



ScienceDirect®

Pathophysiology

Menu



Search in this journal

Volume 25, Issue 4

Pages 253-446 (December 2018)

[Download full issue](#)

[Previous vol/issue](#)

[Next vol/issue](#)

Full text access

Editorial Board

Page ii



[View PDF](#)

Review article ☐ Abstract only

Chitin, chitinases, and chitin lectins: Emerging roles in human pathophysiology

Sally Ziatabar, Jennifer Zepf, Sushama Rich, Ben T. Danielson, ... Robert Stern

Pages 253-262

Article preview [View](#)

Review article ☐ Abstract only

[FEEDBACK](#)

Food additives, food and the concept of 'food addiction': Is stimulation of the brain reward circuit by food sufficient to trigger addiction?

A.Y. Onaolapo, O.J. Onaolapo

Pages 263-276

[Article preview](#) 

[Review article](#)  [Abstract only](#)

The pathophysiology of myocardial infarction-induced heart failure

Daniele Gabriel-Costa

Pages 277-284

[Article preview](#) 

[Review article](#)  [Abstract only](#)

Genetic polymorphism of glutathione S-transferases: Relevance to neurological disorders

Sreenivasulu Dasari, Sailaja Gonuguntla, Muni Swamy Ganjayi, Suman Bukke, ... Balaji Meriga

Pages 285-292

[Article preview](#) 

[Research article](#)  [Abstract only](#)

Mimosa pudica Protects the Testes Against Cadmium-Induced Inflammation and Oligospermia: Potential Benefits in Treatment of Heavy Metal Toxicity

F.M. Onyije, M.C. Nwanze, G.O. Alade, T.O. Alade, ... C.K. Okpora

Pages 293-297

[Article preview](#) 

[Research article](#)  [Abstract only](#)

Kolaviron protects the brain in cuprizone-induced model of experimental multiple sclerosis via enhancement of intrinsic antioxidant mechanisms: Possible therapeutic applications?

Gabriel Olaiya Omotoso, Ileje Inelo Ukwubile, Leviticus Arietarihire, Fatima Sulaimon, Ismail Temitayo Gbadamosi

Pages 299-306

[Article preview](#) 

[Research article](#)  [Abstract only](#)

Royal jelly supplementation reduces skeletal muscle lipotoxicity and insulin resistance in aged obese rats

Samah E.L. Metwally Ibrahim, Ayman Ahmed Kosba

Pages 307-315

Article preview 

Research article ☐ Abstract only

Jobelyn suppresses hippocampal neuronal apoptosis and necrosis in experimental alcohol-induced brain stress

C.A. Oyinbo, F.O. Robert, O.G. Avwioro, P.S. Igbigbi

Pages 317-325

Article preview 

Research article ☐ Abstract only

Potential use of bitter melon (*Momordica charantia*) derived compounds as antidiabetics: *In silico* and *in vivo* studies

Olusola Olalekan Elekofehinti, Esther Opeyemi Ariyo, Moses Orimoloye Akinjiyan, Olanrewaju Sam Olayeriju, ... Joao Batista Teixeira Rocha

Pages 327-333

Article preview 

Research article ☐ Abstract only

SPOP, ZEB-1 and E-cadherin expression in clear cell renal cell carcinoma (cc-RCC): Clinicopathological and prognostic significance

Ola A. Harb, Mariem A. Elfeky, Basant Sh El Shafaay, Heba F. Taha, ... Ahmed Embaby

Pages 335-345

Article preview 

Research article ☐ Abstract only

Correction of coagulopathy in thrombocytopenia and Glanzmann thrombasthenia models by fibrinogen and factor XIII as assessed by thromboelastometry

Ivan Budnik, Boris Shenkman, Olga Morozova, Jurii Andreichyn, Yulia Einav

Pages 347-351

Article preview 

Research article ☐ Abstract only

Melatonin attenuates chronic immobilization stress-induced muscle atrophy in rats: Influence on lactate-to-pyruvate ratios and Na^+/K^+ ATPase activity

Eman S.H. Abd Allah, Ahmed M. Mahmoud

Pages 353-357

Article preview 

Research article  Abstract only

Resveratrol prevents high-fat diet-induced obesity and oxidative stress in rabbits

A. Jimoh, Y. Tanko, A. Ahmed, A. Mohammed, J.O. Ayo

Pages 359-364

Article preview 

Research article  Abstract only

Catechin, quercetin and taxifolin improve redox and biochemical imbalances in rotenone-induced hepatocellular dysfunction: Relevance for therapy in pesticide-induced liver toxicity?

Afolabi C. Akinmoladun, Comfort Odunayo Oladejo, Sunday Solomon Josiah, Courage Dele Famusiwa, ... M. Tolulope Olaleye

Pages 365-371

Article preview 

Research article  Abstract only

Heart remodeling produced by aortic stenosis promotes cardiomyocyte apoptosis mediated by collagen V imbalance

Paula Grippa Sant'Ana, Sabrina Setembre Batah, Patrícia Santos Leão, Walcy Rosolia Teodoro, ... Alexandre Todorovic Fabro

Pages 373-379

Article preview 

Research article  Abstract only

Studies on biofilm formation and virulence factors associated with uropathogenic *Escherichia coli* isolated from patient with acute pyelonephritis

A. Vysakh, Sebastian Jose Midhun, Kuriakose Jayesh, Mathew Jyothis, M.S. Latha

Pages 381-387

Article preview 

Research article  Abstract only

L-arginine attenuates blood pressure and reverses the suppression of angiogenic risk factors in a rat model of preeclampsia

G.O. Oludare, H.D. Jinadu, O.O. Aro

Pages 389-395

Article preview 

Research article ☐ Abstract only

Human apolipoprotein A-I Gly26Arg stimulation of inflammatory responses via NF- κ B activation: Potential roles in amyloidosis?

Nahuel A. Ramella, Isabel Andújar, José L. Ríos, Silvana A. Rosú, ... Guillermo R. Schinella

Pages 397-404

Article preview 

Research article ☐ Abstract only

Peripheral brain-derived neurotrophic factor is reduced in stroke survivors with cognitive impairment

Tasneem M. Hassan, Isyaku U. Yarube

Pages 405-410

Article preview 

Research article ☐ Abstract only

Resveratrol increases serum adiponectin level and decreases leptin and insulin level in an experimental model of hypercholesterolemia

A. Jimoh, Y. Tanko, J.O. Ayo, A. Ahmed, A. Mohammed

Pages 411-417

Article preview 

Research article ☐ Abstract only

Pentoxifylline protects against loss of function and renal interstitial fibrosis in chronic experimental partial ureteral obstruction

Hongbao Ma, Steven Lee, Yan Yang, Puneet Bedi, Shyan-Yih Chou

Pages 419-425

Article preview 

Research article ☐ Abstract only

The relationship between the neck circumference and the carotid intima-media thickness in Amerindians. Potential links to health risks?

Oscar H. Del Brutto, Robertino M. Mera, Juan A. Nader, Mauricio Zambrano, ... Leslie V. Simon

Pages 427-431

Article preview [✓](#)

Research article ☐ Abstract only

Expression of thioredoxin and glutaredoxin in experimental hepatocellular carcinoma—Relevance for prognostic and diagnostic evaluation

Nabil M. Abdel-Hamid, Tarek K. Mahmoud, Shimaa A. Abass, Mamdouh M. El-Shishtawy

Pages 433-438

Article preview [✓](#)

Research article ☐ Abstract only

Does loss of hormonal receptors influence the pathophysiological characteristics of the HER-2 breast cancer phenotype?

Rabab Ahmed Ahmed Mohammed, Moustafa EzEldien M. Radwan, Bashayer Marzoog Alrufayi, Linah Abdulsamad Qari Abdulaziz

Pages 439-444

Article preview [✓](#)

Discussion ☐ No access

Interventricular vessel of the heart and myocardial infarction-induced heart failure

Pekka J. Kuusela

Pages 445-446

[< Previous vol/issue](#)

[Next vol/issue >](#)

ISSN: 0928-4680

Copyright © 2024 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.



All content on this site: Copyright © 2025 Elsevier B.V., its licensors, and contributors. All rights are reserved, including those for text and data mining, AI training, and similar technologies. For all open access content, the relevant licensing terms apply.





Kolaviron protects the brain in cuprizone-induced model of experimental multiple sclerosis via enhancement of intrinsic antioxidant mechanisms: Possible therapeutic applications?

Gabriel Olaiya Omotoso*, Ileje Inelo Ukwubile, Leviticus Arietarhire, Fatima Sulaimon, Ismail Temitayo Gbadamosi

Division of Neurobiology, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

ARTICLE INFO

Article history:

Received 7 February 2018

Received in revised form 20 April 2018

Accepted 26 April 2018

Keywords:

Cuprizone

Demyelination

Kolaviron

Oxidative enzymes

Prefrontal cortex

ABSTRACT

Multiple sclerosis is a demyelinating condition of the central nervous system which commonly affects young adults. Kolaviron, a biflavonoid isolate of *Garcinia kola*, has been used in experimental studies which explored its anti-oxidative, anti-inflammatory and anti-genotoxic properties. This work was aimed at unraveling the possible ameliorative effect of kolaviron on cuprizone-induced demyelination in the prefrontal cortices of Wistar rats. A total of 28 adult male Wistar rats were divided into four groups A–D. Group A received corn oil (Control), group B received 0.2% Cuprizone, group C received kolaviron (200 mg/kg bw), while group D rats were treated concomitantly with both kolaviron and cuprizone. All groups were treated for 42 days, after which behavioral, histological, immunohistochemical and biochemical analyses were carried out on the prefrontal cortices. Cuprizone significantly down-regulated the level of superoxide dismutase, exacerbated lipid peroxidation and, reduced spatial memory. Cuprizone also induced peripheral and central chromatolysis alongside with atrophied astrocytes in the prefrontal cortex. These alterations were significantly prevented in kolaviron-treated rats, as kolaviron sustained the integrity of neuronal and non-neuronal cells. The activity of kolaviron observed in this study was due to its intrinsic antioxidant properties, which enabled it to combat oxidative damage induced by cuprizone, thereby making kolaviron a potential tool in neurodegeneration therapy of demyelination origin.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Multiple sclerosis (MS) is a neurodegenerative disease caused by oxidative, inflammatory and autoimmune processes [1,2]. Its pathologic hallmarks include demyelination, inflammation, axonal loss and gliosis [2], occurring throughout the brain and spinal cord. These abnormalities usually result into neuronal cell death and damage to the white matter [2]. Some of the clinical manifestations of MS include sensory loss, loss of cognitive functions, depression and difficulty in movement [3,4,5]. In many Countries, MS is a leading cause of non-traumatic neurologic disability among young adults [3]. The underlying cause of MS is unknown but is thought to be multifactorial as it involves genetic predisposition to environmental agents well as other immunological, metabolic and biophysical factor [1,4].

Cuprizone (CPZ) [oxalic acid bis (cyclohexylidene hydrazide)] is a selective and sensitive copper-chelating agent which targets mature oligodendrocytes- the brain cells responsible for the production of myelin [6,7]. A consistent pattern of demyelination and depletion of oligodendrocytes population have been observed in the corpus callosum and other brain areas such as the cerebellar peduncles, cortex, and dorsal hippocampal commissures of cuprizone-treated animals [8,9,10]. Meanwhile, demyelinated areas of the brain become populated by reactive astrocytes and microglia coupled with the secretion of a battery of proinflammatory cytokines like TNF- α , interleukin-1 β , interferon and nitric oxide which regulate the demyelination events [11,12]. The underlying mechanism of action of CPZ remains controversial; however, some studies have reported that intoxication of rats with CPZ led to increased production of free oxygen radicals. The occurrence of oxidative stress and the activation of the immune system often lead to effective induction of oligodendrocytes apoptosis and neuronal cell death [8,9].

Kolaviron (Kv), a biflavonoid complex, is a natural antioxidant and anti-inflammatory compound isolated from the seed of *Garcinia*

* Corresponding author.

E-mail address: gabrielolaiya@yahoo.com (G.O. Omotoso).

kola. The plant itself is used locally as a therapeutic agent for treating several diseases due to its antiviral, antibacterial, antifungal and analgesic properties [13,14]. The antioxidant and free radicals-scavenging activities of Kv have been extensively investigated [15–18]. According to Farombi et al. [18], Kv reduces damage to proteins and lipids induced by Fe³⁺/EDTA/ascorbate mixtures as well as prevents lipid peroxidation. In a dose-dependent pattern, kolaviron inhibits intracellular reactive oxygen species (ROS) production caused by hydrogen peroxide in HepG2 cells detected as 2'-7'-dichlorofluorescein diacetate fluorescence [19,20]. Therefore, the ability of kolaviron to act as an antioxidant underscores its potential role in the chemoprevention of chemically-induced demyelination, and this possibility is being explored in the current study. Meanwhile, our earlier study on cerebellar histology showed that kolaviron is protective against cuprizone-induced neurotoxicity [21]. The current study was designed to explore kolaviron's ameliorative ability on cuprizone-induced toxicity in the prefrontal cortex of Wistar rats.

2. Materials and methods

2.1. Animal acquisition and care

All protocols and treatment procedures were done according to the Institutional Animal Care and Use Committee (IACUC) guidelines and as approved by the Faculty of Basic Medical Sciences Ethics Review Committee, University of Ilorin, Nigeria. Twenty eight adult male Wistar rats with an average weight of 180 ± 10 g were purchased from a private animal holding at Flower Garden, Ilorin and used for the research. These rats were kept in the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin. The rats were daily fed with rat pellets procured from Ogo-Oluwa Feeds and Flour Mill Limited, Sango, Ilorin. They had access to tap water *ad libitum*. Proper ventilation was maintained by use of well-spaced and gauzed cages and a hygienic environment was ensured. The rats were allowed to acclimatize for 14 days.

2.2. Kolaviron isolation

The seeds of *Garcinia kola* (5.2 kg) were procured from the same source at Oja-Oba market in Ilorin. The seeds were then authenticated at the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin. It was confirmed by the curator that the seeds were of uniform species and the specimen voucher number in the herbarium is: UILH/001/1217. The seeds were then air-dried at room temperature (28–30 °C) for three weeks. Subsequently, kolaviron was isolated from the dry seed of kola and characterized according to the method of Iwu [22] as modified by Farombi et al. [16]. The early stages of Kolaviron isolation was done at the Laboratories of Pharmacology and Physiology Departments, University of Ilorin. The dried seeds were pulverized with an electric blender into fine powdered seeds and then extracted with light petroleum ether (boiling point (b.p.) 40–60 °C) in soxhlet extractor which was placed in an electric water bath for 24 h. After this, the defatted, dried marc was repacked and extracted with acetone (b.p. 56–60 °C) in the water bath for 14 h. Subsequently, the resultant extract was concentrated and diluted twice its volume with distilled water and then extracted with ethyl acetate (7.250 ml). The concentrated ethyl acetate fraction gave a yellow solid which is referred to as kolaviron. The purity and identity of kolaviron, carried out at the Drug Metabolism Unit, Faculty of Basic Medical Sciences, University of Ibadan, Nigeria, 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 UV light at a wavelength of 254 nm with Rf (ratio to front) values of 0.48, 0.71 and 0.76. The yield of kolaviron preparation in this study was 6.3%. Kolaviron was kept at a temperature of 4 °C before and after each use.

2.3. Method of administration

0.2 g of Cuprizone (Sigma-Aldrich CHEMIE GmbH) was added to 100 g of feeds daily for animals fed with 0.2% cuprizone diet [23], while kolaviron was dissolved (200 mg/kg) in corn oil (Carlina, ALDI Inc. Batavia) as solvent [15]. Administration of treatment solution (corn oil and kolaviron) to rats across all groups was done orally using oral cannula.

2.4. Animal grouping and treatment

The rats were randomly assigned into control and three treatment groups with each group containing 7 rats. The rats in the control group were administered 0.5 ml corn oil (the vehicle for kolaviron oral administration) [24], while the three treatment groups B, C and D received 0.2% cuprizone, 200 mg/kg of kolaviron [17] and both cuprizone and kolaviron for 42 days respectively.

2.5. Behavioural tests

2.5.1. Y-maze spontaneous alternation test

The Y-maze is used in various behavioral tests that commonly assess learning and memory in rodents [25]. Alternation behavior is based on the natural tendency in rodents to explore the maze systematically entering each arm in turn. In the spontaneous alternation task, the animal was placed in a Y maze and allowed to move freely through the maze. Alternation behavior was defined as consecutive entries into each of the three arms without repetition. Therefore, spontaneous alternation was assessed using a Y-maze composed of three equally spaced arms (120°, 41 cm long and 15 cm high). The floor of each arm was made of plywood and 5 cm wide. Each rat was placed in one of the arm compartments and was allowed to move freely until its tail completely enters another arm. The sequence of arm entries was recorded. For each animal in a group the Y-maze testing was carried out for 5 min, three trials and recorded for later analysis with a webcam. The videos were analyzed by a neutral observer to eliminate bias.

2.5.2. Morris water maze test

The rats were placed in a small pool of water which contained an escape platform hidden a few millimeters below the water surface. Visual cue (a platform), was placed in the pool in plain sight of the animal. When released, the rats did swim around the pool in search of this platform within the time frame of 3 min and on subsequent trials the rats were able to locate the platform more rapidly. This improvement in performance occurred because the rat has learned where the hidden platform was located. The latency to find the platform was measured in seconds [26].

2.5.3. Collection and preservation of brain samples for histological, histochemical, immunohistochemical and biochemical studies

Rats in each group were allowed 4 h rest in their cages after the open field test prior to sacrifice. They were thereafter anaesthetized with ketamine (20 mg/kg, intra-peritoneal) and subjected to transcardial perfusion with 50 ml of 0.1 M PBS (pH 7.4) followed by 500 ml of 4% paraformaldehyde (PFA). The brain tissues were excised, rinsed in 0.25 M sucrose 3 times for 5 min each and then post-fixed in 4% PFA for 24 h after which they were stored in 30% sucrose at 4 °C until further processing. The tissues were processed using Haematoxylin & Eosin, cresyl fast violet stains (for

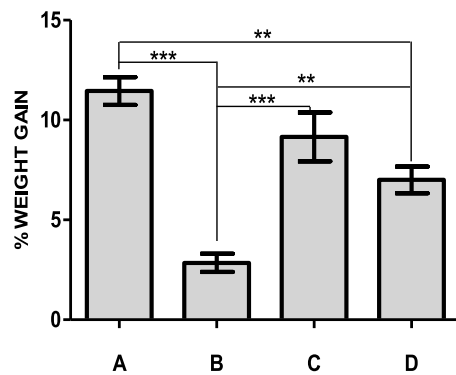


Fig. 1. Mean and standard error of mean of the percentage weight change of experimental animals. A = corn oil [CO] (control), B = cuprizone [CPZ], C = kolaviron [KV], and D = CPZ + KV. ** and *** are p values less than 0.01 and 0.005 respectively.

Nissl bodies demonstration) and anti-glial fibrillary acidic protein antibody (for astrocyte expression). The animals that were used for enzymatic studies were sacrificed by cervical dislocation, to eliminate the interference of ketamine-induced changes in biochemical redox. Their brain tissues were excised, rinsed in 0.25 M sucrose 3 times for 5 min each and placed in 30% sucrose solution and stored at 4 °C. The homogenates were subsequently assayed using specific biochemical kits for blood levels of glutathione peroxidase (GPx), malondialdehyde (MDA), and superoxide dismutase (SOD).

2.5.4. Statistical analysis

All quantitative data were analyzed using GraphPad® prism (version 6) and SPSS (version 20) software. The results of the body weights, spontaneous alternation, Morris water maze, and the oxidative enzymes were analysed using one way ANOVA with Tukey's multiple comparisons test. Significance was set at 95% confidence interval, and the results were represented as mean with standard error of mean.

3. Results

3.1. Kolaviron restores CPZ-induced weight loss

The body weights of the experimental animals were obtained on the first and last day of treatment, representing the initial and final weights respectively. The percentage weight gain was obtained and analyzed. CPZ-treated animals had a significantly lower body weight compared to the control and kolaviron groups ($p < 0.005$ for both). Animals co-treated with Kv and CPZ had significantly higher body weights when compared with the CPZ group ($p < 0.01$). This suggests that kolaviron played a role in sustaining the body weights of Wistar rats against CPZ-induced weight loss. It was also noted that the body weight of animals in the control group was significantly higher than that of the animals that were co-treated with cuprizone and kolaviron ($p < 0.01$) (Fig. 1).

3.2. Kolaviron prevents long and short term memory loss

In the present study, the Wistar rats were subjected to Morris water maze and spontaneous alternation test at the end of the treatment regimen to quantify the spatial and working memory respectively. The escape latency period in the Morris water maze is used as a measure of long term memory index in animal studies [26,27]. CPZ-treated animals presented with a significantly higher escape latency period in the Morris water maze relative to the Control and kolaviron group ($p < 0.005$) (Fig. 2). The percentage correct alternation in the spontaneous alternation test is used to quantify short term memory in animal studies [25]. Expectedly, cuprizone

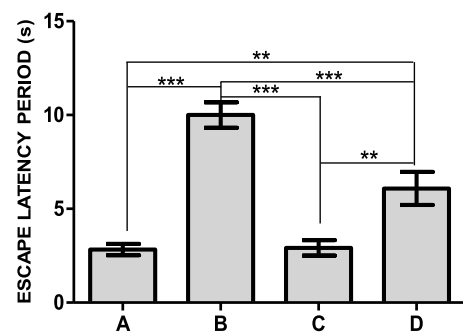


Fig. 2. Escape latency of experimental animals in the Morris water maze: assessment of spatial memory of rats. A = corn oil [CO] (control), B = cuprizone [CPZ], C = kolaviron [KV], and D = CPZ + KV. Group B had a significantly higher escape latency period when compared to all other groups (A, C, and D) ($p < 0.005$). There was a significant difference in the escape latency period of groups A and D ($p < 0.01$) as well as groups C and D ($p < 0.05$), while no significant difference was noticed between groups A and C. ** and *** are p values less than 0.01 and 0.005 respectively.

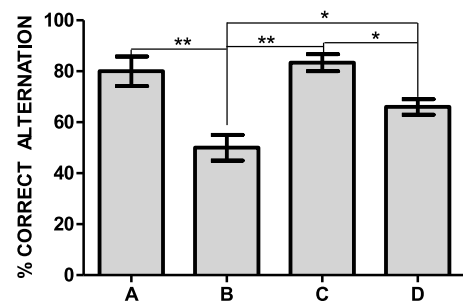


Fig. 3. Percentage correct alternation of experimental animals in the Y-maze: Quantifying the working memory of experimental animals. A = corn oil [CO] (control), B = cuprizone [CPZ], C = kolaviron [KV], and D = CPZ + KV. Group B had a significantly lower percentage correct alternation relative to control ($p < 0.01$), KV ($p < 0.01$), and CPZ + KIV ($p < 0.05$). There was also a significant difference between KV and CPZ + KV groups ($p < 0.05$). * and ** are p values less than 0.05 and 0.01 respectively.

caused a reduction in the percentage correct alternation in animals that received CPZ diet relative to the control and kolaviron groups ($p < 0.01$ for both) (Fig. 3). These findings suggest that CPZ induced a reduction in the memory performance of the experimental animals; however, kolaviron prevented the perturbation of the memory indices in animals that were treated concomitantly with CPZ and kolaviron. Compared with the CPZ-treated rats, those animals treated with both CPZ and Kv had a significantly lower escape latency period ($p < 0.01$) and a comparatively higher percentage correct alternation ($p < 0.05$).

3.3. Frontocortical histomorphology, Nissl profile and astrocyte expression

Histological presentation of the frontal cortices showed mild morphological and histoarchitectural differences across the four experimental groups. The external pyramidal layer of the frontal cortex of animals treated with corn oil and kolaviron presented with typical cellular density and proper cortical delineation of cells. The pyramidal cells in these areas had large cell bodies with adjoining conspicuous neuronal processes and supporting cells all properly situated within their respective intact neuropils. The soma and dendritic processes were properly stained and well distributed. CPZ-treated animals presented with reduced cellular density, neuronal cell size and bitty neuropils with collection of nuclear debris. The vast majority of the pyramidal cells within this layer were poorly stained with fragmented neuropils, as signs of pyknosis. Animals that received a combined treatment of cuprizone and kolaviron had a frontocortical histomorphological presenta-

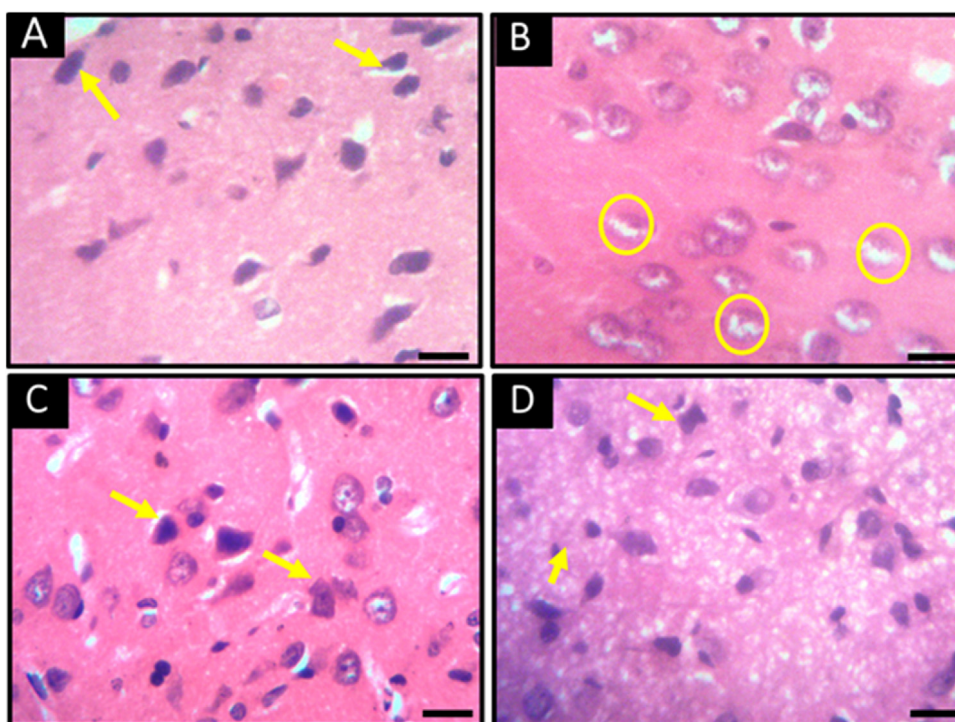


Fig. 4. Representative photomicrographs showing the general cytoarchitecture of the prefrontal cortex (PFC) of Wistar rats. A = corn oil [CO] (control), B = cuprizone [CPZ], C = kolaviron [KV], and D = CPZ + KV. Slides A and C presented with characteristically stained large pyramidal dendrites with apical and basal dendrites (neuronal processes) jutting out of the soma (yellow arrows). These cells possessed intact neuropil with no signs of cellular fragmentation. CPZ-treated animals presented with reduced cellular density and neuronal cell size with collection of nuclear debris. The vast majority of the pyramidal cells within this layer were poorly stained with fragmented neuropils as signs of pyknosis (yellow circles). CPZ + KV-treated animals presented with pyramidal cells in the external pyramidal layer with morphology similar to that seen in the corn oil and KV-treated tissues. Some of the cells in the CPZ + KIV group appeared pyknotic. Haematoxylin and Eosin stain (scale bar = 25 μ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

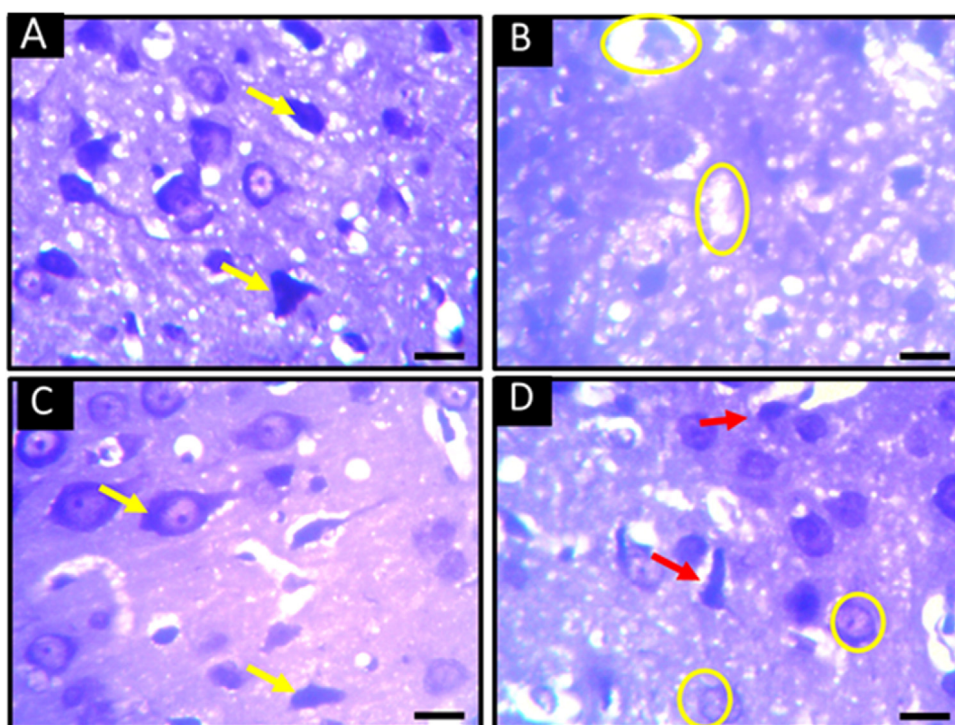


Fig. 5. Representative photomicrographs of the external pyramidal layer of PFC of Wistar rats showing Nissl substances of the neuronal cells. A = corn oil [CO] (control), B = cuprizone [CPZ], C = kolaviron [KV], and D = CPZ + KV. A and C presented with intensive chromatogenic pyramidal cells with prominent neuronal processes (apical and basal dendrites) projecting out of the cell body (yellow arrow). The apical and basal dendrites projecting out of these cells also appeared to be chromatogenic as they were also positively stained for Nissl substances. Conversely, CPZ-treated animals presented with chromatolytic and pyknotic pyramidal cells, indicated by poorly stained cell bodies of the aforementioned cells (yellow circles of Fig. 5B). CPZ + KV presented with mild chromatolytic changes (red arrows). Cresyl Fast Violet stain (scale bar = 25 μ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

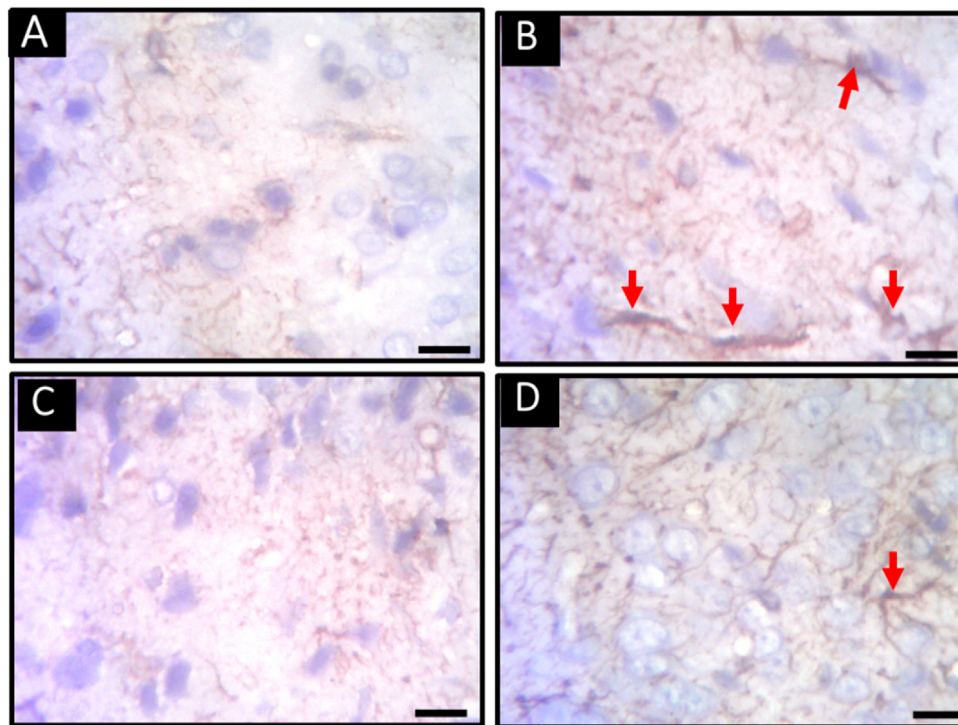


Fig. 6. Representative photomicrographs of the immunohistochemical staining of the astrocytes within the PFC of Wistar rats using anti-GFAP anti-body. A = corn oil [CO] (control), B = cuprizone [CPZ], C = kolaviron [KV], and D = CPZ + KV. A and C presented with normal astrocyte distribution with no activation around the neuronal cells, with generally low astrocytotic densities. CPZ-treated animals presented with hypertrophied astrocytes (red arrows) around the neuronal cells of the frontal cortex with increased astrocytotic density as an indicator of astrocyte activation. CPZ + KV presented with few hypertrophied astrocytes and lower astrocyte density. GFAP stain (scale bar = 25 μ) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

tion that is similar to those of the control and kolaviron-treated animals. Their pyramidal cells had large cell bodies with appreciable cytoplasmic processes that were well seated within their respective neuropils. The Nissl profile of the cells and astrocyte expression within the external pyramidal layer of the frontal cortex of the control and kolaviron-treated animals were normal. The cells were properly stained suggesting intact Nissl bodies in the cells. CPZ-treated animals presented with mild chromatolysis and hypertrophied astrocytes. The cells of the external pyramidal layer were not properly stained, suggesting that CPZ compromised the integrity of the Nissl substance. Kolaviron prevented cuprizone-induced chromatolytic changes and excessive expression of astrocytes in the external pyramidal layer of the frontal cortex of animals that were concomitantly treated with both CPZ and Kolaviron (Figs. 4–6).

3.4. Kolaviron boosts intrinsic antioxidant system to counteract CPZ-induced neural redox imbalance

In the present study, we assayed for the activities of SOD and GPx enzymes as well as lipid peroxidation (using MDA as marker) within the prefrontal cortex to ascertain the effects of CPZ and Kv on reactive oxygen generation in the aforementioned brain region. Kolaviron enhanced the activity of neural SOD activity relative to the control group ($p < 0.01$). CPZ significantly reduced the level of SOD relative to the control ($p < 0.005$) and kolaviron group ($p < 0.005$). Kv normalized the levels of neural SOD in the animals that were concomitantly treated with both CPZ and Kv as we observed a significant increase in SOD level relative to the CPZ-treated rats ($p < 0.01$), despite being lower than that of the control ($p < 0.05$) and kolaviron groups (Fig. 7). A similar pattern was observed with GPx activities. CPZ-treated rats had a significantly lower GPx level relative to control and kolaviron groups ($p < 0.005$

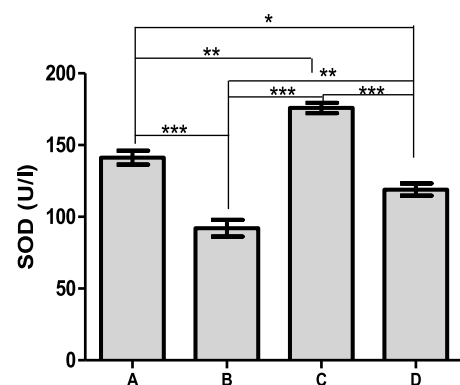


Fig. 7. Activities of superoxide dismutase in the PFC of experimental animals. A = corn oil [CO] (control), B = cuprizone [CPZ], C = kolaviron [KV], and D = CPZ + KV. There was a significant decrease in SOD activities in B compared to A ($p < 0.005$), C ($p > 0.005$) and D ($p > 0.01$). SOD activities in D was also significantly less than those in A and C at $p < 0.05$ and 0.005 respectively. (*, **, and *** are p values less than 0.05, 0.01 and 0.005 respectively).

for both). Kolaviron sustained the level of GPx in rats treated with CPZ and Kv, which had a significantly higher GPx activity relative to CPZ group ($p < 0.05$) (Fig. 8). Lipid peroxidation (MDA) was higher in CPZ-treated animals relative to the control group and kolaviron group ($p > 0.005$ for both), while rats co-treated with CPZ and Kv had a lower level of lipid peroxidation relative to the cuprizone-treated animals ($p > 0.05$) (Fig. 9).

4. Discussion

Body weight reduction during CPZ intoxication has been reported in various experimental studies [29]. This sometimes might be due to poor feeding habit in experimental animals

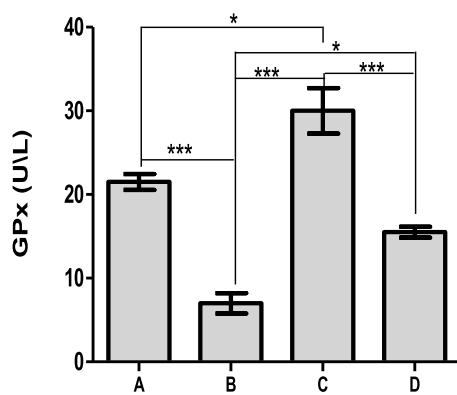


Fig. 8. Activities of Glutathione peroxidase in the PFC of experimental animals. A = corn oil [CO] (control), B = cuprizone [CPZ], C = kolaviron [KV], and D = CPZ + KV. GPx activities in B was significantly lower relative to A ($p < 0.005$), C ($p > 0.005$) and D ($p > 0.005$). GPx activities in D was also significantly lower than C at $p < 0.005$ and 0.005 respectively. The level of GPx in A was significantly lower than C ($p < 0.05$) (* and *** are p values less than 0.05 and 0.005 respectively).

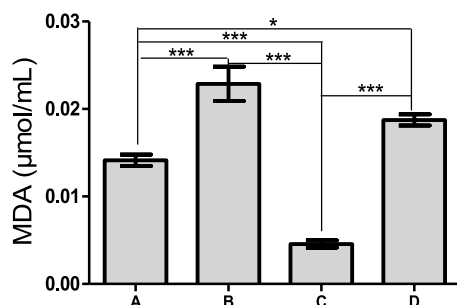


Fig. 9. Activities of malondialdehyde in the PFC of experimental animals. A = corn oil [CO] (control), B = cuprizone [CPZ], C = kolaviron [KV], and D = CPZ + KV. MDA activities in B was significantly higher compared to A ($p < 0.005$), C ($p > 0.005$) and D ($p > 0.01$). MDA activities in D was also significantly higher than those in A and C at $p < 0.005$ and 0.05 respectively (* and *** are p values less than 0.05 and 0.005 respectively).

exposed to CPZ diet; however, normal feeding habit resumes upon withdrawal of CPZ. The mechanism by which CPZ causes weight loss could be due to disruption of normal energy flow [30]. These changes earlier reported were also corroborated by the current study. Body weight maintenance is dependent on eating and dietary patterns [31,32]. Therefore, it can be suggested that the observed body weight reduction in this study is caused by reduced feeding pattern and disruption of normal energy flow. Treatment with Kv following CPZ intoxication resulted in significant elevation of body weight when compared to the CPZ group. The possible mechanism by which Kv preserves the body weight might be due to its ability to prevent the normal disruption of energy flow and its ability to prevent decrease in the eating pattern among rats treated with CPZ.

Damage to the white matter has been linked to emotional and behavioral deficits [26,28]. The effectiveness of treatment can be determined by measuring behavioural outcomes in neurodegeneration versus behavioral outcomes in therapeutic targets. Hence, a correlative test for cellular and neuropathological changes in the prefrontal cortex was done in this present study using the Morris water maze test and the Y-maze test to determine the cognitive, memory and exploratory activities among experimental animals [25–27]. Our findings showed that CPZ intoxication resulted in spontaneous alternation deficits in Y-maze test and significantly increased the escape latency period in the Morris water maze test, which is an indication of memory and cognitive impairment. The impaired performance noticed in the activities of the CPZ-treated rats is suggestive of anterior prefrontal lesions and studies have

shown a link between CPZ intoxication and reduced behavioral performances. Cognitive, memory and exploratory processes are dependent on the intact integrity of the myelin sheath. Kv treatment was protective against behavioral deficits induced by CPZ intoxication. Olajide et al. [33] reported an increase in exploratory activities among rats treated with Kv after induction with NaN_3 neurodegeneration. The mechanism involved in the protective role of Kv against CPZ-induced behavioural deficits in this study might be due to its ability to prevent dysfunctions in mitochondrial biochemical redox and ROS production [34].

Normal metabolic processes often result in the production of ROS [35]. However, there is a dynamic equilibrium that exists between the production of reactive ROS and the antioxidant capacity of the cell [20,36]. Disruption to this equilibrium predisposes the cells to generation of excess free radicals and oxidative stress, which eventually results into cell death and axonal loss [37]. Studies have linked oxidative stress to the pathogenic traits of MS and are suggested to cause alteration in the normal function of the electron transport chain [38,39]. Furthermore, the PFC regions appears to be particularly vulnerable to free radicals as a result of their reduced glutathione content, high proportion of polyunsaturated fatty acids and high demand for substantial quantity of oxygen for their proper metabolic activities [40–42]. SOD functions in the dismutation of superoxides (O^{2-}), a highly reactive oxygen species, into H_2O_2 , which is a less reactive oxygen species. GPx requires glutathione (GSH) as an electron donor for the conversion of H_2O_2 to H_2O and O_2 . Oligodendrocytes have low amounts of GSH and low GPx activity [23].

Biancotti et al. [37] observed earlier a reduction in GSH content during CPZ treatment, suggesting that cuprizone through a cascade of events caused excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which cannot be catered for by endogenous SOD and GPx [37]. These superfluous ROS and RNS could exacerbate the rate of lipid peroxidation by continuous degradation of neuronal cell membrane, thereby gaining access into the intracellular environment and damaging the DNA component of the cell. These structural alterations could be responsible for the observed pyknotic and chromatolytic changes in the histochemical tissue sections.

Furthermore, decrease in SOD level has been implicated in the mechanism through which cuprizone induces demyelination due to the decrease in Cu^+ level which is required for the proper function of SOD [43]. The decrease in GPx levels, which could either increase or reflect oxidative state, has been implicated in the mechanistic pathway by which cuprizone induces demyelination [23]. Xuan et al. [44] documented significant increase in MDA level following 4 weeks of CPZ intoxication in different regions of the brain. However, kolaviron administration in the current work significantly reduced the oxidative stress induced by CPZ intoxication, as evident by the increased levels of SOD and GPx and decreased level of MDA in the prefrontal cortex of rats co-treated with cuprizone and kolaviron. Thus, supplementation with antioxidants which act as free radical scavengers is indispensable for sustaining oligodendroglial functions. It is therefore suggested that CPZ induces its damage via excess production of H_2O_2 , leading to generation of hydroxyl radicals and eventual damage to membrane lipids, while Kv exerts its effect by preventing H_2O_2 production and consequently lipid peroxidation, by scavenging free radicals [4].

Morphological examination of the cuprizone-treated rats revealed cytoarchitectural distortion, pyknosis and neuropil fragmentation in the prefrontal cortex of Wistar rats. Severe central and peripheral chromatolytic changes and extreme reduction in the cellular density, which may be an indication of neuronal necrosis, were also noticed. Furthermore, CPZ intoxication causes the formation of megamitochondria and oxidative stress leading to energy flow disruption and shortage [23]. These energy flow disruption

and depletion causes alteration in the proper functioning of the endoplasmic reticulum resulting into extensive proliferation of ER and a reduction in hydrophobic protein synthesis; these often leads to cellular degeneration and disintegration of oligodendrocytes, perikaryon and myelin sheath [9]. These cytotoxic properties of CPZ may account for the mechanism through which it induces cellular degeneration and white matter demyelination. In this study, rats treated concomitantly with Kv and CPZ showed a cortical morphology which was similar to those observed in the control and Kv treated group. The neuronal morphology in this group showed normal neurons that were highly chromatogenic with normal cellular density, and appreciable axon and dendrites within the neuropil.

Astrocytes have increasingly been implicated in most demyelinating diseases and it is said to be involved in CPZ-induced demyelination [19,23,45]. Activation of astrocytes results in extensive astrogliosis in CPZ intoxication, and astrogliosis persists (although less severe) through remyelination [19,23,45]. Using the anti-I GFAP) antibody, we numerous hypertrophic astrocytes around the neuronal cells with increased density in the prefrontal cortex. These astrocytes were characterized by numerous cytoplasmic vacuoles and possibly, extensive presence of glial filaments which is an indication of astrogliosis. However, rats treated with kolaviron in addition to cuprizone, exhibited similar morphology in the PFC with those of the Control and the kolaviron-treated animals. The ability of the PFC to withstand CPZ-induced damage is as a result of the intervention using Kv, which has conferred substantial cytoprotection on the prefrontal cortex of rats by preventing astrogliosis.

5. Conclusion

Kolaviron significantly counteracted cuprizone-induced neurotoxicity by eliciting significant protective effect on the prefrontal cortex, thereby sustaining the integrity of neuronal and non-neuronal cells. This action was due to its intrinsic antioxidant and anti-inflammatory abilities to combat oxidative and inflammatory damage induced by CPZ and as such may be a potential tool in neurodegeneration therapy of demyelinating origin.

References

- [1] L.F. Barcellos, J.R. Oksenberg, A.J. Green, P. Bucher, J.B. Rimmner, S. Schmidt, et al., Genetic basis for clinical expression in multiple sclerosis, *Brain* 125 (2002) 150–158.
- [2] J.D. Haines, M. Inglese, P. Casaccia, Axonal damage in multiple sclerosis, *Mt. Sinai J. Med.* 78 (2) (2011) 231–243.
- [3] P. Browne, D. Chandraratna, C. Angood, H. Tremlett, C. Baker, B.V. Taylor, et al., Atlas of multiple sclerosis 2013: a growing global problem with widespread inequity, *Neurology* 83 (11) (2014) 1022–1024.
- [4] B.D. Trapp, K.A. Nave, Multiple sclerosis: an immune or neurodegenerative disorder? *Annu. Rev. Neurosci.* 31 (2008) 247–269.
- [5] J.A. Twomey, M.L. Espir, Paroxysmal symptoms as the first manifestations of multiple sclerosis, *J. Neurol. Neurosurg. Psychiatry* 43 (1980) 296–304.
- [6] K. Suzuki, Y. Kikkawa, Status spongiosus of CNS and hepatic changes induced by cuprizone (biscyclohexanone oxalyldihydrazone), *Am. J. Pathol.* 54 (1969) 307–325.
- [7] S. Montes, S. Rivera-Mancia, A. Diaz-Ruiz, L. Tristan-Lopez, C. Rios, copper and copper proteins in Parkinson's disease, *Oxid. Med. Cell. Longev.* 2014 (2014) 147251, <http://dx.doi.org/10.1155/2014/147251>.
- [8] M.M. Hiremath, Y. Saito, G.W. Knapp, J.P. Ting, K. Suzuki, G.K. Matsushima, Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice, *J. Neuroimmunol.* 92 (1998) 38–49.
- [9] M. Kipp, T. Clarner, J. Dang, S. Copray, C. Beyer, The cuprizone animal model: new insights into an old story, *Acta Neuropathol.* 118 (2009) 723–736.
- [10] P.N. Koutsoudaki, T. Skripuletz, V. Gudi, D. Moharreggh-Khiabani, H. Hildebrandt, C. Trebst, et al., Demyelination of the hippocampus is prominent in the cuprizone model, *Neurosci. Lett.* 451 (2009) 83–88.
- [11] J. Correale, M.F. Farez, The role of astrocytes in multiple sclerosis progression, *Front. Neurol.* 6 (2015) 180, <http://dx.doi.org/10.3389/fneur.2015.00180>.
- [12] T. Macháček, L. Panská, H. Dvořáková, P. Horák, Nitric oxide and cytokine production by glial cells exposed in vitro to neuropathogenic schistosome *trichobilharzia regenti*, *Parasit. Vectors* 14 (1) (2016) 579, 9.
- [13] M.M. Iwu, O.A. Igboke, C.O. Okunji, M.S. Tempesta, Antidiabetic and aldose reductase activities of biflavonones of *Garcinia kola*, *J. Pharm. Pharmacol.* 42 (1990) 290–292.
- [14] J.F. Akoachere, R.N. Ndip, E.B. Chenwi, L.M. Ndip, T.E. Njock, D.N. Anong, Antibacterial effect of *Zingiber officinale* and *Garcinia kola* on respiratory tract pathogens, *East Afr. Med. J.* 79 (11) (2002) 588–592.
- [15] O.A. Adaramoye, V.O. Nwaneri, K.C. Anyanwu, E.O. Farombi, G.O. Emerole, Possible anti-atherogenic effect of kolaviron (a *Garcinia kola* seed extract) in hypercholesterolaemic rats, *Clin. Exp. Pharmacol. Physiol.* 32 (1–2) (2005) 40–46.
- [16] E.O. Farombi, S. Shrotriya, Y.J. Surh, Kolaviron inhibits dimethyl nitrosamine-induced hepatotoxicity by suppressing COX-2 and iNOS expression: NF-κB and AP-1 as potential molecular targets, *Life Sci.* 84 (2009) 149–155.
- [17] E.O. Farombi, O. Owolaye, Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*, *Int. J. Environ. Res. Public Health* 8 (2011) 2533–2555.
- [18] E.O. Farombi, P. Moller, L.O. Dragsted, *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.* 20 (2004) 71–82.
- [19] E.P. Eddy, P.C. Howard, G.D. McCoy, H.S. Rosenkranz, Mutagenicity, unscheduled DNA synthesis, and metabolism of 1-nitropyrene in the human hepatoma cell line HepG2, *Canc. Res.* 47 (1987) 3163–3168.
- [20] J.O. Nwankwo, J.G. Tanhteng, G.O. Emerole, Inhibition of aflatoxin B1 genotoxicity in human liver-derived HepG2 cells by kolaviron biflavonoids and molecular mechanisms of action, *Eur. J. Canc. Prev.* 9 (2000) 351–361.
- [21] G.O. Omotoso, O.J. Olajide, I.T. Gbadamosi, F.A. Suleiman, J.O. Oladimeji, Kolaviron ameliorates histomorphological changes associated with cuprizone-induced cerebellar damage, *J. Anat. Sci.* 8 (2) (2017) 131–137.
- [22] M.M. Iwu, Antihepatotoxic constituents of *Garcinia kola* seeds, *Experientia* 41 (1985) 699–700.
- [23] J. Praet, C. Guglielmetti, Z. Berneman, V.L. Annemie, P. Peter, Cellular and molecular neuropathology of the cuprizone mouse model: clinical relevance for multiple sclerosis, *Neurosci. Biobehav. Rev.* 47 (2014) 485–505.
- [24] O.A. Adaramoye, M. Arisekola, Kolaviron, a biflavonoid complex from *Garcinia kola* seeds, ameliorates ethanol-induced reproductive toxicity in male wistar rats, *Niger. J. Physiol. Sci.* 28 (1) (2013) 9–15.
- [25] Wolf, A., Bauer, B., Abner, E.L., Ashkenazy-Frolinger, T., Hartz, A.M.S., A Comprehensive Behavioral Test Battery to Assess Learning and Memory in 129S6/Tg2576 Mice. Reddy, H., ed. *PLoS ONE*. 2016; 11(1):e0147733. doi:10.1371/journal.pone.0147733.
- [26] P. Monfort, S. Erceg, B. Piedrafita, M. Llansola, V. Felipo, Chronic liver failure in rats impairs glutamatergic synaptic transmission and long-term potentiation in hippocampus and learning ability, *Eur. J. Neurosci.* 25 (2007) 2103–2111.
- [27] C.V. Vorhees, M.T. Williams, C.V. Vorhees, M.T. Williams, Morris water maze: procedures for assessing spatial and related forms of learning and memory, *Nat. Protoc.* 1 (2) (2006) 848–858, <http://dx.doi.org/10.1038/nprot.2006.116>.
- [28] J. Chun, H.P. Hartung, Mechanism of action of oral fingolimod (FTY720) in multiple sclerosis, *Clin. Neuropharmacol.* 33 (2010) 91–101.
- [29] Q. Yu, R. Hui, J. Park, Y. Huang, A.W. Kusnecov, C.F. Dreyfus, R. Zhou, Strain differences in cuprizone-induced demyelination, *Cell. Biosci.* 7 (59) (2017), <http://dx.doi.org/10.1186/s13578-017-0181-3>.
- [30] J. Galgani, E. Ravussin, Energy metabolism, fuel selection and body weight regulation, *Int. J. Obes.* 32 (7) (2008) S109–S119, <http://dx.doi.org/10.1038/ijo.2008.246>.
- [31] L.G. Ogden, S. Phelan, J.G. Thomas, J.O. Hill, R.R. Wing, H.R. Wyatt, Dietary habits and weight maintenance success in high versus low exercisers in the national weight control registry, *J. Phys. Activity Health* 11 (8) (2014) 1540–1548.
- [32] D.K. Tobias, C. Zhang, J. Chavarro, S. Olsen, W. Bao, A.A. Bjerregaard, et al., Healthful dietary patterns and long-term weight change among women with a history of gestational diabetes mellitus, *Int. J. Obes.* 40 (11) (2016) 1748–1753.
- [33] O.J. Olajide, B.U. Enaibe, O.O. Bankole, O.B. Akinola, B.J. Laoye, O.M. Ogundele, Kolaviron was protective against sodium azide (NaN₃) induced oxidative stress in the prefrontal cortex, *Metab. Brain Dis.* (2015), <http://dx.doi.org/10.1007/s11011-015-9674-0>.
- [34] E.O. Farombi, M.C. Alabi, T.O. Akuru, Kolaviron modulates cellular redox status and impairment of membrane protein activities induced by potassium bromate (KBrO₃) in rats, *Pharmacol. Res.* 45 (1) (2002) 63–68.
- [35] P. Sharma, A.B. Jha, R.S. Dubey, M. Pessarakli, Reactive oxygen species, oxidative damage, and antioxidant defense mechanism in plants under stressful conditions, *J. Bot.* (2012), <http://dx.doi.org/10.1155/2012/217037>, Article ID 217037, 2012.
- [36] E.M. Frohman, M.K. Racke, C.S. Raine, Multiple sclerosis—the plaque and its pathogenesis, *N. Engl. J. Med.* 354 (2006) 942–955.
- [37] J.C. Biancotti, S. Kumar, J. de Vellis, Activation of inflammatory response by a combination of growth factors in cuprizone-induced demyelinated brain leads to myelin repair, *Neurochem. Res.* 33 (2008) 2615–2628.
- [38] G.G. Ortiz, F.P. Pacheco-Moisés, O.K. Bitzer-Quintero, A.C. Ramírez-Anguiano, L.J. Flores-Alvarado, V. Ramírez-Ramírez, et al., Immunology and oxidative stress in multiple sclerosis: clinical and basic approach, *Clin. Dev. Immunol.* (2013), 708659, <http://dx.doi.org/10.1155/2013/708659>.

- [39] M. Tully, R. Shi, New insights in the pathogenesis of multiple sclerosis—role of acrolein in neuronal and myelin damage, *Int. J. Mol. Sci.* 14 (10) (2013) 20037–20047.
- [40] B. Ozyurt, H. Ozyurt, N. Akpolat, H. Erdogan, M. Sarsilmaz, Oxidative stress in prefrontal cortex of rat exposed to MK-801 and protective effects of CAPE, *Prog. Neuropsychopharmacol. Biol Psychiatry* 31 (4) (2007) 832–838.
- [41] X. Wang, E.K. Michaelis, Selective neuronal vulnerability to oxidative stress in the brain, *Front. Aging Neurosci.* 2 (2010) 12, <http://dx.doi.org/10.3389/fnagi.2010.00012>.
- [42] B.K.Y. Bitanirwe, T.W. Woo, Oxidative stress in schizophrenia: an integrated approach, *Neurosci. Biobehav. Rev.* 35 (3) (2011) 878–893, <http://dx.doi.org/10.1016/j.neubiorev.2010.10.008>.
- [43] H.R. Ghaiaad, M.M. Nooh, M.M. El-Sawalhi, A.A. Shaheen, Resveratrol promotes remyelination in cuprizone model of multiple sclerosis: biochemical and histological study, *Mol. Neurobiol.* 54 (5) (2017) 3219–3229.
- [44] Y. Xuan, G. Yan, R. Wu, Q. Huang, X. Li, H. Xu, The cuprizone-induced changes in 1 H-MRS metabolites and oxidative parameters in C57BL/6 mouse brain: effects of quetiapine, *Neurochem. Int.* (2015) 1–8.
- [45] V. Gudi, S. Gingele, T. Skripuletz, M. Stangel, Glial response during cuprizone-induced de- and remyelination in the CNS: lessons learned, *Front. Cell. Neurosci.* 8 (2014) 73, <http://dx.doi.org/10.3389/fncel.2014.00073>.