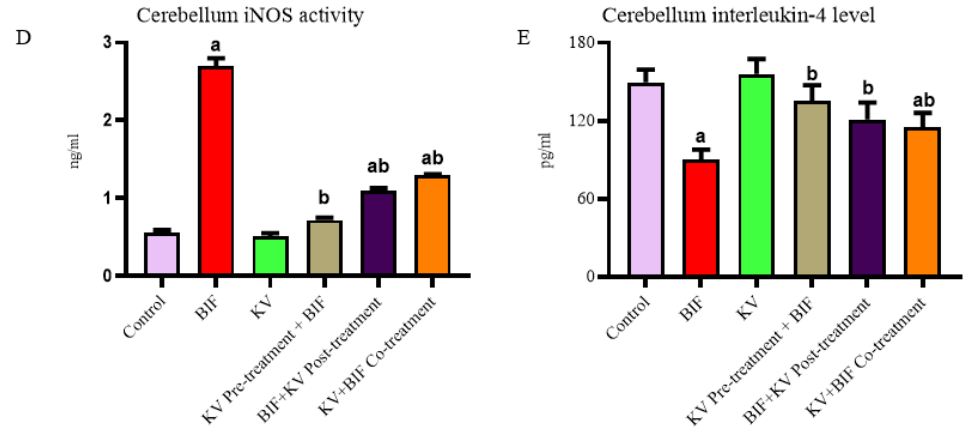


Figure 2. *Effect of kolaviron (KV) on inflammatory mediators in bifenthrin-induced cerebellar neuro toxicity. (A) Tumor necrosis factor alpha (TNF-α), (B) interleukin-1 beta (IL-1β), (C) cyclooxygenase-2 (COX-2), (D) inducible nitric oxide synthase (iNOS), and (E) interleukin-4 (IL-4). Values are expressed as mean ± SD.* a$P < 0.05$ *versus the control group;* b$P < 0.05$ *versus the bifenthrin group;* ab$P < 0.05$ *versus both control and bifenthrin groups.*

3.2. Kolaviron Promotes Apoptotic Balance in Bifenthrin-induced Cerebellar Toxicity

Furthermore, bifenthrin exposure significantly increased cerebellar caspase-3, p38 mitogen-activated protein kinase (p38 MAPK), and nuclear factor kappa B (NF-κB) activities compared with the control group ($P < 0.05$), indicating enhanced apoptotic, stress-responsive, and inflammatory signaling (Figure 4a–c). Kolaviron administration attenuated these elevations, with pretreatment showing particularly marked reductions relative to bifenthrin exposure.

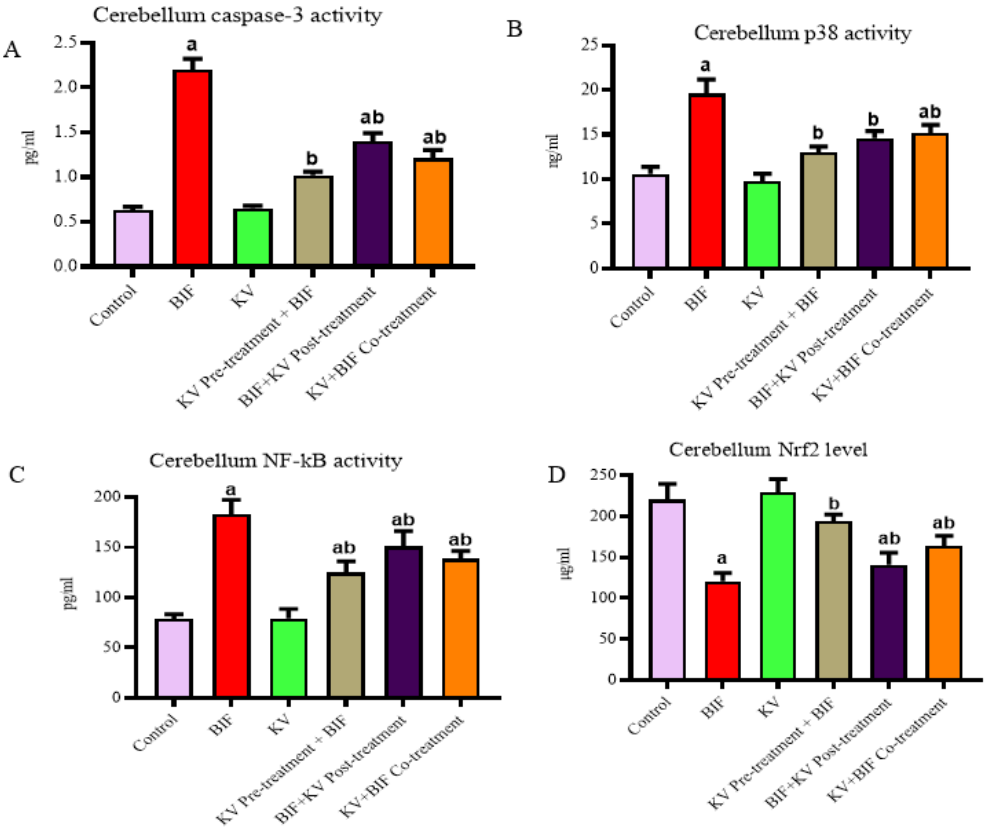


Figure 3. *Effect of kolaviron (KV) on apoptotic- and oxidative stress-related proteins in bifenthrin-induced cerebellar neuro toxicity. (A) Caspase-3, (B) p38 mitogen-activated protein kinase (p38), (C) nuclear factor kappa B (NF-κB), and (D) nuclear factor erythroid 2–related factor 2 (Nrf2). Values are expressed as mean ± SD.* a$P < 0.05$ *versus the control group;* b$P < 0.05$ *versus the bifenthrin group;* ab$P < 0.05$ *versus both control and bifenthrin groups.*

In contrast to the pattern observed for caspase-3, p38 MAPK, and NF-κB, bifenthrin exposure significantly decreased Nrf2 activity compared with the normal control group ($P < 0.05$; Figure 4d). Kolaviron administration increased Nrf2 relative to the bifenthrin-only group, with the greatest restoration observed following kolaviron pretreatment. Post-treatment and co-treatment also improved Nrf2 activity to varying degrees.

3.3. Histopathological Changes in the Cerebellum of Rats Exposed to Bifenthrin and Treated with Kolaviron

Representative photomicrographs of hematoxylin and eosin (H&E)-stained cerebellar sections (Figure 4) showing: Control, preserved cerebellar architecture with normally arranged Purkinje cells; BIF, marked Purkinje cell loss and degeneration, faintly stained nuclei, vacuolation, and dense inflammatory cell infiltration; KV, preserved cerebellar architecture; KV pretreatment + BIF, preservation of Purkinje cells with mild inflammatory cell infiltration; BIF + KV post-treatment, preserved Purkinje cells with occasional faintly stained nuclei and sparse inflammatory cell infiltration; and BIF + KV co-treatment, preservation of Purkinje cells with mild inflammatory cell infiltration. H&E stain; ×400 magnification.

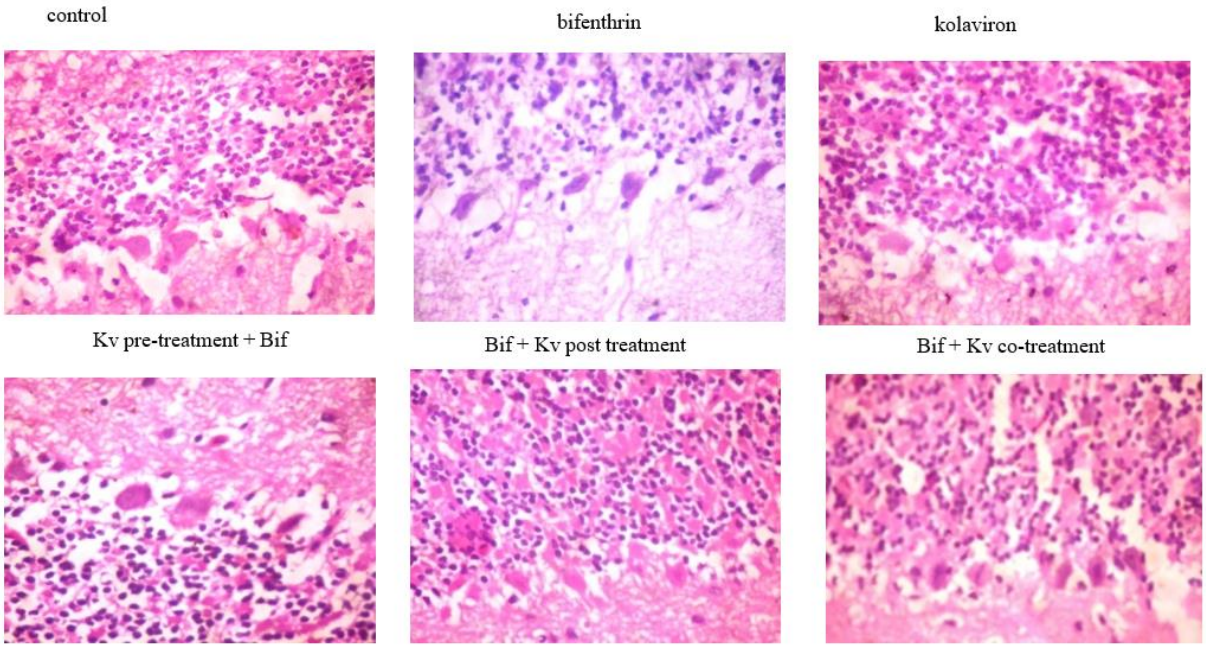


Figure 4. *Representative photomicrographs of cerebellar tissues derived from experimental groups taken at ×400 magnification Hematoxylin and Eosin stain.*

4. Discussion

The present study demonstrates that bifenthrin induces cerebellar toxicity characterized by oxidative stress, inflammation, activation of stress and apoptotic signaling, and structural damage, while kolaviron provides substantial protection against these alterations. Bifenthrin significantly reduced SOD, CAT, GSH, GPx, and GST while increasing MDA, indicating disruption of endogenous antioxidant defenses and enhanced lipid peroxidation. The brain is particularly susceptible to oxidative injury because of its high oxygen consumption, abundant polyunsaturated fatty acids, and relatively limited antioxidant capacity [35]. Thus, depletion of antioxidant defenses coupled with increased lipid peroxidation may constitute an important mechanism underlying bifenthrin-induced cerebellar injury. The ability of kolaviron to restore antioxidant indices and reduce MDA, particularly when administered before bifenthrin exposure, supports its capacity to preserve cellular redox homeostasis.

The oxidative disturbance was accompanied by a marked inflammatory response. Bifenthrin increased TNF-α, IL-1β, COX-2, and iNOS and reduced the anti-inflammatory cytokine IL-4, demonstrating a shift toward a pro-inflammatory cerebellar environment. This response may be closely associated with oxidative stress, since excessive reactive oxygen species can activate redox-sensitive inflammatory pathways [36]. Consistent with this mechanism, bifenthrin increased

NF-κB and p38 MAPK activities. NF-κB regulates several inflammatory mediators, including TNF-α, IL-1β, COX-2, and iNOS, while p38 MAPK is an important mediator of cellular responses to oxidative and inflammatory stress [37]. Kolaviron attenuated these alterations, suggesting that its protective effects extend beyond antioxidant activity to modulation of inflammatory and stress-responsive signaling. The greater effect observed with pretreatment suggests that establishing antioxidant and anti-inflammatory protection before toxicant exposure may be more effective than intervention after injury has developed.

Bifenthrin also increased caspase-3 activity, indicating enhanced apoptotic signaling. Oxidative stress and persistent inflammatory signaling can promote mitochondrial dysfunction and activation of apoptotic pathways [38]; therefore, increased caspase-3 may represent a downstream consequence of the oxidative and inflammatory disturbances induced by bifenthrin. Conversely, kolaviron reduced caspase-3 activity, supporting an anti-apoptotic component of its neuroprotective action.

An important finding was the suppression of Nrf2 activity by bifenthrin and its restoration by kolaviron. Nrf2 is a major regulator of cellular antioxidant and cytoprotective responses [39]. Its reduction following bifenthrin exposure, together with depletion of antioxidant defenses and increased MDA, suggests impairment of endogenous antioxidant signaling. In contrast, restoration of Nrf2 by kolaviron coincided with improved antioxidant status and reduced p38 MAPK, NF-κB, and caspase-3 activities. These coordinated changes suggest that the protective effect of kolaviron may involve enhancement of Nrf2-associated antioxidant defense alongside suppression of oxidative stress-mediated inflammatory and apoptotic signaling. However, direct involvement of these pathways would require further confirmation using pathway-specific inhibitors or molecular approaches.

The histopathological findings corroborated the biochemical and molecular observations. Bifenthrin caused marked Purkinje cell loss and degeneration, vacuolation, faint nuclear staining, and inflammatory cell infiltration, demonstrating substantial cerebellar structural injury. In contrast, the control and kolaviron-only groups maintained normal cerebellar architecture. Kolaviron pretreatment, post-treatment, and co-treatment substantially preserved Purkinje cells and reduced inflammatory infiltration, with pretreatment showing particularly pronounced protection. The correspondence between preserved cerebellar morphology and improvements in oxidative, inflammatory, and apoptotic indices provides complementary evidence for the neuroprotective effect of kolaviron.

5. Conclusion

Conclusively, these findings indicate that bifenthrin-induced cerebellar toxicity is associated with disruption of redox homeostasis, suppression of Nrf2 activity, activation of p38 MAPK/NF-κB-associated inflammatory signaling, and increased caspase-3-mediated apoptotic activity, culminating in structural cerebellar damage. Kolaviron attenuated these alterations, with pretreatment generally providing the most consistent protection. The findings therefore suggest that kolaviron may protect the cerebellum against bifenthrin-induced neurotoxicity through coordinated antioxidant, anti-inflammatory, and anti-apoptotic actions.

Abbreviations

ANOVA	Analysis of Variance
ARE	Antioxidant Response Element
BIF	Bifenthrin
CAT	Catalase
COX-2	Cyclooxygenase-2
DMSO	Dimethyl Sulfoxide
EI–MS	Electron Ionization Mass Spectrometry
ELISA	Enzyme-Linked Immunosorbent Assay
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
GST	Glutathione S-Transferase
H&E	Hematoxylin and Eosin
HPLC	High-Performance Liquid Chromatography
HO-1	Heme Oxygenase-1
IL-1β	Interleukin-1 Beta
IL-4	Interleukin-4
IL-6	Interleukin-6
iNOS	Inducible Nitric Oxide Synthase
KV	Kolaviron
MDA	Malondialdehyde
NF-κB	Nuclear Factor Kappa B
NQO1	NAD (P) H Quinone Oxidoreductase 1
Nrf2	Nuclear Factor Erythroid 2-Related Factor 2
p38 MAPK	P38 Mitogen-Activated Protein Kinase
PBS	Phosphate-Buffered Saline
ROS	Reactive Oxygen Species
SD	Standard Deviation
SOD	Superoxide Dismutase
TLC	Thin-Layer Chromatography
TNF-α	Tumor Necrosis Factor Alpha
NMR	Nuclear Magnetic Resonance

Author Contributions

Ujong Peter Ujong: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft

Mbang Edet Ibor: Formal Analysis, Investigation, Validation, Writing – review & editing

Precious Takim Mgbe: Data curation, Investigation, Resources

Edward Odey Emuru: Data curation, Formal Analysis, Investigation, Methodology, Validation

Runyi Bassey Ben: Investigation, Methodology, Validation, Visualization

Vivian Peter-Ujong: Methodology, Project administration, Supervision, Validation, Writing – review & editing

Conflicts of Interest

The authors declare no conflict of interest.

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