Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 8 (2)

Kolaviron ameliorates histomorphological changes associated with Cuprizone-induced Cerebellar Damage

Omotoso GO, Olajide OJ, Gbadamosi IT, Suleiman FA, Oladimeji JO
Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

Corresponding Author: Omotoso GO
E-mail: gabrielolaiya@yahoo.com; +234 703 050 5707

ABSTRACT
Cuprizone is a copper chelator and a drug of choice in studing demyelination/remyelination in the central nervous system. This study assessed the effect of *Kolaviron,* on cuprizone-induced damage to the cerebellum of Wistar rat. Twenty-four adult male Wistar rats were grouped into 5: Group A received 0.5 ml of normal saline for 6 weeks; Group B received 0.5 ml of corn oil for 6 weeks; Group C was treated with 0.2% of cuprizone for 3 weeks followed by treatment with 200 mg/kg of *Kolaviron* for another 3 weeks; Group D received 200 mg/kg *Kolaviron* for 3 weeks followed by 0.2% cuprizone from another 3 weeks; while Group E received 0.2% cuprizone for 6 weeks. Meanwhile, 0.5 ml of corn oil was used as a vehicle for *Kolaviron*. The body and brain weight of the rats showed significant decrease in all treated groups when compared to the control groups. Histological demonstration showed varying degrees of architectural distortions, including depletion of Nissl bodies, disruption of cortical cell layers and depletion of myelin, which were more pronounced in the cerebellar cortex of cuprizone-treated rats.
Kolaviron offered mild cytoprotection to the cerebellar histomorphology of cuprizone-treated rats. Further studies would ascertain the effectiveness of *Kolaviron* in mitigating cerebellar lesions in well-established demyelination.

Keywords: *Kolaviron;* cuprizone; cerebellum; demyelination; histomorphology

INTRODUCTION
Kolaviron is a fraction of the deffated ethanol extract of *Garcinia Kola* seeds, containing Garcinia biflavonoids (GB) GB1, GB2, kolaflavanone and kolaflavone as its major components[1]. It has been suggested that kolaviron is a likely inhibitor of acetylcholinesterase (AChE) activity. Dysregulated cholinergic neurotransmitter systems have been implicated in the pathophysiology of a variety of neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD) and Schizophrenia[2]. *Kolaviron* has shown ample beneficial health effects in animal models of these diseases and also in the prevention of hepatotoxicity induced by several toxins. The protective effects of *Kolaviron* against insults from various xenobiotics have been attributed to its antioxidant properties. *Kolaviron* reduced damage to lipids and proteins induced by $Fe3^{+}$/EDTA ascorbate mixtures ex vivo[3]. In the plasma and liver, *Kolaviron* lowered biomarker of protein oxidation (2-aminoadipic semialdehyde) and also lipid oxidation marker (malondialdehyde) in the liver. In addition, the role of *Kolaviron* in the chemoprevention of chemically-induced geno-toxicity is demonstrated by its inhibitory effect on H_2O_2-induced reactive oxygen species (ROS) production in HepG2 cells [4]. *Kolaviron* also improved antioxidant status by enhancing antioxidant gene expressions and scavenging ROS in atrazine-induced cytotoxicity of rat leydig cells.

Kolaviron showed protection against oxidative stress-related injuries in the liver, testes and spermatozoa [5]. The inhibitory actions of *kolaviron* on inflammatory mediators such as, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), nuclear factor kappa B (NF-kB) and activator protein-1 (AP-1) in rats treated with dimethylnitrosamine is another indication of its protective effect on drug-induced hepatotoxicity [6]. Previous studies have suggested the use of *Kolaviron* as a prophylactic agent in the protection against atherosclerosis due to its antioxidative effects on serum lipoprotein oxidation both *in vitro* and *in vivo* [7,8], and the study by Adaramoye *et al*.[7] noted that *Kolaviron* has anti-atherogenic effects in rats fed on high cholesterol diet. The possible mechanisms of protection were suggested to involve metal chelation, anti-oxidative and scavenging of radical species [9].

Cuprizone (bis-cyclo- hexanone oxaldihydrazone) is a copper chelator used as a model to induce consistent demyelination in rats by causing cell death to oligodendrocytes. Spontaneous remyelination can however be observed as early as 4 days after withdrawal of the neurotoxin [10], thus making the cuprizone model excellent for studying factors which can prevent

demyelination and stimulate remyelination. It is not known why administration of cuprizone only leads to a specific cell death in the oligodendrocytes, although cuprizone causes inhibition of the copper-dependent mitochondrial enzymes cytochrome oxidase and monoamine oxidase [11]. Thus, a plausible hypothesis is that disturbance in energy metabolism leads to apoptosis in the oligodendrocytes, which causes demyelination. The demyelination was previously believed to predominantly affect the corpus callosum and superior cerebellar peduncles [11]. However, recent studies, which have used immunohistochemical techniques, demonstrated extensive cortical and cerebellar demyelination in mice fed with cuprizone [12]. Although adequate demyelination is expected in most part of the brain after a 6 week cuprizone-induction, extensive reactive gliosis coupled with oligodendrocyte apoptosis has been demonstrated in the cerebellum as early as 3rd week of cuprizone induction [13].

Thus, this pathological pattern may resemble the extensive sub-pial demyelination found in multiple sclerosis (MS) [14], which was previously observed by Lucchinetti *et al*. [15] as resembling the type 3 or type 4 lesions in multiple sclerosis. Lucchinetti and colleagues [15] further characterised four different patterns of demyelination in MS patients, where pattern 3 is characterised by oligodendrogliopathy and pattern 4 seems to refiect primary oligodendrocyte damage with secondary demyelination. Both patterns are more reminiscent of a virus- or toxin-induced demyelination than autoimmunity. Cuprizone-induced demyelination is associated with a microglia-macrophage response; however, the cuprizone model differs from MS in that the blood–brain barrier (BBB) remains intact [16]. Cuprizone intoxication was also found by Groebe and colleagues [17] to induce an almost complete demyelination of cerebellar nuclei. Furthermore, axonal loss is also observed both in multiple sclerosis and in the cuprizone model of demyelination [18].

The objective of this study was to assess the neuroprotective effect of *Kolaviron* on cuprizone-induced toxicity in the cerebellar cortex of adult Wistar rats.

MATERIALS AND METHODS

Experimental Animals

A total of 20 adult male Wistar rats were used for this study. Following the approval of the ethical review committee, the animals were housed in a wire gauzed cage in the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin. Proper ventilation was maintained by the use of wire gauze wooden cage. The rats were fed on pelleticised growers mash and water provided throughout the duration of the experiment. The animals were allowed to acclimatise for two weeks prior to the commencement of the study.

Chemicals and Reagents

Cuprizone was purchased from Sigma-Aldrich® (Germany); petroleum ether, acetone and ethyl acetate were purchased from Lab Trade Scientific Chemical Store, Ilorin. They were of analytical grade and purest quality available.

Plant Material and Compound Extraction

Garcinia kola (Guttiferae heckel) seeds were purchased from Oja-Oba market in Ilorin, Kwara State Nigeria. *Kolaviron* was extracted from the fresh dried seeds of the Kola (5 kg) and characterised according to a previously described method [9]. Briefly, powdered seeds (1.87 kg) were extracted with light petroleum ether (40–60°C) in a soxhlet extractor for about 3 hrs per round. The defatted, dried marc was repacked and then extracted with acetone. The extract was concentrated and diluted to twice its volume with distilled water and extracted with ethyl acetate (4.5 litres). The concentrated ethyl acetate fraction gave a yellow solid known as *Kolaviron*. The purity and identity of *Kolaviron* was determined by subjecting it to thin-layer chromatography (TLC) using Silica gel GF 254-coated plates and, solvent mixture of methanol and chloroform in a ratio 1:4 v/v. The separation revealed the presence of three bands which were viewed under ultra violet light at a wavelength of 254 nm with RF values of 0.48, 0.71 and 0.76 [7]. The yield of the preparation was 5.5%.

Animal Treatment

The rats were randomly divided into five groups of 4 animals each. Group A, the control, received 0.5 ml of normal saline for 3 weeks; Group B, received 0.5 ml of corn oil (vehicle for *Kolaviron*) for 3 weeks; Group C was treated with 0.2% of cuprizone for 3 weeks followed by treatment with 200 mg/kg of *Kolaviron* for another 3 weeks (CU+KV); Group D received 200 mg/kg *Kolaviron* for 3 weeks followed by 0.2% cuprizone for another 3 weeks (KV+CU), while Group E received 0.2% cuprizone for 6 weeks.

Histopathological Techniques

Twenty-four hours after the last drug administration, the rats were euthanized using 1 mg/kg of ketamine intramuscularly and subjected to transcardial perfusion with 50 ml of normal saline followed by 4% paraformaldehyde (PFA). The brain tissues were excised, weighed and post-fixed in 4% PFA. Histological and histochemical demonstrations of cerebellar cortical cytoarchitecture were carried out in paraffin-embedded sections which were stained with Haematoxylin and Eosin (H and E), Cresyl Fast Violet (CFV) and luxol fast blue (LFB).

Statistical Analysis

The data obtained from the body weight and the cerebellum to brain weight ratio (CBR) were subjected to statistical analysis using the GraphPad Prism® software (Version 6). The values were plotted using ANOVA with Tukey's multiple comparisons test. Data obtained were presented as mean ± standard error of mean, with determination of level of significance at p values less than 0.05, 0.01 or 00.05. The outcomes were represented in bar charts with error bars to show the

mean and standard error of mean respectively.

RESULTS

Kolaviron Counterbalances Cuprizone-induced Weight Loss

Rats administered with cuprizone only had a significant weight loss ($p < 0.005$) when compared with those given normal saline and corn oil. *Kolaviron*- treated rats (Groups C and D) had significantly higher body weights relative to the cuprizone group ($p<0.05$), but lower than saline and corn oil groups ($p<0.005$) (Figure 1). In a similar manner, there was a significant reduction in the CBR in the CU-only group relative to the saline and corn oil groups ($p<0.005$) as well as the CU+KV and KV+CU groups ($p<0.01$) (Figure 2). The CBR of the CU+KV and KV+CU groups was comparatively lower than those of the saline and corn oil groups ($p<0.005$).

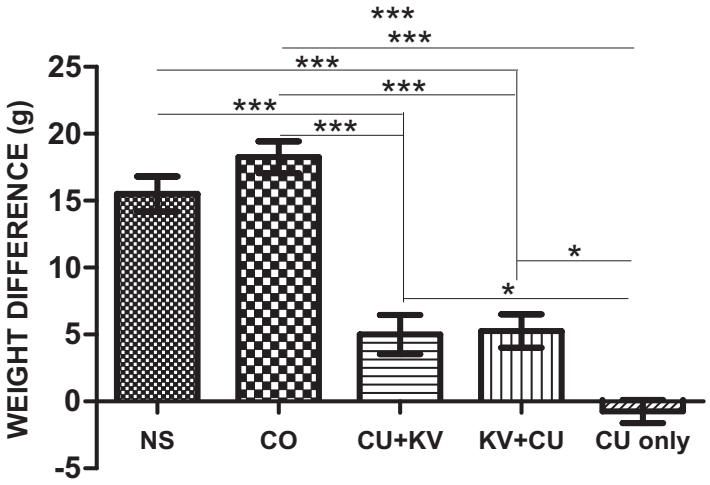

Figure 1: Graph showing the changes in the body weight of experimental animals. There was a significant reduction in the body weight of the cuprizone-treated groups (* and *** are p values < 0.05 and 0.005 respectively). NS= normal saline; CO= corn oil; CU+KV= 3 weeks cuprizone followed by 3 weeks *Kolaviron*; KV+CU= 3 weeks kolaviron followed by 3 weeks cuprizone; CU only= cuprizone only.

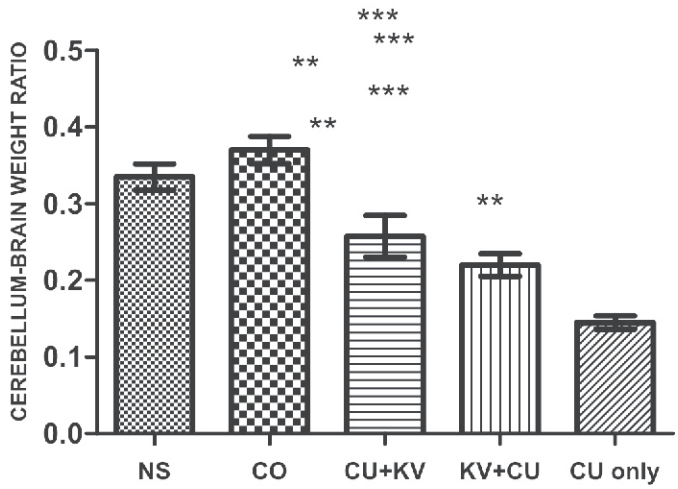

Figure 2: Graph showing the cerebellum to brain weight ratio (CBR) in experimental animals. There was a significant reduction in the CBR of the cuprizone-treated groups (** and *** are p values < 0.01 and 0.005 respectively). NS= normal saline; CO= corn oil; CU+KV= 3 weeks cuprizone followed by 3 weeks *Kolaviron*; KV+CU= 3 weeks kolaviron followed by 3 weeks cuprizone; CU only= cuprizone only.

Histological and Histochemical Observations

The histological presentation of the cortical layers of the cerebellum of rats treated with normal saline and corn oil showed three distinct layers, which includes the outer molecular layer, the middle Purkinje cell layer and the inner granular layer (Figures 3 and 4). The cellular arrangement in the aforementioned groups was characterized by Purkinje cells with conspicuous cell bodies and dendrites that were protruding deep into the molecular layers. The granular layer of the cerebelli of rats in these groups comprised small granule neurons which were compactly disposed. The CU+KV and KV+CU groups presented with Purkinje cell with dendrites that were projecting into the molecular layer. The cellular density in the CU+KV and KV+CU groups was reduced when compared to the saline and corn oil groups (Figures 3 and 4). The Purkinje cell layer of the animals that received only cuprizone was degenerated as the cell bodies and dendrites of the Purkinje were sparse when compared to the other groups. The granule cells of the granular layer in this group were sparingly distributed.

The Nissl substances in the cerebellar cortex of the saline and corn oil groups were deeply stained, while there were extreme chromatolytic changes in the cerebellar cortex of the CU-only group (Figure 4). These chromatolytic changes were more pronounced in the Purkinje cell layer, indicated by the poor staining of the Nissl substance in this layer. In a similar manner, the myelin stain (LFB) of the CU-only group was faint, especially in the medulla of the cerebellum, compared to the CU+KV and KV+CU groups (Figure 5).

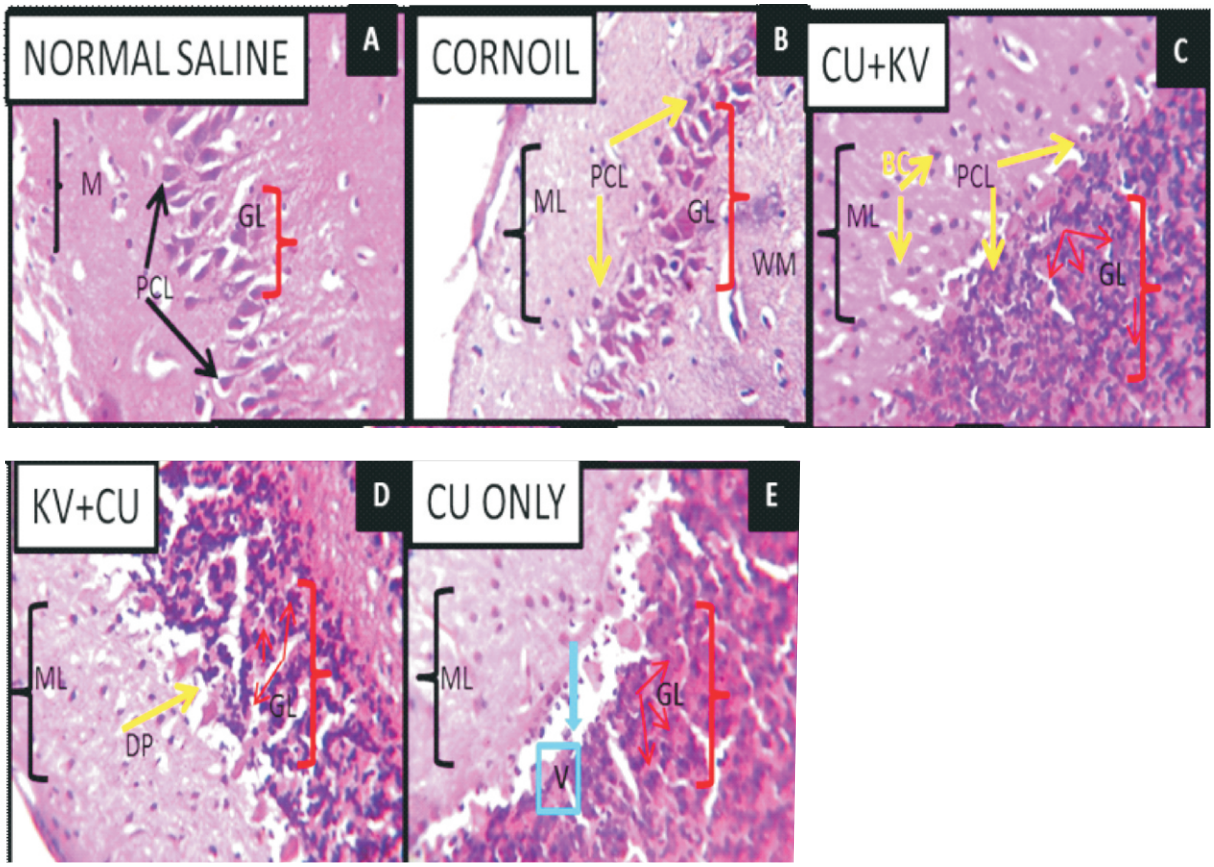

Figure 3: Representative photomicrographs of the cerebellum of Wistar rats. A and B were saline and corn oil groups respectively, showing distinct layer of Purkinje cells; C (cuprizone + *kolaviron*) showed numerous cells highly concentrated in the granular layer (red arrow), including Basket cells (BC), with distinct molecular layer (ML, yellow arrow); D (*kolaviron* + cuprizone) showed distorted and diminished Purkinje layer of cells (DP) (yellow arrow), sparse cell deposition in the granular layer (red arrow), while E (cuprizone-only), showed a very severe distortion in the Purkinje layer, scanty deposition of cell in the granular layer (GL) and large vacuolation in the Purkinje layer (V, blue arrow); H and E x400.

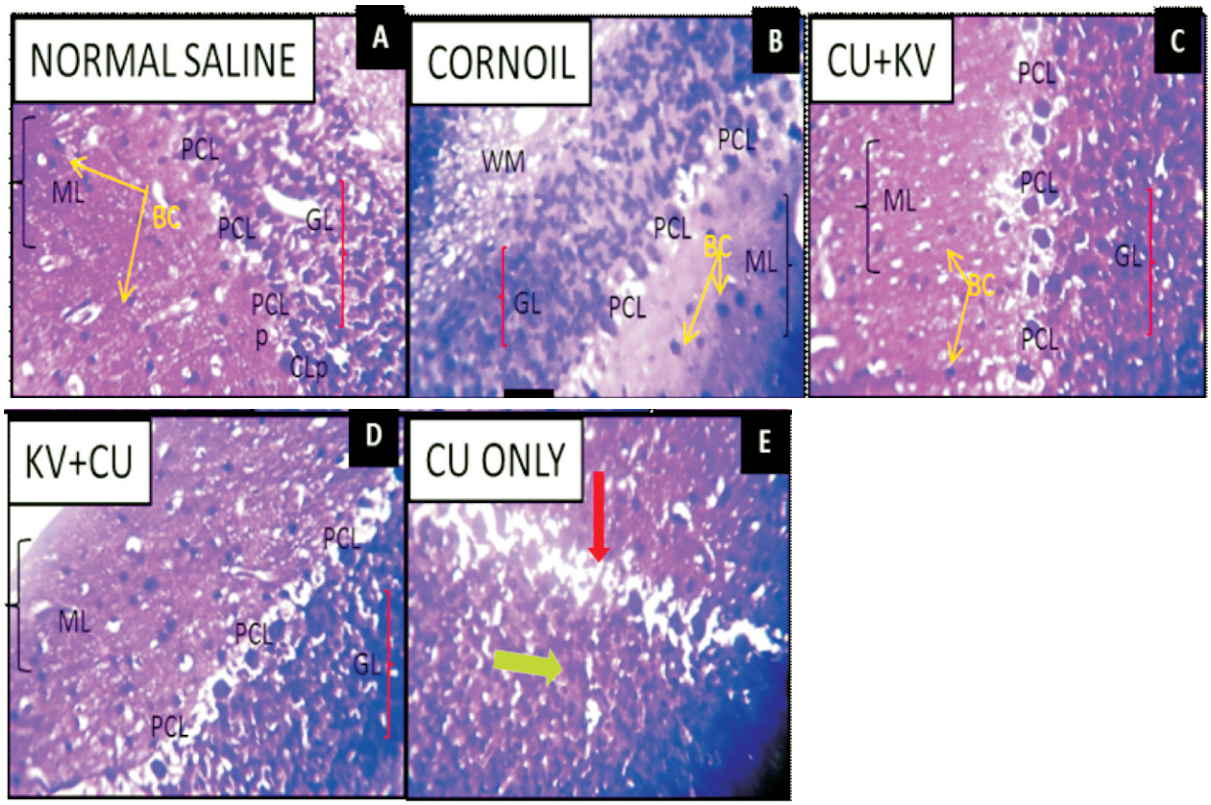

Figure 4: Representative photomicrographs of cerebellum of Wistar rats. A (normal saline) and B (Corn oil) showed proper staining of abundant deposition of Nissl substance in the granular layer (GL), BC= basket cells, WM=white matter; C (cuprizone + *Kolaviron*) showed significant cell concentration in the granular layer and reduced number of cells in the purkinje layer (PLC); D (*Kolaviron* and cuprizone) showed scanty, poorly stained Nissl substance in the granular layer and diminished purkinje cell layer (PLC) and less cellular concentration when compared to group C; while Group E (Cuprizone only) showed the presence of distorted purkinje layer with vacuolation (red arrow), scanty cell deposition in the granular layer (green arrow); CFV x400.

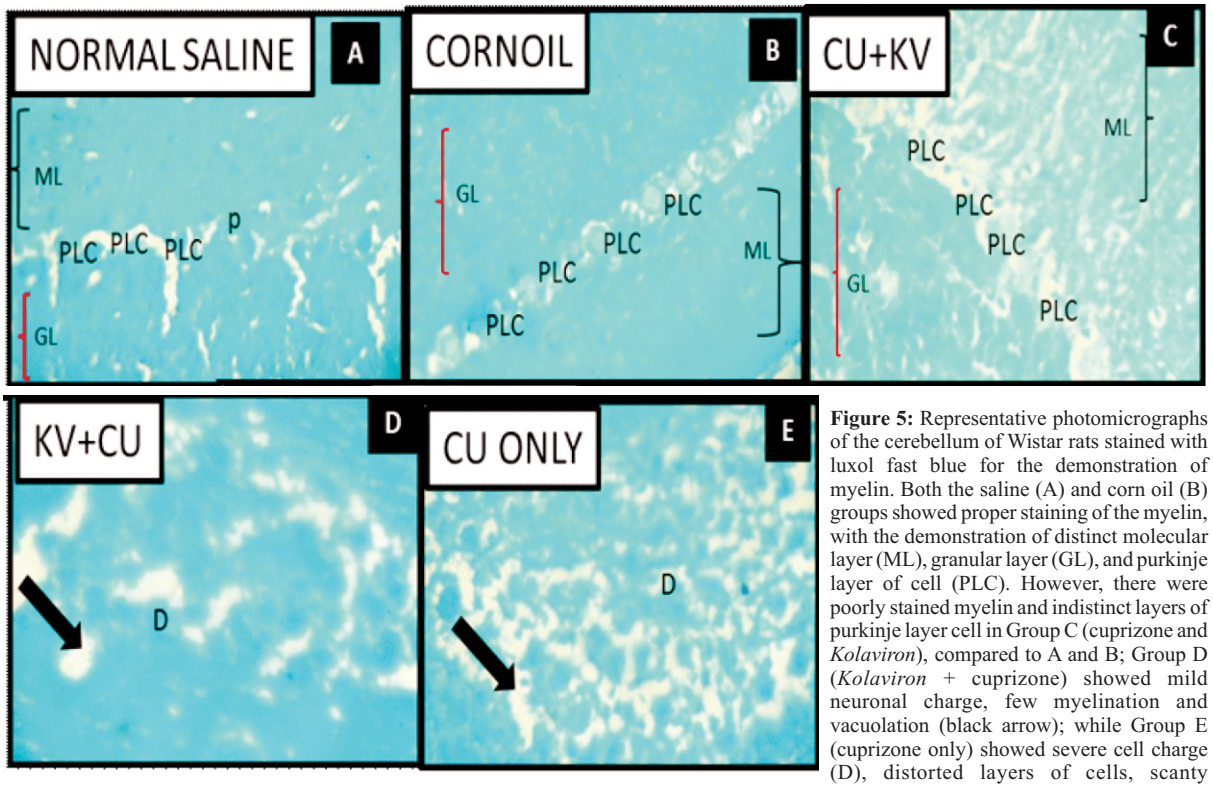

Figure 5: Representative photomicrographs of the cerebellum of Wistar rats stained with luxol fast blue for the demonstration of myelin. Both the saline (A) and corn oil (B) groups showed proper staining of the myelin, with the demonstration of distinct molecular layer (ML), granular layer (GL), and purkinje layer of cell (PLC). However, there were poorly stained myelin and indistinct layers of purkinje layer cell in Group C (cuprizone and *Kolaviron*), compared to A and B; Group D (*Kolaviron* + cuprizone) showed mild neuronal charge, few myelination and vacuolation (black arrow); while Group E (cuprizone only) showed severe cell charge (D), distorted layers of cells, scanty myelination and numerous vacuolation (black arrow); x400.

DISCUSSION

Cuprizone is a neurotoxicant which causes the death of oligodendrocytes, the myelinating cells of the central nervous system, in an action that is somewhat associated with the induction of oxidative stress [19]. In the current study, the rats exposed to cuprizone produced adequate demyelination necessary to evaluate the potential intervention of new neuroprotective agents on cerebellar damage. Although adequate and extensive demyelination is usually seen in most part of the brain after a 6-week cuprizone treatment, extensive reactive gliosis accompanied by oligodendrocyte apoptosis can be demonstrated in the cerebellum as early as 3 weeks into the treatment [13].

All the experimental rats in this study treated with cuprizone had reduced body weights, and this was much more pronounced in rats that received cuprizone for 6 weeks. Administration of *Kolaviron* 3 weeks before or after cuprizone exposure did not enhance body weight to attain the near Control values. Earlier reports have however shown that *Kolaviron* causes reduction in body weights [20]. Reduction in cerebellar weights was proportional to the body weights. A reduction in the cerebellum-brain ratio indicates reduced cerebellar weight either as a result of neurodegeneration or demyelination. However, administration of *Kolaviron* after a 3-week cuprizone exposure appreciably prevented marked reduction in CBR when compared to rats that received *Kolaviron* prior to cuprizone administration. This could be attributed to the process of remyelination that resumes immediately after cuprizone withdrawal, and *Kolaviron* could probably be synergistic.

As observed by previous authors, cuprizone exposure causes decrease in body weight, brain weight and behavioural deficits [21]. The reduced body weight could have been caused by reduced feeding appetite generally, and the reduction in brain weight could have ensued by an alteration in mitochondrial function of the cerebellum, thus eliciting a display of hypoxia-like pattern of tissue injury which seems to be induced by a dysfunction in the complex of respiratory chain [22]. In such condition as reported in multiple sclerosis patients, oligodendrocyte follows a caspase-independent pathway [23], thus resulting in reduced activity of the various complexes of the respiratory chain in the mitochondria of cuprizone-treated groups [24]. Impaired mitochondrial respiratory chain results in excessive production of reactive oxygen species and consequent damage to various cellular components including deoxyribonucleic acid (DNA). Meanwhile, extensive DNA damage triggers cell dysfunction and death which in turn leads to reduced brain and body weight due to cuprizone exposure as observed in rats that received both cuprizone and *Kolaviron* [25].

No significant change was seen in the histomorphology of the cerebellar cortical layers of rats given Kolaviron post-cuprizone treatment when compared to the Control. Animals that received cuprizone after prophylactic Kolaviron showed mild cerebellar degeneration of the Purkinje layer and scanty cell deposition in the granular layer. Although these changes were not as pronounced as those seen in the cuprizone-only group, this could still interfere with motor and exploratory drive functions of these rats.

The microarchitecture of the cerebellum of rats exposed to cuprizone only showed evidence of demyelination, with the presence of large vacuolation, more than what was seen in animals exposed to Kolaviron before or after cuprizone exposure. Also, the intensity of Nissl staining was least in the cuprizone-only group followed by the group that received prophylactic Kolaviron treatment. This shows that administration of Kolaviron might not prevent the depletion of Nissl bodies prior to the onset of demyelination, but may be able to limit the extent of damage after the onset of the condition. This, however, requires further studies as the effect of remyelination cannot be ruled out.

Cuprizone-induced brain injury interferes with motor activity and other motor functions, such as loss of fine movement, maintenance of equilibrium and loss of regulation of muscle tone which are modulated by the spinal cord and brain stem mechanisms involved in postural control [26]. Mutter and colleagues [27] had reported a decrease in dopamine concentration in the cerebellum of mice ingested with neurotoxin for a week which resulted in motor function disorder. Because neurotransmitter dopamine release is also dependent on calcium transport, cuprizone exposure induces severe impairment to the dopaminergic system, acting both directly on neurotransmitter synthesis and indirectly on calcium transport. As a consequence, the signal conduction and transmission in neuronal circuits of the CNS and peripheral nervous system could be seriously impaired [28].

Conclusion

Kolaviron exhibits mild beneficial effects when administered after the onset of demyelination. However, further studies are necessary to ascertain the synergy, if any, between the effects of Kolaviron during this period and remyelination that sets in after cuprizone withdrawal. Furthermore, studies on concurrent use of cuprizone and Kolaviron on a longer term are necessary to determine the place of Kolaviron in therapeutic intervention of ongoing demyelinating conditions.

REFERENCES

1. Iwu M. Antihepatoxic constituents of Garcinia kola seeds. Experientia; 1985; 41(5): 699-700.
2. Ijomone MO, Obi AU. Kolaviron, isolated from Garcinia kola, inhibits acetylcholinesterase activities in the hippocampus and striatum of Wistar rats. Annals of Neuroscience 2013; 20(2): 42–46.
3. Farombi EO, Møller P, Dragsted LO. Ex-vivo and in

vitro protective effects of kolaviron against oxygen-derived radical-induced DNA damage and oxidative stress in human lymphocytes and rat liver cells. Cell Biol Toxicol. 2004; 20(2):71-82.
4. Nwankwo JO, Tahnteng JG. Inhibition of aflatoxin B1 genotoxicity in human liver-derived HepG2 cells by kolaviron biflavonoids and molecular mechanisms of action. Eur J Cancer Prev. 2000; 9(5):351-61.
5. Farombi EO, Adedara IA, Oyenihi AB, Ekakitie E, Kehinde S. Hepatic, testicular and spermatozoa antioxidant status in rats chronically treated with Garcinia kolaseed. J Ethnopharmacol. 2013; 146(2):536-42.
6. Farombi EO, Shrotriya S, Surh YJ. Kolaviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-kappaB and AP-1. Life Sci. 2009;84(5-6):149-55
7. Adaramoye OA, Nwaneri VO, Anyanwu KC, Farombi EO, Emerole GO. Possible anti- atherogenic effect of kolaviron (a Garcinia kola seed extract) in hypercholesterolaemic rats. Clinical Experimental Pharmacology and Physiology; 2005;32(1- 2): 40-46.
8. Farombi EO, Nwaokeafor IA. Anti-oxidant mechanisms of kolaviron: studies on serum lipoprotein oxidation, metal chelation and oxidative membrane damage in rats. Clinical and Experimental Pharmacology and Physiology 2005;32:667–674.
9. Farombi EO, Adepoju BF, Ola-Davies OE, Emerole GO. Chemoprevention of aflatoxin B1-induced genotoxicity and hepatic oxidative damage in rats by kolaviron, a natural biflavonoid of Garcinia kola seeds Europe Journal of Cancer Preview; 2005;14:207–214.
10. Lindner M, Heine S, Haastert K, Garde N, Fokuhl J, Linsmeier F, Grothe C, Baumgärtner W, Stangel M. Sequential myelin protein expression during remyelination reveals fast and efi cient repair after central nervous system demyelination. Neuropathology of Applied Neurobiology 2008; 34:105–14.
11. Matsushima GK, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Patholology; 2001;11:107-16.
12. Xiao L, Xu H, Zhang Y, Wei Z, He J, Jiang W, Li X, Dyck LE, Devon RM, Deng Y, Li XM. Quetiapine facilitates oligodendrocyte development and prevents mice from myelin breakdown and behavioral changes. Molecular Psychiatry 2008;13:697–708.
13. AJ, Thompson P, J. Demyelination and remyelination in anatomically distinct regions of the corpus callosum following cuprizone intoxication. Neurosci Res. 2012;72(1): 32–42.
14. Bø L, Vedeler CA, Nyland HI, Trapp BD, Mørk SJ. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. Journal of Neuropathology and Experimental Neurology 2003; 62:723–32.
15. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Annual Neurology; 2003; 47:707–17.
16. McMahon EJ, Suzuki K, Matsushima GK. Peripheral macrophage recruitment in cuprizone-induced CNS demyelination despite an intact blood–brain barrier. Journal of Neuroimmunology 2002; 130:32–45.
17. Groebe A, Clarner T, Baumgartner W, Dang J, Beyer C, Kipp M. Cuprizone treatment induces distinct demyelination, astrocytosis, and microglia cell invasion or proliferation in the mouse cerebellum. Journal of Neuroscience 2009; 8: 163-174
18. Irvine K-A, Blakemore WF. Age increased axon loss associated with primary demyelination in cuprizone-in- duced demyelination in C57BL /6 mice. Journal of Neuroimmunology 2006; 175(1-2):69–76.
19. Haider L, Fischer MT, Frischer JM, Bauer J, Höftberger R, Botond G, Esterbauer H, Binder CJ, Witztum JL, Lassmann H. Oxidative damage in multiple sclerosis lesions. Brain 2011; 134(7):1914-24.
20. Obi AU, Nwoh PU. Effect of kolaviron, a major constituent of Garcinia kola in the histology of the hypothalamus, pituitary gland and testes, using adult male Wistar rat as a model organism. Foresnsic Medicine and Anatomy Research 2014; 12:80-87
21. Carlton WW. Response of mice to the chelating agents sodium diethyldithiocarbamate, alpha-benzoinoxime, and biscyclohexanone oxaldihydrazone. Toxicology and Applied Pharmacology 1966; 8: 512-521
22. Lucchinetti C, , Rodriguez M, Lassmann H. Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. Brain Pathology 1996; 6(3):259-74.
23. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Annual Neurology 2004; 55(4):458-68.
24. Pasquini LA, Calatayud CA, Bertone Uña AL, Millet V, Pasquini JM, Soto EF. The neurotoxic effect of cuprizone on oligodendrocytes depends on the presence of pro-inflammatory cytokines secreted by microglia. Neurochem Res. 2007;32(2):279-92.
25. Turrens JF. Mitochondrial formation of reactive oxygen species. Journal of Physiology 2003; 552(Pt 2):335-44.
26. Iwasa K, Yamamoto S, Takahashi M, Suzuki S, Yagishita S, Awaji T, Maruyama K, Yoshikawa K (2014): Prostaglandin F2α FP receptor inhibitor reduces demyelination and motor dysfunction in a cuprizone-induced multiple sclerosis mouse model. Prostaglandins Leukot Essent Fatty Acids 91(5):175-82.
27. Mutter J, Curth A, Naumann J, Deth R, Walach H (2010): Does inorganic mercury play a role in Alzheimer's diseasefi A systematic review and an integrated molecular mechanism. Journal of Alzheimers Disease 22(2):357-74.
28. Mameli O, Caria MA, Solinas A, Tavera C, Tocco M, Flore C, Sanna Randaccio F (2001): Neurotoxic effect of lead at low concentrations. Brain Reserach Bulletin; 55(2):269–75.