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Research Article

Kolaviron and *Bryophyllum pinnatum* Attenuate $AlCl_3$-Induced Memory Impairment by Modulating Oxidative Stress, Astrogliosis, and Bcl-2/Nrf2 Signaling

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Abstract

Background: Chronic exposure to aluminium chloride ($AlCl_3$) damages the hippocampus and impairs cognition through oxidative stress, reactive astrogliosis, and neuronal apoptosis. Phytochemicals with antioxidant and anti-inflammatory activity are increasingly considered as candidate neuroprotectants, yet evidence supporting combined plant-derived interventions that target these convergent pathways remains scarce. Purpose: This study examined whether Kolaviron, a biflavonoid complex from Garcinia kola seeds, and an ethanolic extract of Bryophyllum *pinnatum* (CRA), administered individually or in combination, attenuate $AlCl_3$-induced hippocampal injury and cognitive impairment in Wistar rats, and whether their protective actions converge on the Nrf2 signalling pathway. Methods: Seventy adult male Wistar rats were randomly assigned to seven groups of ten: vehicle control, $AlCl_3$ (100 mg/kg), Kolaviron alone (200 mg/kg), CRA alone (600 mg/kg), $AlCl_3$ + Kolaviron, $AlCl_3$ + CRA, and $AlCl_3$ + Kolaviron + CRA. All agents were administered by oral gavage daily for fourteen days. Spatial learning and memory were assessed using the Morris water maze. Hippocampal homogenates were assayed for superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). Haematoxylin and eosin staining was used to evaluate cytoarchitecture, and immunohistochemistry quantified glial fibrillary acidic protein (GFAP), B-cell lymphoma 2 (Bcl-2), and nuclear factor erythroid 2-related factor 2 (Nrf2) expression in the CA3 subfield. Data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Results: $AlCl_3$ exposure prolonged escape latency, lowered SOD and CAT activities, raised MDA, produced neuronal loss with pyknotic and vacuolated cells, increased GFAP immunoreactivity, and reduced both Bcl-2 and Nrf2 expression in CA3. Co-treatment with Kolaviron or CRA reversed each of these alterations ($p < 0.05$ versus $AlCl_3$ alone), and the combined regimen produced the most consistent restoration across behavioural, biochemical, histological, and immunohistochemical endpoints, frequently returning values close to control levels. Conclusion: Kolaviron and B. *pinnatum* protect the rat hippocampus against $AlCl_3$-induced damage by restoring antioxidant defences, attenuating astrogliosis, preserving

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Bcl-2 expression, and activating Nrf2 signalling, with the combination conferring broader protection than either agent alone. These findings support further investigation of these phytochemicals as candidates against environmental neurotoxicant-induced neurodegeneration.

Keywords

Aluminum, Oxidative Stress, Nrf2 Pathway, Hippocampus, Kolaviron

1. Introduction

The global prevalence of neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), continues to rise, posing a significant public health challenge. There is mounting evidence that chronic exposure to environmental neurotoxicants is strongly linked to neurodegeneration [1]. Aluminium chloride ($AlCl_3$), a ubiquitous environmental pollutant found in drinking water, food, and pharmaceuticals, readily accumulates in the hippocampus, a region critical for learning and memory, causing structural damage, impaired neurogenesis, and cognitive deficits in animal models [2].

A key mechanism of aluminium neurotoxicity is oxidative stress [3, 4]. Although not redox-active, aluminium disrupts antioxidant defences, creating an imbalance between reactive oxygen species (ROS) and antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), resulting in lipid peroxidation reflected by elevated malondialdehyde (MDA) levels [3]. Chronic oxidative stress drives neuronal dysfunction and death [5] and prompts neuroinflammation, marked by glial cell activation. Glial fibrillary acidic protein (GFAP), a marker for astrocyte activation, signals chronic neuroinflammation when elevated [6, 7]. The neuroinflammatory environment sustained by activated glial cells accelerates neurodegeneration [8, 9].

$AlCl_3$ also induces neuronal apoptosis, as shown by pyknotic nuclei and widespread degeneration in hippocampal regions [2, 10]. This pro-apoptotic effect is linked to decreased anti-apoptotic proteins like B-cell lymphoma 2 (Bcl-2). The nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway, the main regulator of antioxidant and anti-inflammatory defences, provides key neuroprotection. Nrf2 activation increases antioxidant enzymes, inhibits pro-inflammatory pathways, and can boost Bcl-2. Dysregulation of Nrf2 can contribute to chronic neurodegeneration [11].

Kolaviron, a biflavonoid complex from Garcinia kola seeds, and Bryophyllum *pinnatum* ethanolic extract have exhibited antioxidant and anti-inflammatory properties in various disease models [7, 12]. Kolaviron has been shown to modulate the Nrf2 pathway [13]. Bryophyllum *pinnatum* contains diverse bioactive constituents including quercetin, kaempferol glycosides, and bufadienolides, which may contribute to neuroprotection through direct radical scavenging, metal ion chelation, and anti-inflammatory activity [12, 14]. Since aluminium-induced neurotoxicity involves multiple concurrent pathological processes, a combination of agents targeting these pathways through different mechanisms may offer broader neuroprotection than either agent alone [11]. By testing each compound individually and in combination, this study design allows determination of whether either agent is independently effective and whether their combination produces additive benefits.

This study aims to elucidate the neuroprotective mechanisms of Kolaviron and B. *pinnatum* extract against $AlCl_3$-induced neurotoxicity in Wistar rats, hypothesising that these extracts will ameliorate $AlCl_3$-induced pathological alterations by: (1) restoring hippocampal antioxidant capacity; (2) attenuating neuroinflammation through reduced GFAP immunoreactivity; (3) preventing neuronal cell death by upregulating Bcl-2; and (4) improving neuronal integrity. Cognitive performance was assessed using the Morris water maze.

2. Materials and Methods

2.1. Animals and Ethical Statement

Seventy (70) adult albino Wistar rats were housed in clean plastic cages under standard laboratory conditions (12-hour light/dark cycle, ~25–28°C, with free access to standard rodent chow and water) and acclimatised for 14 days prior to experimentation. Animals were randomly divided into seven groups of 10 rats each, and all treatments were administered for 14 consecutive days. All procedures were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Ethical approval was obtained from the Faculty of Basic Medical Sciences Ethical Committee, University of Cross River State (Approval number: CRUT/FBMC/REC/23/003), following the Declaration of Helsinki principles.

2.2. Chemicals and Reagents

Aluminum chloride ($AlCl_3$) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Kolaviron (KV) was prepared from Garcinia kola seeds following the established extraction

protocol described by Farombi et al. [15] and administered at 200 mg/kg body weight. An ethanolic extract of B. *pinnatum* (family Crassulaceae, hereafter referred to as CRA) was prepared from dried leaves via cold maceration in 70% ethanol and administered at 600 mg/kg. All other reagents were of analytical grade.

2.3. Experimental Protocol

The 70 rats were randomly assigned to seven groups (n = 10 per group):

Group 1 (Control): Vehicle only
Group 2: $AlCl_3$ (100 mg/kg) only
Group 3: Kolaviron (200 mg/kg) only
Group 4: CRA extract (600 mg/kg) only
Group 5: $AlCl_3$ (100 mg/kg) + Kolaviron (200 mg/kg)
Group 6: $AlCl_3$ (100 mg/kg) + CRA extract (600 mg/kg)
Group 7: $AlCl_3$ (100 mg/kg) + Kolaviron (200 mg/kg) + CRA extract (600 mg/kg)

All treatments were administered once daily by oral gavage for 14 days. Dosage selection and treatment duration were based on prior studies [2, 14, 16, 17].

2.4. Morris Water Maze (MWM) Test

Spatial learning and memory were assessed using the Morris water maze (MWM) [18]. A circular pool (~120 cm diameter) was filled with opaque water containing a hidden escape platform (~1 cm below the surface). Each rat received 3–4 trials per day with an inter-trial interval of ~5 min. Escape latency was recorded; a decrease over successive trials indicated learning. Behavioural tests were conducted between 07: 00–11: 00 h, and observers were blinded to treatment groups.

2.5. Animal Sacrifice and Tissue Collection

Twenty-four hours after the last treatment, rats were deeply anaesthetised (100 mg/kg ketamine with 10 mg/kg xylazine) and euthanised by cervical dislocation. Brains were excised and dissected on ice. For histological and immunohistochemical analyses, one hemisphere was fixed in 10% neutral-buffered formalin for at least 48 hours. For biochemical assays, the hippocampus was rapidly isolated, flash-frozen, and stored at –80°C. Hippocampal homogenates were prepared in ice-cold 50 mM Tris–KCl buffer (pH 7.4) and centrifuged at 10,000 × g for 15 minutes at 4°C [15, 19].

2.6. Tissue Processing and Immunohistochemical Analysis

Fixed brain tissues were processed using standard paraffin-embedding procedures. Serial coronal sections (~5 µm) were stained with haematoxylin and eosin (H&E) [20]. For immunohistochemistry, sections underwent heat-mediated antigen retrieval in citrate buffer (0.01 M, pH 6.0), endogenous peroxidase quenching (0.3% H_2O_2), and blocking with normal serum. Sections were incubated overnight at 4°C with primary antibodies: anti-GFAP (1: 1000, ThermoFisher #16825-1-AP), anti-Bcl-2 (1: 200, Novus Biologicals #NB100-56098), and anti-Nrf2 (1: 200, Thermo Fisher #PA1-38312). Horseradish peroxidase (HRP)-conjugated secondary antibody detection was followed by 3,3′-diaminobenzidine (DAB) chromogen visualization and haematoxylin counterstaining. Negative controls omitting primary antibody confirmed specificity [21, 22].

2.7. Image Acquisition and Analysis

Stained slides were examined under a digital bright-field microscope (OMAX 40–2000X). Photomicrographs of the hippocampus (CA3) were captured at ×400 magnification. Immunopositive cells were quantified using ImageJ software (NIH, USA) "Cell Counter" plugin across five non-overlapping fields per section (≥3 sections per animal), following the method of Ijomone and Nwoha [23].

2.8. Biochemical Assays

SOD activity was determined by the method of Misra and Fridovich [24] as adopted by Ebokaiwe et al. [19]. CAT activity was measured following the protocol of Claiborne [25]. Lipid peroxidation was estimated by the thiobarbituric acid reactive substances (TBARS) method [19]. All assays were performed in triplicate and normalised to protein concentrations determined by the Lowry method.

2.9. Statistical Analysis

Data were analysed using GraphPad Prism (version 8.0) and presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used; $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of Kolaviron and/or *B. pinnatum* on Spatial Learning and Memory

One-way ANOVA revealed significant changes in spatial learning and memory ($F_{6,28} = 11.81$; $p < 0.0001$). $AlCl_3$-exposed rats exhibited significantly prolonged escape latencies compared to controls ($p < 0.05$). Both Kolaviron and *B. pinnatum* significantly shortened escape latency in $AlCl_3$-treated rats ($p < 0.05$). The combined treatment produced the greatest improvement, with escape times approaching control values. Neither Kolaviron-alone nor *B. pinnatum*-alone affected escape latency compared to controls (Figure 1).

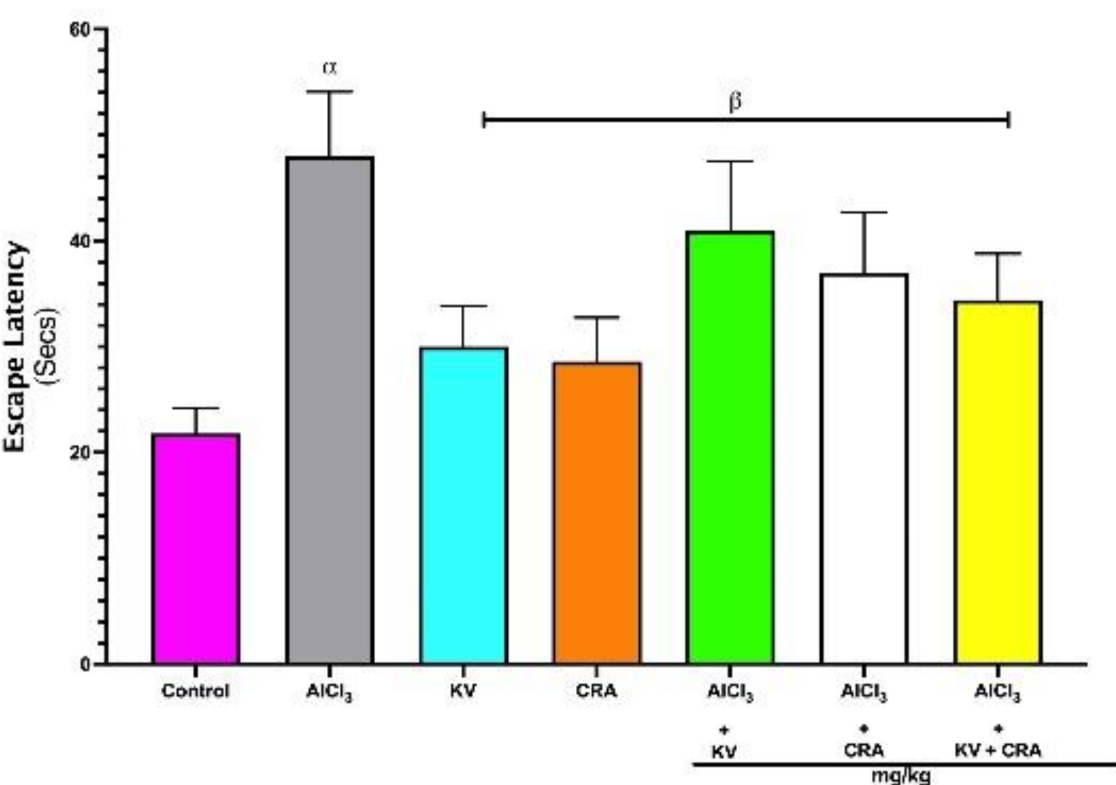
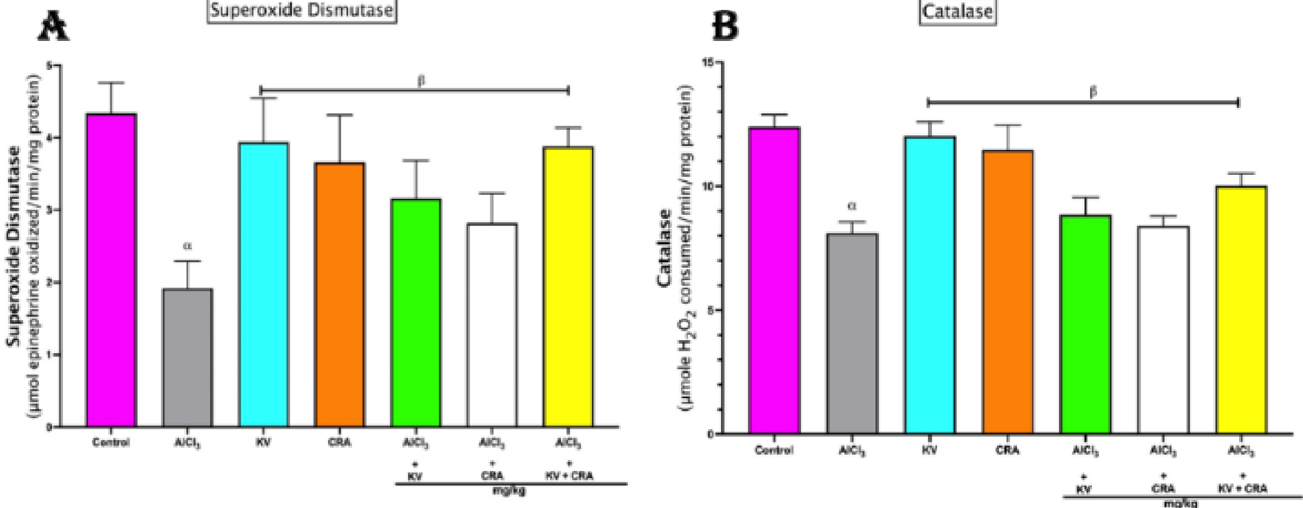


Figure 1*. Escape Latency of rats exposed to Aluminium Chloride (AlCl3) and treated with Kolaviron and/or Crassulaceae extract. Bars represent mean ± SEM; n=5. α indicates significant difference from control (p<0.05); β indicates significant difference from AlCl3 only group (p<0.05). One-way ANOVA followed by Tukey post-hoc test. (AlCl3 - Aluminium Chloride, KV - Kolaviron, CRA - Crassulaceae).*

3.2. Effect of Kolaviron and/or *B. pinnatum* on Antioxidant Status and Lipid Peroxidation

ANOVA revealed significant differences in SOD ($F_{6,28}$ = 14.63; p < 0.0001), CAT ($F_{6,21}$ = 34.48; p < 0.0001), and MDA ($F_{6,28}$ = 12.95; p < 0.0001). $AlCl_3$ significantly reduced SOD and CAT activities and elevated MDA compared to controls (p < 0.05). Co-treatment with Kolaviron or *B. pinnatum* significantly restored SOD and CAT activities and reduced MDA levels (p < 0.05). The combination was particularly effective, nearly normalising all markers. Neither extract alone altered these parameters relative to controls (Figure 2).



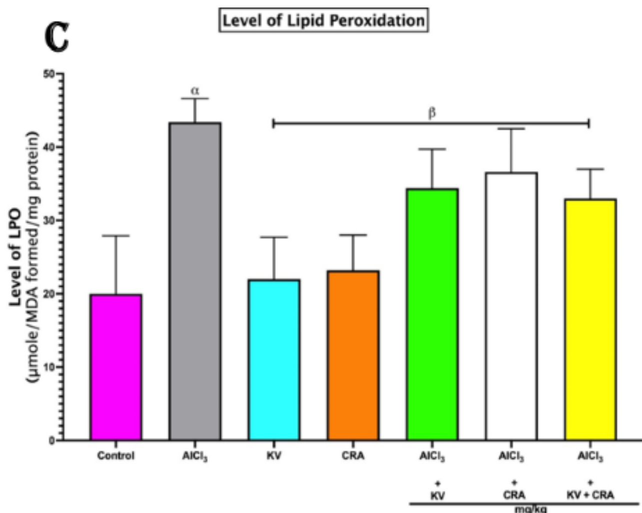


Figure 2. Influence of Kolaviron and/or Crassulaceae extract on antioxidant status and lipid peroxidation in the brain tissue of rats exposed to Aluminium Chloride ($AlCl_3$). Bars represent mean ± SEM; n=5. α indicates significant difference from control (p<0.05); β indicates significant difference from $AlCl_3$ only group (p<0.05). One-way ANOVA followed by Tukey post-hoc test. ($AlCl_3$ - Aluminium Chloride, KV - Kolaviron, CRA - Crassulaceae).

3.3. Attenuating Effects of Kolaviron and *B. pinnatum* on $AlCl_3$-induced Neuropathology in the Hippocampus

Control rats (Figure 3A) showed normal hippocampal cytoarchitecture with densely packed pyramidal neurons. $AlCl_3$-only rats (Figure 3B) displayed extensive neuronal loss, pyknotic nuclei, eosinophilic cytoplasm, and vacuolation. Treatment groups (Figure 3C, 3D) showed preserved hippocampal morphology with higher neuronal density and fewer degenerating cells. The combined treatment group (Figure 3G) showed the greatest protection, approaching normalcy. Extract-only groups (Figure 3E, 3F) displayed normal histology.

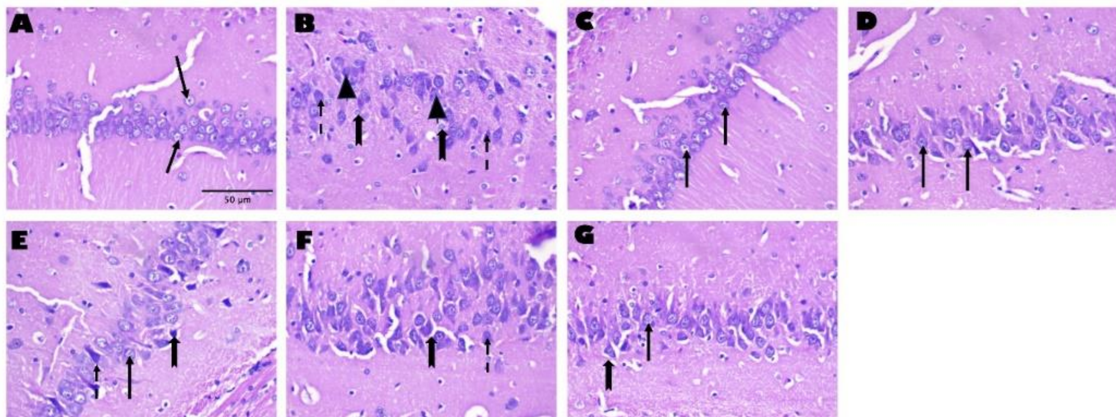


Figure 3. Hematoxylin and eosin (H&E) staining of the hippocampus (CA3) in rat brain tissue sections following exposure to Aluminium Chloride ($AlCl_3$) and treatment with Kolaviron and/or Crassulaceae extract. Magnification: 400×; Scale bars: 50µm. Arrows: intact neurons; Dashed arrow: neurons with prominent eosinophilic cytoplasm; Notched arrows: neuronal swelling and/or vacuolation; Arrow head: Pyknotic nuclei.

3.4. Effect of Kolaviron and/or *B. pinnatum* on GFAP Immunoreactivity

GFAP immunoreactivity was significantly different across groups ($F_{6,28} = 31.99$; $p < 0.0001$). $AlCl_3$ provoked increased GFAP immunoreactivity with hypertrophic astrocytes (Figure 4B). Kolaviron and *B. pinnatum* co-treatments significantly reduced GFAP expression compared to $AlCl_3$-only ($p < 0.05$). The combined treatment showed the strongest reduction, with GFAP levels near control (Figure 4H). Neither extract alone increased GFAP staining.

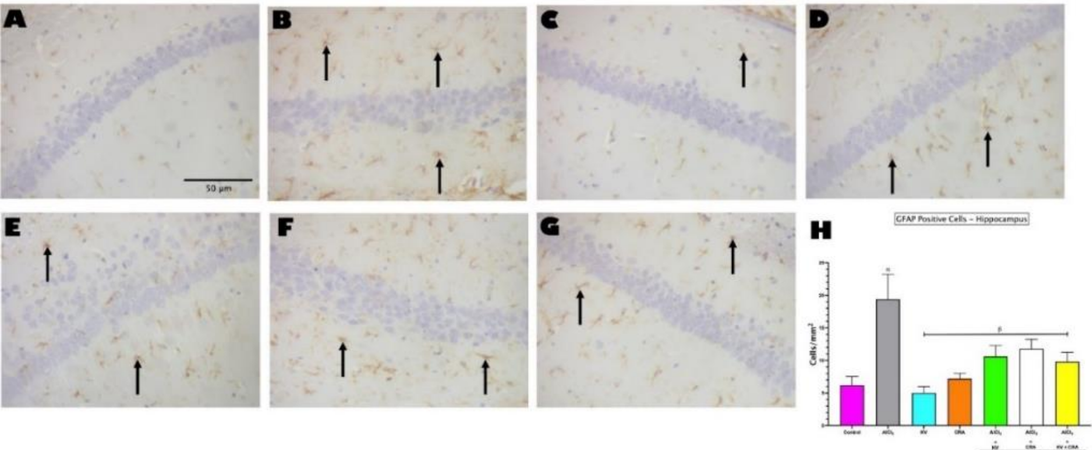


Figure 4. *Photomicrograph showing immunohistochemical staining of GFAP-positive Cells in the hippocampus (A-G). Magnification: 400 x; Scale bars: 50µm. Black arrows indicate GFAP-positive Astrocytes. H shows Image J analysis of GFAP-positive cells in the Hippocampus (CA3). Bars represent mean ± SEM; n=5. α indicates significant difference from control (p<0.05); β indicates significant difference from $AlCl_3$ only group (p<0.05). One-way ANOVA followed by Tukey post-hoc test. ($AlCl_3$ - Aluminium Chloride, KV - Kolaviron, CRA - Crassulaceae).*

3.5. Effect of Kolaviron and/or *B. pinnatum* on Nrf2 Immunoexpression

Nrf2 immunoreactivity differed significantly across groups ($F_{6,28} = 49.36$; $p < 0.0001$). $AlCl_3$ greatly diminished Nrf2 immunostaining, with predominantly faint cytoplasmic staining (Figure 5B). Co-treatment with Kolaviron or *B. pinnatum* restored Nrf2 expression, with many neurons displaying intense nuclear immunostaining (Figure 5E, 5F). The combined therapy elicited the most pronounced Nrf2 response, fully restoring levels to control values ($p < 0.05$ vs. $AlCl_3$-only; Figure 5H).

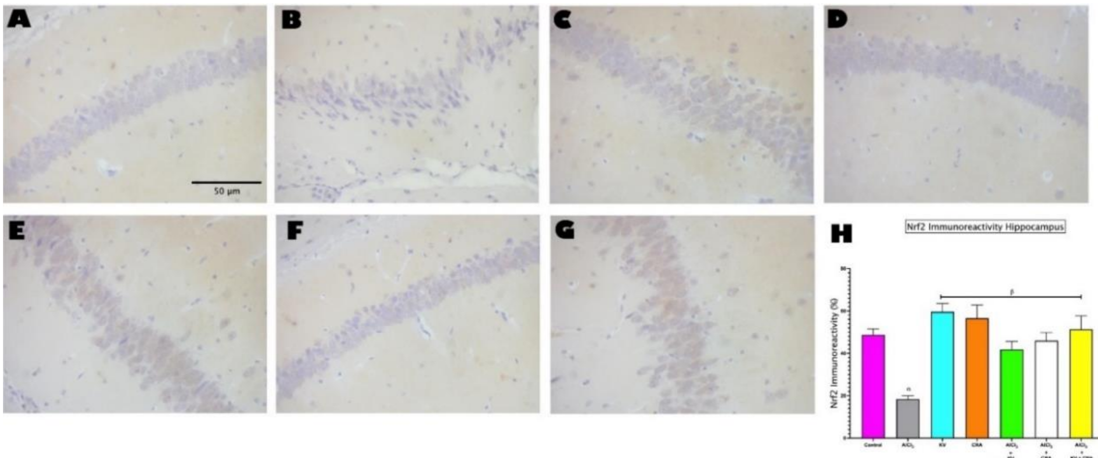


Figure 5. *Photomicrograph showing Immunoreactivity of Nrf2 in the hippocampus (A-G). Magnification: 400 ×; Scale bars: 50µm. H shows Image J analysis of Nrf2 Immunoreactivity in the Hippocampus (CA3). Bars represent mean ± SEM; n=5. α indicates significant difference from control (p<0.05); β indicates significant difference from $AlCl_3$ only group (p<0.05). One-way ANOVA followed by Tukey post-hoc test. ($AlCl_3$ - Aluminium Chloride, KV - Kolaviron, CRA - Crassulaceae).*

3.6. Effect of Kolaviron and/or *B. pinnatum* on Bcl-2 Immunoexpression

Bcl-2 levels differed significantly across groups ($F_{6,28} = 5.347$; $p = 0.0009$). $AlCl_3$ drastically reduced Bcl-2 immunoreactivity (Figure 6B). Both Kolaviron and *B. pinnatum* co-treatments maintained significantly higher Bcl-2 levels than $AlCl_3$-only ($p < 0.05$). The combined treatment restored Bcl-2 to near-normal levels, with no significant difference from controls (Figure 6H).

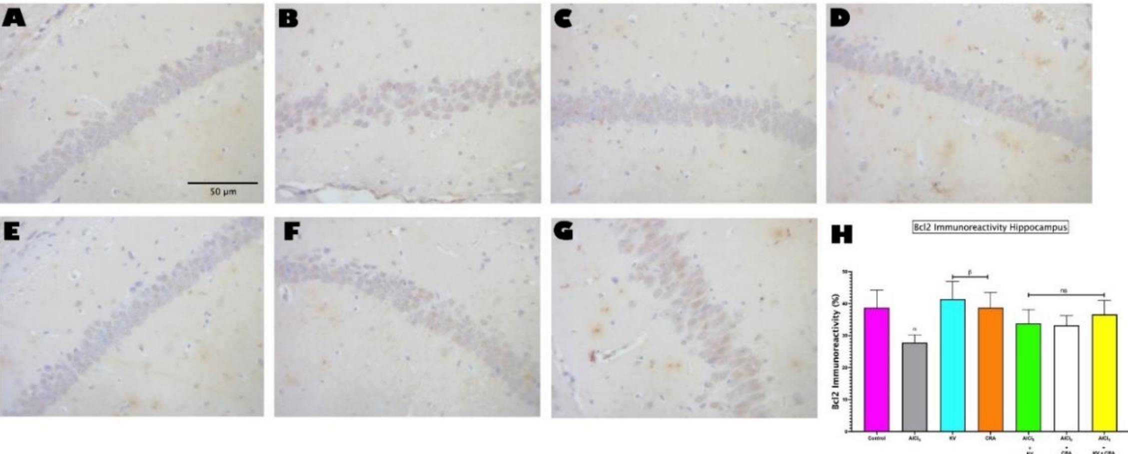


Figure 6. *Photomicrograph showing Immunoreactivity of Bcl2 in the hippocampus (A-G). Magnification: 400 ×; Scale bars: 50µm. H shows Image J analysis of Bcl2 Immunoreactivity in the Hippocampus (CA3). Bars represent mean ± SEM; n=5. α indicates significant difference from control (p<0.05); β indicates significant difference from* $AlCl_3$ *only group (p<0.05). One-way ANOVA followed by Tukey post-hoc test. (*$AlCl_3$ *- Aluminium Chloride, KV - Kolaviron, CRA - Crassulaceae).*

4. Discussion

$AlCl_3$ exposure impaired hippocampus-dependent spatial learning, as evidenced by prolonged escape latencies in the Morris water maze. Both Kolaviron and *B. pinnatum* significantly ameliorated these cognitive deficits, with co-treated rats approaching control performance. Kolaviron has been shown to counteract lipopolysaccharide-induced cognitive impairment through its antioxidative properties, and its neuroprotective effects against aluminium-induced neurotoxicity suggest utility in disorders associated with heavy metal exposure.

Biochemical analyses confirmed oxidative stress as central to $AlCl_3$ neurotoxicity. $AlCl_3$ exposure depressed hippocampal SOD and CAT activities and elevated MDA, reproducing the antioxidant enzyme depletion and lipid peroxidation consistently reported in aluminium-intoxicated rodents [26]. Kolaviron and *B. pinnatum* co-treatment restored enzyme activities and lowered MDA, with the combination producing near-complete normalisation. These effects are mechanistically complementary: Kolaviron activates Nrf2/ARE-dependent transcription of phase II antioxidants including HO-1 [27], while the flavonoid constituents of *B. pinnatum* directly scavenge ROS and chelate trivalent metal cations through their hydroxyl and keto functions.

Histopathological examination revealed severe CA3 neurodegeneration after $AlCl_3$, with pyramidal cell loss, pyknosis and vacuolar degeneration. This pattern reproduces findings from aluminium models in which chronic $AlCl_3$ exposure reduces pyramidal cell-body density specifically in CA1 and CA3 alongside cytoskeletal and metabolic protein dysregulation [28]. Kolaviron and *B. pinnatum* preserved cytoarchitecture, with more intact pyramidal somata and fewer pyknotic profiles, reflecting coupled antioxidative and anti-apoptotic activity. Preservation of CA3 integrity carries functional weight, since the recurrent CA3-CA3 network supports pattern completion and rapid encoding of episodic memory, and loss of these neurons disrupts the autoassociative circuitry on which hippocampal-dependent learning depends [29].

Immunohistochemistry showed marked upregulation of GFAP in $AlCl_3$-treated rats, with hypertrophic astrocyte morphology indicative of reactive astrogliosis. Aluminium drives this response through cell-cycle entry and proliferative expansion of GFAP-positive astrocytes, an effect dose-dependently linked to long non-coding RNA-mediated regulation of GFAP expression [30]. Kolaviron and *B. pinnatum* reduced GFAP immunoreactivity and restored a thinner, more ramified astrocyte phenotype. Quercetin, the dominant flavonoid in *B. pinnatum*, has been shown to prevent astrocyte uptake of pathological protein aggregates and to block the downstream astrogliosis and synaptic dysfunction that follows [31]. By interrupting the feed-forward loop linking ROS, cytokines and reactive glia, both treatments preserved neuronal integrity in the CA3 subfield.

The Nrf2 pathway emerged as central to the observed neuroprotection. $AlCl_3$ suppressed Nrf2 in hippocampal neurons, consistent with the notion that Nrf2 downregulation contributes to neurodegeneration [1, 32]. Our quantification focused on nuclear Nrf2 immunoreactivity, representing the functionally active pool. Upon activation, Nrf2 dissociates from Keap1, translocates to the nucleus, and binds antioxidant response elements [11]. The marked increase in nuclear Nrf2-positive neurons in treated groups indicates genuine pathway activation. Kolaviron's restoration of Nrf2 aligns with prior studies identifying it as an Nrf2 activator [13, 33]. *B. pinnatum* flavonoids are also known Nrf2/ARE modulators [12]. The combined regimen produced the highest Nrf2 activation, reflecting

additive effects. Nrf2 activation confers enhanced ROS clearance, increased glutathione synthesis, and suppression of pro-inflammatory gene expression. Nrf2 crosstalk with inflammatory pathways can inhibit NF-κB and inflammasome activation [6], partly explaining the reduced astrogliosis observed.

$AlCl_3$ significantly downregulated Bcl-2, tilting the balance towards pro-apoptotic signalling. Bcl-2 preserves mitochondrial integrity and prevents Cytochrome C release; its loss is consistent with prior observations of aluminium-induced apoptosis [2, 10]. Both Kolaviron and *B. pinnatum* preserved Bcl-2 expression, with the combination restoring Bcl-2 to near-normal levels. Kolaviron has demonstrated anti-apoptotic effects in neurodegenerative models [7]. Nrf2 activation may directly augment Bcl-2 expression, as Bcl-2 is an Nrf2-responsive gene with antioxidant response elements in its promoter [33]. The parallel increases in Nrf2 and Bcl-2 in treated groups are consistent with this relationship.

Bcl-2 and Nrf2 pathways converge at the level of transcriptional control. Under basal conditions, Keap1 sequesters Nrf2 for ubiquitin-mediated degradation. Oxidative stress releases Nrf2, allowing nuclear translocation and binding to AREs with small Maf proteins [11]. The Bcl-2 promoter contains a functional ARE at −3148 to −3140 that is directly bound by Nrf2. This upregulates Bcl-2, limiting cytochrome c release and caspase-3/7 activation [34]. Thus, Nrf2 coordinates antioxidant defence while restraining intrinsic apoptosis. Our findings align with this transcriptional framework. $AlCl_3$ reduced hippocampal Nrf2 and Bcl-2 in CA3, with elevated MDA, reduced SOD and CAT, increased GFAP, and neuronal loss. These changes reflect a shared upstream lesion, aluminium-induced oxidative stress that suppresses Nrf2-ARE signalling. Loss of Nrf2 lowers both antioxidant enzyme expression and Bcl-2 transcription. This leaves the hippocampus vulnerable to oxidative injury and apoptosis. Restoration by Kolaviron and *B. pinnatum* indicates reactivation of this common regulatory axis. Kolaviron is a biflavonoid complex comprising GB1, GB2, and kolaflavanone. In BV2 microglia, it stabilises Nrf2, promotes nuclear translocation, enhances ARE activity, and induces HO-1; Nrf2 knockdown abolishes these effects [27]. Its catechol-rich structure supports Keap1 cysteine modification and Nrf2 release [11]. Nrf2 upregulation by Kolaviron is also observed in the MPTP model [13]. Taken with our data, Kolaviron likely acts upstream at Keap1. This drives Nrf2-dependent transcription of antioxidant enzymes and Bcl-2. *Bryophyllum pinnatum* acts via a chemically distinct but convergent pathway. Its extract is rich in quercetin and kaempferol glycosides, with minor bufadienolides [14]. Quercetin stabilises Nrf2, promotes nuclear accumulation, and drives ARE-dependent transcription, alongside antioxidant and metal-chelating actions [35]. Chelation is critical in $AlCl_3$ toxicity, limiting Al^{3+} bioavailability before neuronal uptake. This complement intracellular Nrf2 activation. Consistent restoration of antioxidant status and reduced aluminium burden has been observed in $AlCl_3$-treated models [14]. This complementary action explains the additive effect of co-administration. Kolaviron activates intracellular Nrf2, while *B. pinnatum* provides metal chelation and membrane-level radical control. Both converge on Nrf2-dependent transcription of antioxidant enzymes and Bcl-2. They act on the same pathway from distinct upstream points. The CA3 profile reflects this, with maximal Nrf2, elevated Bcl-2, reduced GFAP, and improved behaviour. This pattern is consistent with dual engagement of a single protective axis.

5. Conclusion

Kolaviron and *Bryophyllum pinnatum* extract provide substantial neuroprotection against aluminium-induced hippocampal injury by activating Nrf2 signalling, counteracting oxidative stress, reducing reactive astrogliosis, and upregulating Bcl-2, resulting in preserved hippocampal cytoarchitecture and reversal of cognitive deficits. Although this study offers proof-of-concept in an acute toxicity model, limitations include the translational challenges of rodent models and the lack of long-term toxicity or pharmacokinetic data. Advancement toward therapeutic application will require human clinical trials and detailed molecular investigations.

Abbreviations

AD	Alzheimer's Disease
$AlCl_3$	Aluminium Chloride
ANOVA	Analysis of Variance
ARE	Antioxidant Response Element
ARRIVE	Animal Research Reporting of In Vivo Experiments
Bcl-2	B-cell lymphoma 2
CA3	Cornu Ammonis 3 (Hippocampal Subfield)
CAT	Catalase
CRA	Crassulaceae (Bryophyllum *Pinnatum* Ethanolic Extract)
DAB	3,3′-Diaminobenzidine
GFAP	Glial Fibrillary Acidic Protein
H&E	Haematoxylin and Eosin
HRP	Horseradish Peroxidase
Keap1	Kelch-like ECH-associated Protein 1
KV	Kolaviron
MDA	Malondialdehyde
MWM	Morris Water Maze
NF-κB	Nuclear Factor Kappa B
NIH	National Institutes of Health
Nrf2	Nuclear Factor Erythroid 2-related Factor 2
PD	Parkinson's Disease
ROS	Reactive Oxygen Species
SEM	Standard Error of the Mean
SOD	Superoxide Dismutase
TBARS	Thiobarbituric Acid Reactive Substances

Author Contributions

Rademene Sunday Oria: Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing

Bolaji Samuel Mesole: Conceptualization, Formal Analysis, Investigation, Resources, Writing – review & editing

Ujong Gabriel Otu: Conceptualization, Formal Analysis, Investigation, Resources, Supervision, Writing – review & editing

Kingsley Ekpe Akpang: Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing

Saviour God'swealth Usin: Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing

Cynthia Nyen Tangban: Formal Analysis, Investigation, Methodology, Resources, Supervision, Writing – original draft

Sesugh Ugese: Formal Analysis, Investigation, Resources

Comfort Andokie Ugi Ugbong: Formal Analysis, Investigation, Resources

Chidera Michael Nwodo: Formal Analysis, Investigation, Resources

Oluchi Priscilla Uwazurike: Formal Analysis, Investigation, Resources

Chinecherem Marvelous Obidinanwa: Formal Analysis, Investigation, Resources

Margaret Ikanobi Michael: Formal Analysis, Investigation, Resources

Data Availability Statement

All data are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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