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# Kolaviron Attenuates Cuprizone-Induced Hippocampal Structural and Biochemical Impairment in a Rat Model of Schizophrenia-associated Neuropathology

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## ABSTRACT

Schizophrenia and related psychiatric disorders are often characterized by hippocampal dysfunction and demyelination. Cuprizone (CPZ), a copper chelator, is commonly used to induce neurotoxicity and oxidative stress in rodent models to mimic these dysfunctions. This study investigated the neuroprotective potential of Kolaviron (Kv), a biflavonoid complex from *Garcinia kola*, against CPZ-induced hippocampal damage. Twenty-eight male Wistar rats were divided into four groups: Control (corn oil), CPZ (0.2% diet), Kv (200 mg/kg), and Kv+CPZ. Following 42 days of treatment, hippocampal health was assessed through biochemical assays for oxidative stress (SOD, GPx, and MDA), and histological evaluation using H&E, Cresyl Fast Violet (Nissl profiling), and GFAP immunohistochemistry. The results show that CPZ administration significantly decreased antioxidant enzyme activities (SOD and GPx) and increased lipid peroxidation (MDA levels) in the hippocampus. Pyknotic changes and disorganized pyramidal neurons were evident in the CA1-CA3 regions, and significant chromatolysis. Furthermore, increased GFAP-positive reactive astrocytes were seen as a result of astrogliosis induced by CPZ. Conversely, concurrent administration of Kolaviron significantly mitigated these effects, restoring antioxidant levels, reducing MDA, and preserving the morphological integrity of pyramidal neurons and astrocytes. In conclusion, Kolaviron exhibits significant neuroprotective properties against cuprizone-induced hippocampal impairment. Its ability to suppress oxidative stress, maintain Nissl substance, and prevent reactive astrogliosis suggests that Kolaviron may be a viable therapeutic candidate for managing neurodegenerative and psychiatric disorders associated with hippocampal oxidative damage.

**Keywords:** kolaviron, cuprizone, hippocampus, oxidative stress, neuroprotection

## INTRODUCTION

The hippocampus has a distinct role in memory encoding and retrieval. It enables the brain to differentiate associations between items and record event sequences, making it necessary for the formation of relational, episodic, and historical memory[1]. Each human hippocampus has about 10 million neurons[2]. The hippocampus is organized into five regions: cornu ammonis (CA) sectors 1-4 and dentate gyrus (DG). About 90% vast majority of hippocampal neurons are massive, pyramidal-shaped glutamatergic neurons (principal cells). The remaining 10% of hippocampal neurons are non-pyramidal, GABAergic neurons[3,4]. The two types of hippocampal neurons generate a complex balance of excitation and inhibition. The pyramidal cell layer contains the majority of hippocampal neurons, while the other two layers of the hippocampus (the stratum oriens and the stratum radiatum/lacunosum/moleculare) contain few neurons. The hippocampus was activated in an object identification memory test in research using intracranial event-related potential (ERP) recordings[5]. There is convincing evidence for hippocampal dysfunction in amnesia, dementia, and epilepsy. In contrast, although hippocampal structural and functional abnormalities have been reported in schizophrenia, the cellular and biochemical mechanisms underlying these alterations remain incompletely understood. Memory impairment in schizophrenia has been associated with hippocampal dysfunction. In schizophrenia, there is insufficient γ-aminobutyric acid (GABA)ergic inhibition of

hippocampal neurons[6,7]. The aberrant expression of GABAergic genes and proteins, as well as abnormal hippocampal activity, reported in studies of glucose metabolism and blood flow, provide significant evidence for this concept[8,9].

Cuprizone is a well-known copper chelator that targets mature oligodendrocytes, which are in charge of neuromyelination[10-12]. The mechanism through which cuprizone exerts this action has been somewhat elucidated. It has been shown to promote demyelination in the brain by generating oxygen radicals, which leads to oxidative stress and death in adult oligodendrocytes[13]. Cuprizone has been shown to increase hydrogen peroxide and malondialdehyde levels, as well as apoptosis in oligodendrocytes, with a corresponding decrease in SOD and GPx activity in the brain of mice [12,14].

Kolaviron is a defatted ethanol extract from the seeds of *Garcinia Kola* (GK). It is a mixture of three compounds - Garcinia biflavonoid GB1, GB2, and Kola flavanone in a ratio of 2:2:1[15]. Various studies have highlighted the numerous health benefits of kolaviron. The seed also has anti-inflammatory, antimicrobial, antidiabetic, and antiviral[15] as well as antiulcer properties[16]. Its antioxidant properties limit the oxidative conversion of amino acids by reactive oxygen species to other damaging fatty acid products[17]. A few works have explored the antioxidative potential of kolaviron in preventing neurotoxicity. It was also reported that kolaviron inhibited acetylcholinesterase activities in the brain, and therefore suggested that it could have a place in the management of neurodegenerative disorders, especially those associated with cholinergic dysfunction[18]. The protective roles of kolaviron in mitigating neurotoxicity, especially via its antioxidant activities, have also been well documented[19,20]. Furthermore, previous studies have demonstrated the neuroprotective effects of Kolaviron against cuprizone-induced neurotoxicity, including its ability to attenuate oxidative stress and preserve neuronal integrity in the prefrontal cortex[13]. However, the effects of Kolaviron on cuprizone-induced hippocampal structural and biochemical alterations have received comparatively limited attention. Given the central role of the hippocampus in cognitive processing and its vulnerability to oxidative and cellular injury, further characterization of the hippocampal response to cuprizone and the potential protective effects of Kolaviron is warranted. Therefore, the present study investigated whether Kolaviron could attenuate cuprizone-induced oxidative stress, neuronal structural alterations, Nissl substance loss, and reactive astrogliosis in the hippocampus of male Wistar rats. We hypothesized that cuprizone would induce hippocampal oxidative and structural injury, whereas Kolaviron co-treatment would mitigate these alterations.

## MATERIALS AND METHODS

Twenty-eight male Wistar rats, 9 weeks old, were purchased from a private animal holding at Flower Garden, Tanke, Ilorin. Animals were acclimatized for 7 days in the animal holding facilities of the Faculty of Basic Medical Sciences, University of Ilorin, Nigeria, where they had easy access to chow and water. Ethical approval was sought for and obtained from the College of Health Sciences Ethical Committee, University of Ilorin, Nigeria. Animal handling and protocols were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978). *Garcinia kola* seeds were procured from a market in Ilorin, Nigeria, and verified at the herbarium of the Botany Department, Faculty of Life Sciences, University of Ilorin with verification number 'UILH/001/1217'. Cuprizone, 3′3′-Diaminobenzidine tetrachloride and methenamine silver intensification kits salts were procured from Sigma-Aldrich (Germany). Phosphate-buffered solution (PBS; pH 7.0) was freshly prepared. Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Malondialdehyde (MDA) assay kits were purchased from Abcam®, USA. Rat anti-GFAP was acquired from Cell Signaling Technologies, Massachusetts, USA.

### Isolation and identification of Kolaviron from *Garcinia kola*

The seeds were air-dried at room temperature (28-30°C) for 3 weeks. Subsequently, Kolaviron was isolated from the dry seed of kola and characterized according to the method described by Farombi in 2009[21]. The dried seeds were pulverized into fine powder using an electric blender. Extraction from powdered seeds was done by using light petroleum ether (boiling point (bp) 40-60 ºC) in a Soxhlet extractor placed in an electric water bath for 24 h. Thereafter, the defatted, dried marc was repacked and extracted with acetone (b.p 56-60°C) in a water bath for 14 h. The resultant extract was then concentrated and diluted twice its volume with distilled water and then extracted with ethyl acetate (7.250ml). The concentrated ethyl acetate fraction gave a yellow solid, which is referred to as Kv. The purity and identity of Kv were determined by subjecting it to thin-layer chromatography using silica gel GF 254-coated plates and a solvent mixture of methanol and chloroform in a ratio of 1: 4 v/v. The separation revealed the presence of 3 bands, which were viewed under UV light at a wavelength of 254 nm with RF values of 0.48, 0.71, and 0.76. The total yield of Kv was 7.3%, and it was kept at a temperature of 4°C before and after each use.

**Animal grouping and treatment**

Kv was dissolved (80 mg/mL) in corn oil (Carlini, ALDI Inc., Batavia), a solvent that totally dissolves it and allows for oral administration. Administration of treatment solutions (corn oil and Kv) to rats across all groups was done orally using a modified oral cannula. Rats were randomly grouped into four (4) labeled groups A-D (n = 7). A received corn oil (0.5ml) daily for 42 days; B consumed a CPZ (0.2%) containing diet for 42 days; C received Kv (200 mg/kgBw) daily for 42 days; and D received Kv (200 mg/kgBw) and consumed a CPZ (0.2%) containing diet for 42 days.

**Tissue preparation**

Following the final administration of the treatment, animals (n=2) designated for histological and immunohistochemical analyses were euthanized with ketamine (20mg/kg) and subjected to transcardial perfusion. A flush of 50 mL of 0.4 M phosphate-buffered saline (PBS; pH 7.4) was followed by 500 mL of 4% paraformaldehyde (PFA). The brain was then excised, rinsed in 0.25 M sucrose three times for 5 min each, and post-fixed in 4% PFA for 24 h, after which the tissues were stored in 30% sucrose at 4 °C until further processing. Rats used (n=5) for biochemical studies were sacrificed by cervical dislocation; then excised, rinsed in 0.25 M sucrose 3 times for 5min each, and placed in 30% sucrose at 4 ° C. Coronal sections were made to expose the hippocampus, following which they were processed routinely to obtain paraffin wax-embedded blocks for histology and antigen retrieval immunohistochemistry. Histological demonstration of hippocampal cytoarchitecture and Nissl profiling were carried out in paraffin wax-embedded sections, which were stained in hematoxylin and eosin(H&E) and Cresyl Fast Violet using the methods described by Fischer and Bancroft, respectively[22, 23].

**Immunohistochemistry**

Serial sections of the hippocampus (15 μm) were taken from paraffin blocks, with protein cross-linkages removed in the sections by applying 0.1%trypsin for 20 min at room temperature to activate the antigens. Hydrogen peroxide was used to block endogenous peroxidase, while 5% bovine serum albumin (BSA) was used to reduce nonspecific protein reactions. Diluted primary antibody was added to each slide (500 mL) and incubated overnight at 4 ° C. Anti-GFAP primary antibodies dilution was done in blocking buffer (10% calf serum with 1% BSA and 0.1% Triton X-100 in0.1 M PBS): was diluted at 1: 100. Following this, secondary biotinylated antibody was desalted and diluted in PBS (pH 8.0) prior to its application on tissue sections. Incubation with secondary antibody was done in a humidity chamber for 30 min at room temperature. Immunogenic reaction was developed using 3′3 ′ DAB and intensified using a methenamine silver kit (used according to the manufacturer's instructions). The sections were counterstained in hematoxylin, and subsequently treated in 1% acid alcohol to reduce the counterstain intensity. Histology and IHC images were acquired using an Olympus binocular research microscope (Olympus, New Jersey, USA) connected to an Amscope Camera (5.0 MP).

**Preparation of tissue sample for biochemical analyses**

Hippocampal tissue homogenate was prepared with ice-cold 0.25 M sucrose (Sigma) with an automated homogenizer at 4 ° C. The tissue homogenate was centrifuged for 10 min in a microfuge at 12,000 rpm to obtain the supernatant containing organelle fragments and synaptosomes. The supernatants were aspirated into plain labeled glass cuvettes placed on ice. SOD, MDA, and GPx activities were assayed according to the manufacturer's instructions in the assay kit pack.

**Data analysis**

All quantitative data were analyzed using GraphPad Prism version 6. Data were expressed as mean ± SEM. Differences among the four experimental groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparisons test. Statistical significance was set at $p < 0.05$.

## RESULTS

The levels of SOD and GPx enzymes, which are at the forefront of the CNS's antioxidant defense system, were measured in the hippocampus (Fig. 1 & 2). The level of SOD was lower in the cuprizone group compared to the Kolaviron group ($p < 0.005$) and the control ($p < 0.005$); the group co-treated with cuprizone and Kolaviron had a significant reduction compared to the control ($p < 0.05$) and the Kolaviron-treated group (p 0.005), but significantly higher than the SOD activity in the cuprizone-treated rats ($p < 0.01$). Similarly, GPx activity was reduced in the cuprizone group when compared to the control ($p < 0.005$), the Kolaviron group ($p > 0.05$), and the cuprizone and Kolaviron group ($p < 0.005$). These findings suggested that cuprizone caused an increase in the production of reactive oxygen species, which could lead to oxidative stress in the brain. We also measured MDA, a lipid peroxidation marker.

Cuprizone treatment resulted in increased lipid peroxidation when compared to the control ($p < 0.005$) and the Kolaviron groups ($p > 0.05$), as expected; treatment with Kolaviron following cuprizone intoxication significantly decreased lipid peroxidation. These findings suggest that the increased reactive species generation caused by cuprizone resulted in increased lipid peroxidation in the hippocampus.

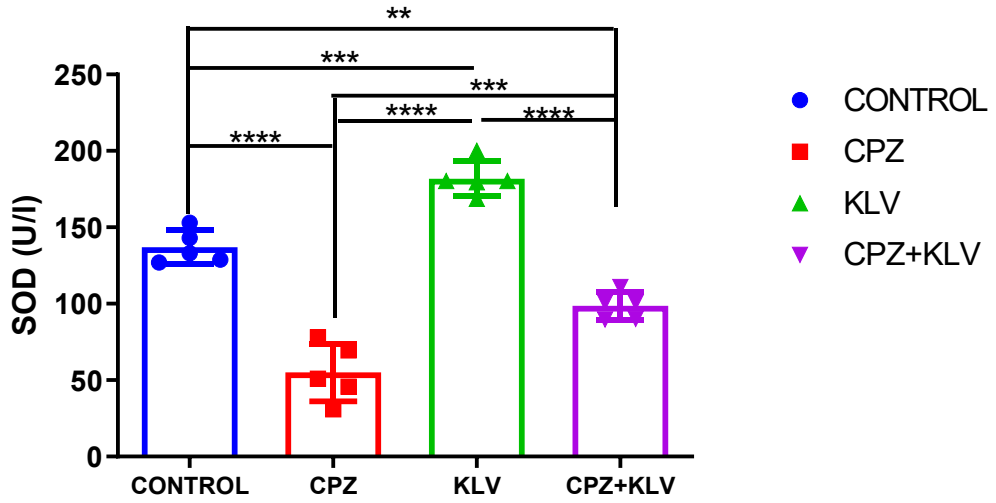


**Figure 1:** Hippocampal SOD expression in rat lysates across treatment groups shows similar SOD activities in the control (A) and Kv (C) groups, but a significant increase in SOD expression in the Kv group when compared to the control. The CPZ group (B) exhibits a significant decrease in SOD expression and activities. However, rats treated concurrently with Kv and CPZ (D) exhibit an up-regulation of SOD activities and expression that is similar to the control; however, there is a significant difference when compared to the control and Kv groups. Values are expressed as the mean ± SE (n = 5 per group and * = $p < 0.05$; ** = $p < 0.01$).

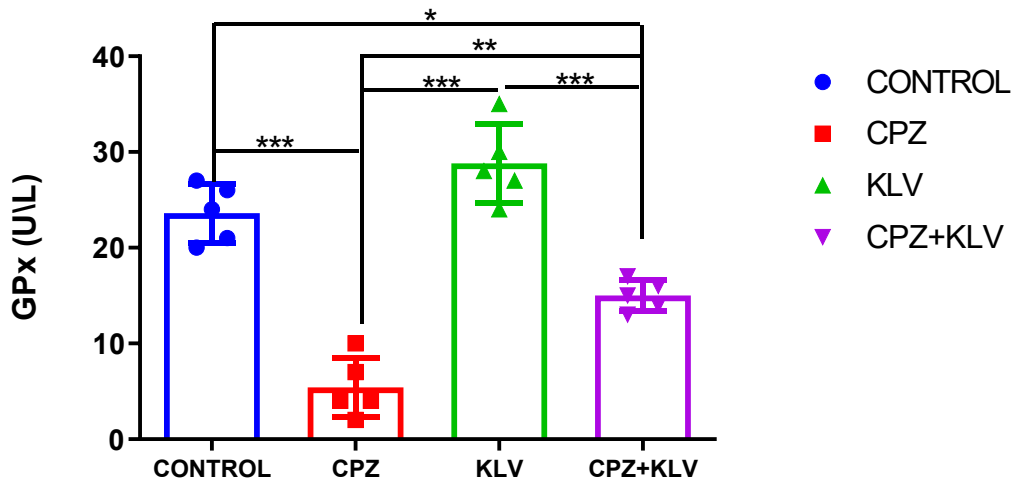


**Figure 2:** The expression of GPx in hippocampus lysates across treatment groups shows a similar level of GPx within the hippocampal homogenate; however, there was a slightly significant elevation of GPx in the Kv group when compared to the control. The hippocampal homogenate of rats intoxicated with CPZ showed a highly significant decrease in GPx expression. When Kv and CPZ were administered concurrently, there was a significant increase in GPx levels in hippocampus lysates when compared to the CPZ-intoxicated group. Values are expressed as the mean ± SE (n = 5 per group and * = $p < 0.05$; ** = $p < 0.01$).

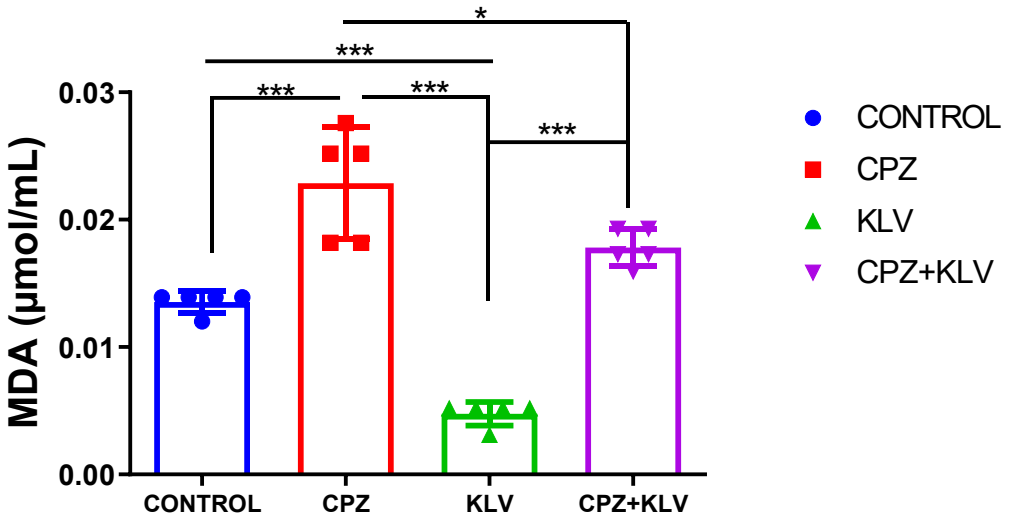


**Figure 3:** MDA expression in the hippocampus differed between treatment groups. Hippocampal lysates from rats treated with Kv show significantly lower levels of MDA expression when compared to other groups. When compared to the other treatment groups, CPZ treatment resulted in a highly significant up-regulation of MDA levels in the hippocampus homogenate. Kv treatment in conjunction with CPZ intoxication results in a significant decrease in MDA levels in the hippocampus. Values are expressed as the mean ± SE (n = 5 per group and * = $p < 0.05$; ** = $p < 0.01$).

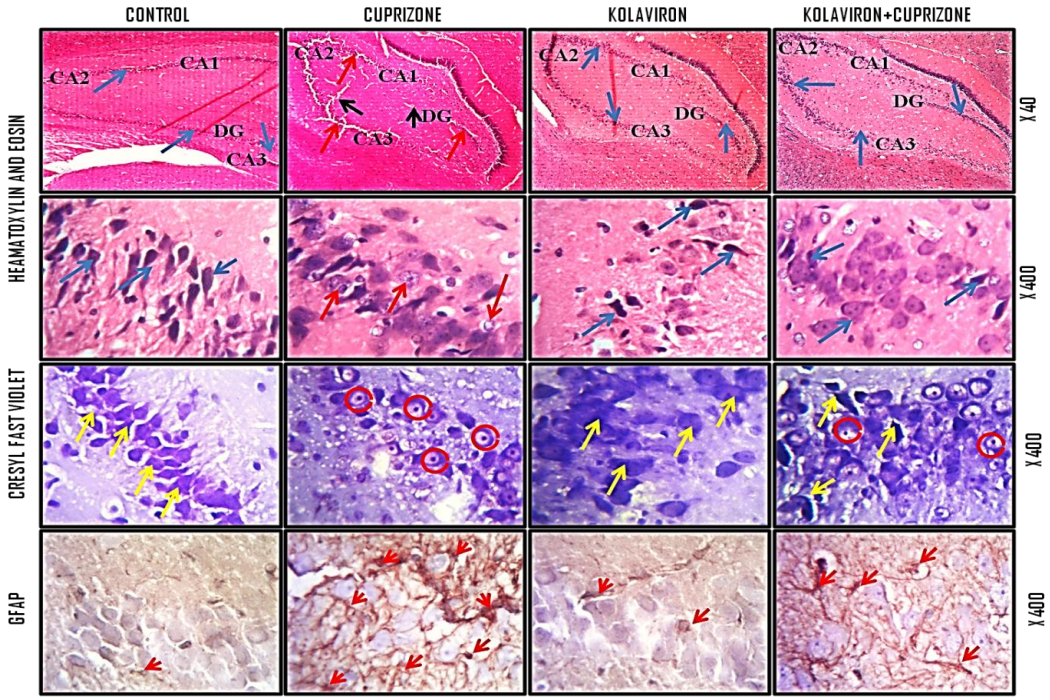


**Figure 4:** Representative photomicrographs of general histology and morphology at low and high magnification, Nissl profiling, and astrocytic distribution in the hippocampus across treatment rats using (H&E and CFV) stains and anti-rat GFAP immunohistochemistry techniques. General histology reveals normal hippocampal morphology in the control and Kv-treated groups, as cells in these groups are in normal arrays (blue arrows), characterized by the distinct arrangement of pyramidal neurons within the cornu ammonis (CA1–CA3) and the dentate gyrus granule cells (DG). However, cuprizone (CPZ) causes disorganization, neuronal degeneration, and pyknotic changes (red arrows). Cresyl fast violet staining shows strong Nissl substance in control and Kv groups, but marked chromatolysis and faint staining after CPZ, which is partly restored by Kv treatment. GFAP immunostaining shows sparse astrocytes in control and Kv groups, marked astrogliosis after CPZ, and reduced astrocytic activation in the Kv + CPZ group, indicating a protective effect of Kv against CPZ-induced hippocampal injury.

**Kolaviron attenuates cuprizone-induced hippocampal neuronal degeneration and chromatolysis**

The characteristic presentations of histological and histochemical properties of the hippocampus in this study were demonstrated using H&E and Cresyl Fast Violet (CFV) staining techniques, respectively. Fig. 4 shows the general histology, which reveals normal hippocampal morphology in the control and Kv-treated groups, as cells in these groups are in normal arrays, characterized by the distinct arrangement of pyramidal neurons within the cornu ammonis (CA1–CA3) to the dentate gyrus granule cells (DG). Pyramidal neurons in these groups have normal cell bodies with dendritic and axonal processes that are well expressed across hippocampal neuropil at higher magnification. The hippocampus of rats intoxicated with CPZ shows several pyknotic changes, including disorganized pyramidal and granular neurons (red arrows) within the disorganized and fragmented cellular layers. In addition, pyramidal neurons and neuroglia in CA1-CA2 exhibit degenerative changes such as clustered cell bodies, extruded cytoplasmic contents, indistinct nuclear demarcation, short projections, and fusion of adjacent neuronal membranes (red arrows). However, treatment with Kv after CPZ intoxication significantly improves hippocampus cytoarchitecture, as the layers are well arranged with normal pyramidal neurons, as seen in the control groups.

In both the control and Kv groups, Cresyl Fast Violet staining demonstrated prominent Nissl substance within pyramidal cells and neuroglia in the CA1-CA2. Furthermore, the granular and molecular layers are well-stained and organized. The hippocampal sections of rats in this group showed highly chromatolytic pyramidal cells and neuroglia within the CA1-CA2 layers, indicating that CPZ intoxication had a highly deleterious effect. In addition, the granular and molecular layers appeared to be lightly stained. When compared to the group that was only intoxicated with CPZ, treatment with Kv improves the chromatogenic properties of the pyramidal cells and neuroglia within the CA1-CA2, as hippocampal sections of this group show characteristics that are similar to the control and Kolaviron groups.

**Cuprizone-induced hippocampal astrogliosis was counteracted by Kolaviron**

In this study, the GFAP immunohistochemistry method was used to demonstrate astrocytic morphology and distribution in the hippocampus of Wistar rats. In the CO (A) and Kv (B), GFAP-immunopositive cells are sparse around pyramidal neurons and between layers within the CA1-CA2, with regular processes, distribution, and sizes within the neuropil. However, in CPZ (B)-treated rats, astrocytic densities are increased, with reactive astroglia in the granule cell layer and hypertrophic cells in the hippocampal layers. Again, the expression of astroglia in the hippocampus of rats treated with Kv concurrently with CPZ (D) demonstrates improved astrocytic morphology and distribution similar to the control and groups. Both groups have normal astrocyte processes, cellular distribution, and size.

## DISCUSSION

The significant reduction in hippocampal superoxide dismutase (SOD) activity recorded in the CPZ-treated group indicates increased oxidative stress and impaired antioxidant defense mechanisms. This finding aligns with previous studies demonstrating that cuprizone administration induces excessive reactive oxygen species (ROS) production, overwhelming endogenous antioxidant systems and decreasing SOD activity in the hippocampus[13].

Conversely, the upregulation of SOD activity in the Kv-treated group likely shows an adaptive cellular response to counteract oxidative stress, as SOD expression increases under acute oxidative challenge[13,24]. Such increases typically indicate activation of intrinsic antioxidant defense mechanisms. The restoration of SOD activity in the combined Kv + CPZ group toward control levels suggests a protective effect of Kv against CPZ-induced oxidative damage, consistent with findings where antioxidant treatments reversed CPZ-induced SOD reductions and preserved neuronal integrity[13].

The significant reduction in hippocampal glutathione peroxidase (GPx) activity in the CPZ-treated group indicates compromised antioxidant defense. This is consistent with cuprizone intoxication inducing excessive ROS production, leading to depletion of endogenous antioxidant enzymes including GPx[13,25,26]. GPx plays a crucial role in cellular defense by catalyzing hydrogen peroxide and lipid peroxide reduction, thereby protecting neuronal cells from oxidative damage[27]. The observed GPx decrease in the CPZ group suggests increased hippocampal neuronal susceptibility to oxidative injury.

The slight GPx elevation in the Kv-treated group may reflect adaptive upregulation of antioxidant defenses in response to mild oxidative challenge, associated with activation of endogenous protective mechanisms maintaining redox homeostasis. The significant GPx increase in the Kv + CPZ group compared to CPZ alone suggests Kv exerts protective effects against CPZ-induced oxidative stress. Similar findings reported antioxidant treatments restoring GPx levels following CPZ-induced depletion, reducing oxidative damage and improving neuronal integrity[13].

The observed MDA increase in CPZ-treated rat hippocampus indicates enhanced lipid peroxidation and consequently oxidative stress. MDA is a well-established end-product of polyunsaturated lipid peroxidation and a reliable biomarker of oxidative membrane damage. In the cuprizone model, oxidative stress arises primarily from mitochondrial dysfunction and excessive ROS production, disrupting redox homeostasis and promoting lipid peroxidation. Experimental studies consistently show cuprizone intoxication increases MDA levels alongside antioxidant defense depletion, confirming its role in inducing brain oxidative injury [13, 26,28].

The hippocampus is particularly susceptible to oxidative damage due to high metabolic demand and abundant oxidizable lipids. Elevated hippocampal MDA levels reflect significant neuronal membrane damage, potentially contributing to impaired synaptic function and cognitive deficits associated with CPZ toxicity. Cuprizone-induced demyelination links to mitochondrial impairment and altered oxidative metabolism, further exacerbating ROS generation and lipid peroxidation[26].

The significantly reduced MDA levels in the Kv-treated group suggest Kv possesses intrinsic antioxidant properties limiting lipid peroxidation, possibly through free radical scavenging, ROS generation inhibition, or endogenous antioxidant system enhancement. Similar findings reported that antioxidant or neuroprotective interventions attenuate cuprizone-induced oxidative damage by reducing MDA concentrations and restoring redox balance[13].

Importantly, decreased MDA levels in the Kv + CPZ group compared to CPZ-only indicate Kv's protective effect against cuprizone-induced oxidative injury, suggesting Kv mitigates lipid peroxidation and stabilizes neuronal membranes through oxidative stress pathway modulation. Such effects align with previous reports demonstrating that therapeutic interventions targeting oxidative stress can reverse cuprizone-induced MDA increases and improve neuronal integrity[28].

The alterations in SOD, GPx, and MDA levels collectively indicate oxidative imbalance in the hippocampus following CPZ intoxication. Significant SOD and GPx reduction alongside marked MDA increase in the CPZ-treated group reflects impaired antioxidant defense and enhanced lipid peroxidation. This aligns with reports that cuprizone induces mitochondrial dysfunction and excessive ROS generation, depleting endogenous antioxidant enzymes and increasing membrane lipid damage[13,25,26]. On the other hand, SOD and GPx upregulation with MDA reduction in the Kv-treated group suggests enhanced intrinsic antioxidant defenses and attenuated lipid peroxidation, indicative of improved redox homeostasis. Enzymatic antioxidants like SOD and GPx play critical roles in neutralizing ROS and preventing oxidative damage to cellular components[27.] The restoration of SOD and GPx activities alongside decreased MDA levels in the Kv + CPZ group relative to CPZ demonstrates Kv's protective effect against CPZ-induced oxidative stress, suggesting Kv mitigates ROS generation, preserves antioxidant enzyme function, and limits lipid peroxidation, thereby maintaining hippocampal neuronal integrity.

Furthermore, histological and histochemical findings in this study showed the shrinkage or condensation of a cell with increased nuclear compactness or density after cuprizone administration. There was disorganization of the pyramidal and granular neurons within some fragmented layer of the hippocampus as a result of the damage caused by cuprizone. Also, the toxic damage caused by cuprizone can be attributed to the degenerative changes that have occurred in the pyramidal neurons and neuroglia in CA1-CA2, which may be due to the extrusion of cytoplasmic contents. Cuprizone administration was also responsible for the clustering of the cell bodies and fusion of adjacent neural membranes with short projections.

However, the findings in this study suggest that treatment with Kolaviron after Cuprizone administration significantly improved the cytoarchitecture of the hippocampus. Neuronal layers appeared well organized, and pyramidal neurons exhibited relatively normal morphology similar to those observed in control animals. Kolaviron is a biflavonoid complex derived from the seeds of Garcinia kola and has been reported to possess strong antioxidant and anti-inflammatory properties[28,29]. Previous experimental studies have demonstrated that Kolaviron reduces oxidative damage and enhances antioxidant enzyme activity in various tissues, thereby protecting against toxic insults[28,30]. Kolaviron has also been shown to mitigate neurobehavioral deficits through activation of the Nrf2/ARE antioxidant pathway[31,32].

In the present study, Cresyl fast violet (CFV) stain was used to observe the chromatogenic properties of the pyramidal cells and neuroglia. The stain revealed highly chromatogenic pyramidal cells and neuroglial cells specifically within the CA1 and CA2 layers. This deleterious effect is a result of cuprizone intoxication. Kolaviron treatment increased chromatogenic intensity of pyramidal cells and neuroglia within CA1-CA2, indicating improved neuronal viability. This protective effect of Kolaviron can be attributed to its antioxidant properties, as similar histological improvements were observed when Kolaviron reduced CA1 neuronal loss in Alzheimer's disease models[29].

Astrocytic reactivity refers to the functional and morphological changes that occur in astrocytes as a result of various brain lesions, as well as the expression of certain astrocyte structural proteins,

including glial fibrillary acidic protein (GFAP)[25]. Cuprizone treatment increased astrocytic density, characterized by cells immunopositive for GFAP, according to the findings of this research. Astrocytes were found in the granule cell layer as a result of cuprizone's neurotoxic effects. This astrogliosis aligns with previous reports of cuprizone-induced demyelination and reactive gliosis in the hippocampus[30].

The expression of astroglia in the hippocampus of rats treated with Kv simultaneously with CPZ showed better astrocytic morphology and distribution, comparable to the control group. The astrocyte processes, cellular distribution, and size are all normal in both groups. Treatment with Kolaviron protected hippocampal neurons, as the cells in the treated group demonstrated greater histological integrity compared to the CPZ-only group. Kolaviron's neuroprotective effect is most likely owing to its antioxidant properties, which reduced GFAP reactivity and preserved neuronal architecture in similar neurotoxicity models[29,30].

## CONCLUSION

The present study demonstrates that Kolaviron effectively attenuates cuprizone-induced neurotoxicity in the hippocampus of Wistar rats. By mitigating oxidative stress and limiting glial activation, Kolaviron may protect hippocampal neurons from metabolic and inflammatory insults associated with cuprizone-induced demyelination.

**Conflict of interest**: Authors declare no conflict of interest

**Author's contribution:** LOA, JIE: Conception or design of the work; IIU, NJ: data acquisition; OEO, TOA: analysis and interpretation; MMD, KRO, KOA: drafting and substantively revising the work.

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