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Kolaviron protects against cognitive deficits and cortico-hippocampal perturbations associated with maternal deprivation in rats

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Abstract: Prolonged separation of pups from their mother in early postnatal period can interfere with normal growth and development, resulting in different behavioral changes similar to features of schizophrenia in man. This study explored the cytoprotective action of kolaviron, a biflavonoid, on the prefrontal cortex and hippocampus of maternally deprived Wistar rats. Eight months old female rats were time-mated, and after delivery their pups were randomly assigned into four groups; group A received 0.5 ml of normal saline, group B received kolaviron orally (200 mg/kg/bw) on postnatal days (PND) 21–35, group C were maternally deprived on PND 9 for 24 hours, while group D were also maternally deprived on PND 9 for 24 hours, and then received kolaviron orally (200 mg/kg/bw) on PND 21–35. Behavioral studies (open field test, Morris water test, and Y-maze test) were conducted after the experiment prior to sacrifice. Some of the rats were anesthetized with ketamine and perfusion-fixed with 0.1 M phosphate buffered saline and 4% paraformaldehyde, while others were sacrificed by cervical dislocation for enzyme studies. The hippocampus and prefrontal cortex were excised from the brain and processed for tissue histology, histochemistry, and enzymatic analysis. Results revealed behavioral deficits, oxidative stress, degenerative changes, and astrocytosis in the prefrontal cortex and hippocampus of maternally deprived rats, but intervention with kolaviron caused significant improvement in neurobehavior, morphology, and neurochemistry in these brain areas. We concluded that kolaviron could protect the brain against neurological consequences of nutritional and environmental insults arising from maternal separation in early postnatal period.

Key words: Maternal deprivation, Behavior, Kolaviron, Morphology

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Introduction

There is a worldwide growing concern on the burden of neurological disorders in both the developed and developing countries [1]. Some of these conditions which are initiated during the neurodevelopmental stages may not manifest any clinical signs until later in adolescence and adulthood [2]. The trouble of bearing these disorders whose onset begins early in life, both in terms of mortality and morbidity is quite huge; hence research efforts, such as the use of neurodevelopmental models, focus on determining the mechanisms involved and how to curtail the consequences of the disorders [2, 3].

Maternal deprivation (MD) in rodents is an essential neurodevelopmental model for studying different behavioral changes similar to the features of schizophrenia and has been extensively used [3, 4]. Unfavorable early life experiences can modify brain development and afterward accentuate the risk of psychiatric disorders, such as schizophrenia [2, 5, 6]. Stress in early life potentially impairs essential processes in brain

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development, including neurogenesis, migration, differentiation, synaptogenesis, myelination, gliogenesis, and apoptosis [2, 7].

Animal model of MD is based on exposure to stress in early postnatal life. It has repeatedly been shown that early perinatal stress can cause various short- and long-term disturbances in cognitive, emotional, and other behavioral performances [8, 9]. Schizophrenia is a neurodevelopmental disorder manifested as cortical and subcortical volumetric and microstructural abnormalities [10]. A multifaceted disorder such as schizophrenia has no recognized cure, necessitating the search for certain plant derivatives with medicinal properties. Antioxidants in the body guard brain cells and offer numerous benefits important to brain health and function [11]. They have the capability to hunt free radicals, making them risk-free. A good number of dietary supplements from plant sources have antioxidant properties.

Garcinia kola (of the family *Guittiferae*) is a widespread oral masticatory agent commonly consumed in parts of Nigeria and West African Countries, due to its nutritional benefits and acclaimed medicinal values [12]. *Garcinia kola* exhibits a wide range of biological and pharmacological activities, such as anti-inflammatory, antibacterial and anti-fungal properties. Extracts from the roots, barks, seeds, and fruits of *Garcinia kola* are used in the production of syrups and infusions in traditional medicine as cough suppressant, and in the treatment of liver pathologies [13, 14].

Kolaviron is a natural antioxidant and anti-inflammatory bioflavonoid isolated from *Garcinia kola* [15-17]. It is used as a management regimen for several infections and disease conditions due to its acclaimed antiviral, antibacterial, antifungal, anti-inflammatory and antioxidant activities [18, 19]. Kolaviron minimizes oxidative stress and tissue damage by reducing the rate of lipid peroxidation and production of oxygen radicals [18, 20]. The radical scavenging activity of kolaviron is exclusively important in limiting oxidative tissue damage in neural tissue, which is usually due to the selective susceptibility of the neural cells to reactive oxygen species (ROS) created during a deregulated energy metabolism process [21, 22]. The anti-oxidative potential of kolaviron is yet to be explored in combating a severe psychiatric condition such as schizophrenia. Thus, this study aimed to determine the beneficial role of kolaviron in tackling the associated neuropathological and neurological deficits using an MD rat model.

Materials and Methods

Animal acquisition and care

All protocols and treatment procedures were carried out according to the Institutional Animal Care and Use Committee (IACUC) guidelines and as approved by the University Ethical Review Committee, University of Ilorin, Nigeria. Thirty adult female Wistar rats (8 months old) and eight adult male Wistar rats (10 months old) were obtained from Veterinary Department of the University of Ilorin. These rats were kept in the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin. The rats were on a daily basis fed with rat pellets procured from Ogo Oluwa Feeds and Flour Mill, Sango, Ilorin. The rats had access to tap water *ad libitum*. Proper ventilation was maintained by the use of well-spaced and gauzed cages and a hygienic environment was ensured. Acclimatization was for 2 weeks. The oestrus cycle of the female rats was determined through vaginal smear, in order to guide mating [23]. The female rats were thereafter exposed to male rats for mating to occur.

Kolaviron isolation

The seeds of *Garcinia kola* were bought at Oja-Oba market in Ilorin, and authenticated. They were air-dried at room temperature (28°C–30°C) for 3 weeks. Subsequently, kolaviron was isolated from the dry seed of kola and characterized according to the method of Iwu [24] as modified by Farombi et al. [25]. The extraction and isolation of kolaviron was carried out at the Central Research Laboratory of the University of Ilorin. The dried seeds were pulverized into fine powdery form and extracted with light petroleum ether. The product was defatted, repacked, and extracted with acetone. The resulting extract was then concentrated, diluted, and extracted with ethyl acetate. The concentrated ethyl acetate fraction gave a yellow solid, the kolaviron, whose purity and identity can be determined by subjecting it to thin layer chromatography using silica gel GF 254 coated plates and solvent mixture of methanol and chloroform in a ratio 1:4 v/v.

Method of MD and kolaviron administration

MD was carried out on postnatal day (PND) 9 by separating the mother rats from her pups and this lasted for a period of 24 hours, after which both the mother and pups were brought back again in the same cage and re-established breastfeeding [4]. Kolaviron was dissolved in corn oil (Carlini, ALDI Inc., Batavia, IL, USA) to allow for oral administration [26].

Animal grouping and treatment

After weaning on PND 21, the young rats were randomly assigned into four groups each comprised four rats from different mothers. The first group received 0.5 ml of normal saline (control) from PND 21 to PND 35; the second group received kolaviron (200 mg/kg/day) from PND 21 to PND 35; the rats in the third group were initially separated from their mother (MD) on PND 9 for 24 hours, and were untreated between PND 21 to 35; while the fourth group which earlier was maternally deprived at PND 9 received 200 mg/kg/day of kolaviron [20, 27] from PND 21 to PND 35. Kolaviron was administered orally via a cannula. The duration of treatment between PND 21 to 35 amounted to 15 days.

Open field test

Open field test was conducted as described by Gould et al. [28]. The open field apparatus was a 100 cm×100 cm wooden box with 38 cm high walls (opened from tip) which was kept in an isolated room with normal lighting and temperature. The floor of the arena was divided by straight lines into squares of 10 cm each. A video recording system was stationed at an angle to capture the movement of rats within the box arena. Twelve hours after the last administration of kolaviron, each group of rats was taken to the open field arena in separate cages while making sure there was no agitation to avoid stress on the animals. Subsequently, each rat was placed in the open field arena, away from the others and their explorative movement was measured for 10 minutes using the video recorder. This was repeated for rats across the groups. Prior to each exercise, the apparatus was wiped with methylated spirit to abolish the odor of the previously tested rat. A neutral observer stayed away from the apparatus during each test. Following the completion of the exercise, the video was analyzed by the neutral observer who counted the number of squares explored by each rat.

Morris water maze test

Neural mechanisms of spatial learning and memory were evaluated using the Morris water maze [29]. Animals acquire information about spatial location and reach hidden platform in circular pool filled with water. Thus, a decrease in the time to reach the hidden platform suggests learning. The Morris water maze apparatus consists of a circular water tank (110 cm diameter and 60 cm height) filled with water (26±2°C) to a depth of 30 cm. The pool was divided into four hypothetical quadrants, designated as N (North), E (East), W (West), S (South). A black round platform of 10 cm diameter was placed 2 cm below the surface of the water in a constant position in the middle of the south-west quadrant in all trials. For each rat, the test was carried out for 5 minutes by a neutral observer four times and recorded and the videos were analyzed by a neutral observer.

Y-maze spontaneous alternation test

The Y-maze spontaneous alternation test is used as a behavioral test to assess learning and memory in rodents [30]. Alternation behavior is based on the spontaneous tendency in rodents to walk around the maze systematically entering each arm in turn. In the spontaneous alternation task, each rat was placed in a Y maze and allowed to move freely in the maze. Alternation behavior was defined as successive entries into each of the three arms with no repetition. Therefore, spontaneous alternation was assessed using a Y-maze composed of three equally spaced arms (41 cm long and 15 cm high). The floor of each arm was made of plywood which was 5 cm wide. Each rat was placed in one of the arm compartments and was allowed to move freely until the rat completely enters another arm. The sequence of arm entries was recorded. For each animal in a group the Y-maze testing was done for 5 minutes by a neutral observer three times and recorded. The videos were analyzed by a neutral observer.

Tissue collection and processing

Four hours after the behavioral tests were concluded some of the rats were anesthetized with ketamine (20 mg/kg, intraperitoneal) and subjected to transcardial perfusion with 0.1 M phosphate buffered saline (PBS) and 4% paraformaldehyde (PFA). The brain tissues were thereafter excised, rinsed in 0.25 M sucrose thrice for 5 minutes each and post-fixed in 4% PFA for 24 hours. Paraffin wax sections were obtained for histology and immunohistochemistry. The prefrontal cortex and hippocampus were excised from the fixed brain and dehydrated in ascending grades of alcohol (50%, 70%, 90%, and 100%). The tissues were then cleared in xylene twice for 15 min each. Infiltration and embedding was done with paraffin wax in Leica hot air oven at 56°C with tissues eventually embedded in paraffin wax at similar orientations. Tissue sections were obtained serially using a rotary microtome (Leica RM2245) and then mounted on glass slides. Sections were taken at 30 μm for histology and 15 μm for immunohistochemistry.

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Hematoxylin and eosin staining

Brain sections were stained using the method of Pearse [31] as modified by Fischer et al. [32]. This method was carried out to demonstrate the general cytoarchitecture of the prefrontal cortex and hippocampus. Slides containing paraffin sections were placed in a slide holder and deparaffinization and rehydration of sections were done in the following reagents respectively: thrice for 3 minutes in xylene, thrice for 3 minutes 1:1 xylene with 100% ethanol, once for 3 minutes in 95% ethanol, once in 80% ethanol for 3 minutes and then once in deionized H_2O for 5 minutes, while excess water was blotted from the slide holder before taking them into hematoxylin. Subsequently, hematoxylin staining was done with the following procedure: once for 3 minutes with hematoxylin, once in tap water for 5 minutes (to allow the stain to develop); slides were dipped 12 times in acid ethanol, rinsed twice with tap water for 1 minute, then rinsed once in deionized water for 2 minutes and left overnight. Excess water was blotted from the slide holder before putting into eosin.

For eosin staining and dehydration, the following procedures were followed: slides were placed once for 30 seconds in eosin (up to 45 seconds for older batch of eosin) then thrice for 5 minutes in 95% ethanol, followed by thrice for 5 minutes in 100% ethanol (excess ethanol was blotted before putting into xylene) and then thrice for 15 minutes in xylene. Following these, the slides were coverslipped using distrene plasticizer in xylene (DPX) as mountant (one drop of DPX was placed on the slide using a glass rod). Coverslips were angled to let them fall gently on the slide. Thereafter, the slides were dried overnight in the hood.

Cresyl fast violet staining method

This method was carried out to demonstrate the integrity of Nissl bodies in the hippocampus and prefrontal cortex [33]. The reagents used for this procedure included Cresyl fast solution (1 g), distilled water (100 ml), and acetic acid (0.25 ml). Slides containing paraffin sections were placed in a slide holder and deparaffinization and rehydration of sections were done in the following reagents respectively: 5 minutes in xylene, 3 minutes in 100% ethanol, 3 minutes in 90% ethanol, 3 minutes in 70% ethanol, 3 minutes in 50% ethanol, 5 minutes in deionized H_2O and excess water was blotted from the slide holder before taking them into Cresyl fast violet solution for 20–30 minutes, 30 minutes in 96% ethanol, 15 minutes in xylene. The slides were coverslipped with DPX.

Immunoperoxidase technique for astrocyte expression

Enzymatic antigen retrieval method with trypsin (catalog ID ab970, Abcam, Cambridge, MA, USA) was used [34]. The trypsin working solution (0.05%) was prepared by placing 1 ml of 0.5% trypsin stock with 1 ml of 1% of calcium chloride stock solution and 8 ml of distilled water (the pH was adjusted to 7.8 with 1 M NaOH and stored at 4°C. Endogenous peroxidase blocking was done using hydrogen peroxide, while 5% bovine serum albumin (BSA) was used to reduce nonspecific protein reactions. Slides were drained for a few seconds and wiped around the sections with tissue paper. The diluted primary antibody (anti-glial fibrillary acidic protein) was added to each slide and incubated overnight at 4°C. Primary antibody dilution (dilution 1:1,000, Cell Signaling) was done in blocking buffer (10% calf serum with 1% BSA and 0.1% Triton X-100 in 0.1 M PBS). Subsequently, slides were rinsed twice for 5 minutes in 0.1 M PBS (0.025% Triton) with gentle agitation. The slides were then treated with biotinylated secondary antibody (goat anti-rabbit 1:400) and incubated for 2 hours at room temperature, thereafter rinsed thrice for 5 minutes in PBS. Color intensification was done using the chromogen 3,3′-diaminobenzidine (1:1,000 dilution) for 10 minutes at room temperature. Slides were then rinsed in running tap water for 5 minutes. Counterstaining was done in hematoxylin and 1% acid alcohol, while the sections were dehydrated and cleared in ethanol and xylene respectively, and thereafter mounted with DPX.

Superoxide dismutase enzyme assay

Some of the rats were sacrificed by cervical dislocation to assess the activity of superoxide dismutase (SOD) enzyme. The tissues were placed in 0.25 M sucrose solution and homogenized. Tissue homogenate was collected in a 5 ml sample bottle and centrifuged at 3,000 rpm for 15 minutes using a centrifuge (Model 90-1; Jiangsu Jinyi Instrument Tech, Jiangsu, China). The supernatant was collected with Pasteur pipettes into sample bottles and placed in a freezer at –4°C. SOD was assayed [35] using spectrophotometric techniques. The reaction mixture (3 ml) contained 2.95 ml carbonate buffer, 0.02 ml of homogenate and 0.03 ml of 2 mM SOD substrate in 0.005 N HCl, used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate and 0.02 ml of water. The absorbance was read at regular interval of 1 minute for 5 minutes at 480 nm.

Cell count

Activated astrocytes and chromatolytic cell counts were determined from captured prefrontal cortical and hippocampal images using Image J (NIH, Bethesda, MD, USA). For each section, the count was determined using cellular morphology and layer-dependent cell densities) for eight different fields of view.

Data analysis

All quantitative data were analyzed using GraphPad version 6 and SPSS version 20 (IBM Corp., Armonk, NY, USA) software, using one-way ANOVA followed with Tukey's multiple comparisons test. Significance was set at $P<0.05$, $P<0.01$, $P<0.005$, $P<0.001$ (95%, 99%, 99.5%, 99.99% confidence interval, respectively). The results were represented in bar charts with error bars to show the mean and standard error of mean respectively.

Results

General observation and weight change

Observation of the pups in all the groups after delivery until postnatal day 35 revealed noticeable changes, with significant impairment in their activities, such as movement in maternally deprived pups compared to other groups. Body weights increase in all the groups was initially gradual up to PND 9 and 10 when rapid weight increase occurred especially in the control and kolaviron groups until PND 31, followed by a slight increase till the termination of the experiment (Fig. 1). Weight increase was more in the control compared to the kolaviron group, and least in the maternally deprived rats. However, maternally deprived rats that were treated with kolaviron had a higher weight increase compared to those that did not receive kolaviron.

Behavioral observation

Behavioral studies were conducted to determine the effect of MD, with or without kolaviron intervention, on neurocognitive functions. Wistar rats were subjected to Morris water maze, Y maze, and open field tests. The escape latency period

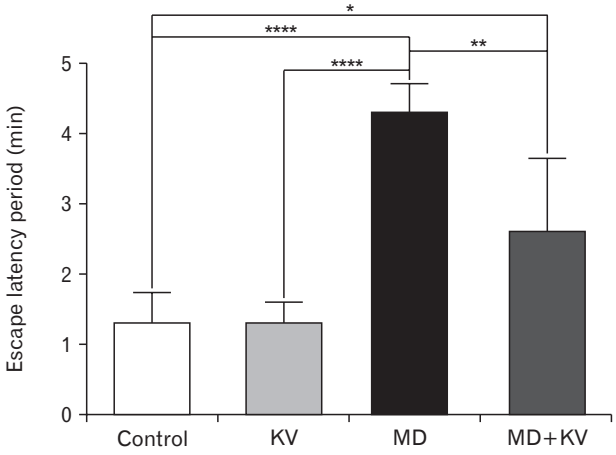

Fig. 2. Escape latency period using the Morris water maze for the assessment of spatial memory of rats. KV, kolaviron-treated group; MD, maternally deprived group; MD+KV, maternally deprived+kolaviron group. Maternally deprived rats had a significantly higher escape latency period compared to control (*$P<0.05$, **$P<0.01$, and ****$P<0.0001$ respectively).

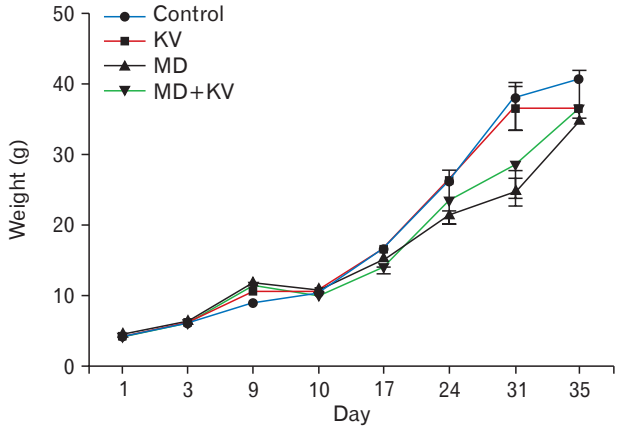

Fig. 1. Bodyweight of pups from postnatal days 1 to 35; there was more increase in weight of rats in the control and kolaviron-treated group (KV group) compared to maternal deprivation groups (MD group). MD+KV, maternal deprivation+kolaviron.

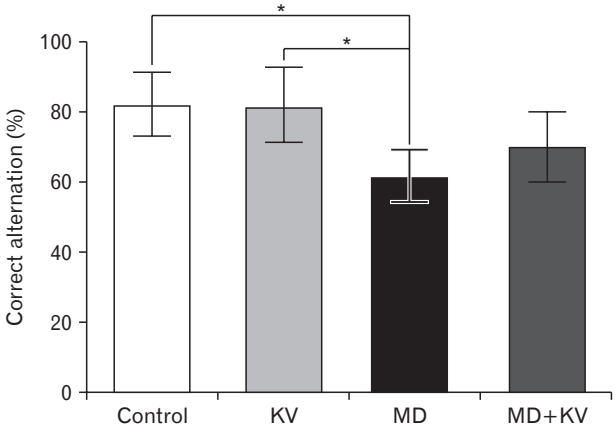

Fig. 3. Percentage correct alternation in the Y-maze test. KV, kolaviron-treated group; MD, maternally deprived group; MD+KV, maternally deprived+kolaviron group. MD group had a significantly lower percentage correct alternation relative to the control (*$P<0.05$) and kolaviron group (*$P<0.05$).

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in the Morris water maze was used as a measure of long-term memory index in this study. Maternally deprived rat presented with a significantly higher escape latency period relative to the control and kolaviron group (P<0.001) (Fig. 2), while rats that received kolaviron after deprivation also had higher escape latency period compared to the control and kolaviron-treated groups though significantly lower than the maternally deprived rats (P<0.01). The percentage correct alternation in the spontaneous alternation test was used to quantify short-term memory. There was a reduction in the percentage correct alternation in maternally deprived rats relative to the control and kolaviron groups (P<0.01) (Fig. 3). Rats treated with kolaviron after deprivation had a higher percentage correct alternation compared to maternally deprived rats that did not receive the intervention (P>0.05). Open field tests revealed locomotory and exploratory activities and anxiety states. There was no significant difference in the number of lines crossed and the rearing frequency in the kolaviron group compared to control. High frequency of lines crossed and rearing indicated low level of anxiety in rats in these groups. However, there was significant reduction in number of lines crossed and rearing frequency in maternally deprived rats when compared with kolaviron-treated rats and the control (P<0.001); this however improved in rats that had kolaviron intervention after deprivation (Figs. 4, 5).

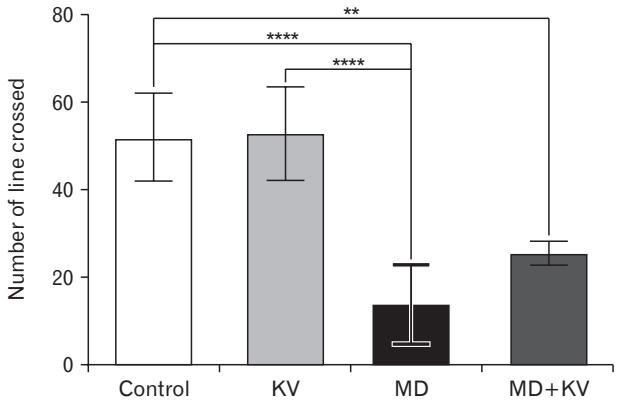

Fig. 4. Number of lines crossed by rats in the open field test. KV, kolaviron-treated group; MD, maternally deprived group; MD+KV, maternally deprived+kolaviron group. MD rats had significant reduction in number of lines crossed compared to control and KV group (**P<0.01 and ****P<0.0001, respectively).

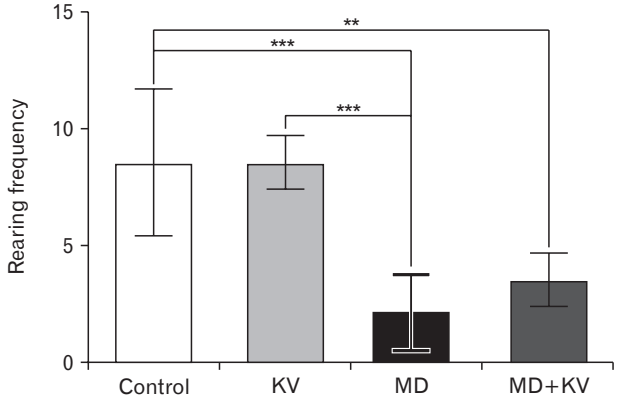

Fig. 5. Rearing frequency of animals in the open field test. KV, kolaviron-treated group; MD, maternally deprived group; MD+KV, maternally deprived+kolaviron group. MD rats had a significant reduction in rearing frequency compared to the control and KV group (**P<0.01 and ***P<0.005, respectively).

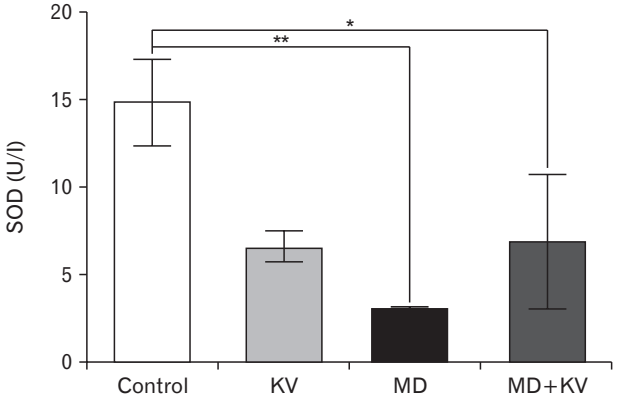

Fig. 6. Superoxide dismutase (SOD) levels in the hippocampus. KV, kolaviron-treated group; MD, maternally deprived group; MD+KV, maternally deprived+kolaviron group. There was a significant decrease of SOD level in MD group compared to control group (P<0.01). (*P<0.05 and **P<0.01, respectively).

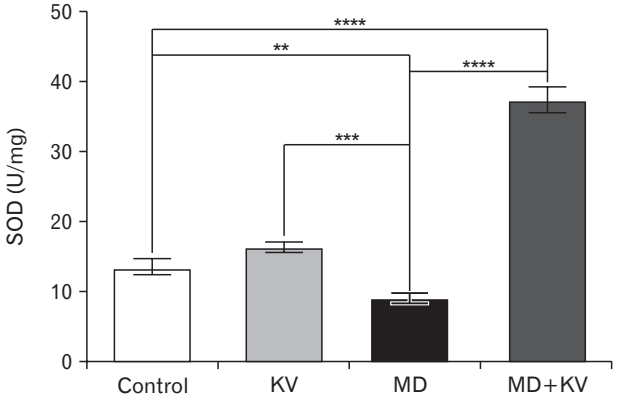

Fig. 7. Superoxide dismutase (SOD) activity in the prefrontal cortex. KV, kolaviron-treated group; MD, maternally deprived group; MD+KV, maternally deprived+kolaviron group. There was a significant decrease in SOD activity in maternally deprived group compared to control group (P<0.01) and KV group (P<0.005); MD+KV group had a significantly higher SOD level compared to the control and MD groups (**P<0.01, ***P<0.005, and ****P<0.0001, respectively).

Kolaviron counterbalances MD-induced oxidative stress in the prefrontal cortex and hippocampus

Hippocampal SOD level was significantly elevated in the control compared to the maternally deprived rats with or without kolaviron intervention (MD, $P<0.01$; MD+KV, $P<0.05$), though rats that received kolaviron after deprivation had a higher SOD level compared to those that received no kolaviron (Fig. 6). In the prefrontal cortex, SOD level was depressed significantly in maternally deprived rats when compared with the control ($P<0.01$) and kolaviron-treated

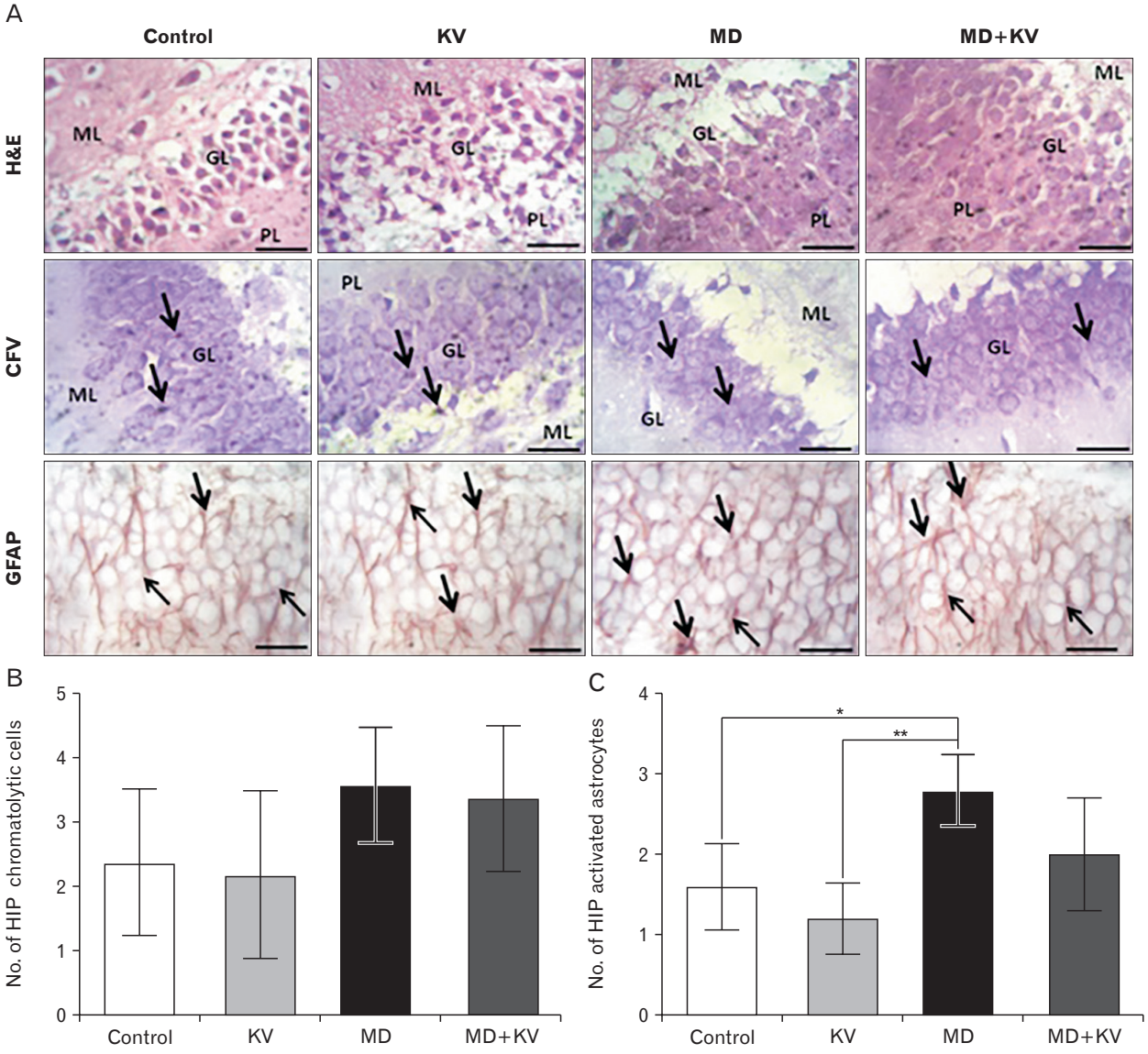

Fig. 8. (A) Representative photomicrographs of the hippocampus stained with hematoxylin and eosin (H&E), cresyl fast violet (CFV), and anti-glial fibrillary acidic protein ([GFAP] for astrocyte expression), showing the molecular layer (ML), granular layer (GL), and polymorphic layer (PL) of the dentate gyrus. Control and kolaviron-treated group (KV group) revealed more population of granule cells (CFV, arrows) in the GL with no chromatolytic cells, while the granule cells in maternally deprived group (MD group) appeared sparsely populated compared to the control and KV group. Numerous reactive/hypertrophied astrocytes were seen in maternally deprived hippocampal section compared with the control and kolaviron-treated sections, while astrocyte expression was only moderate in maternally deprived+kolaviron group (MD+KV group). Scale bars=25 μm. (B) In the hippocampal (HIP) tissue, the number of chromatolytic cells was more in maternally deprived rats compared to the control, KV group and the rats treated with kolaviron after maternal deprivation (MD+KV), though the difference was not statistically significant ($P>0.05$). MD+KV group had a slightly lower number of chromatolytic cells compared to MD group, but not significant ($P>0.05$). (C) The number of activated astrocytes was least in KV group compared to control and other treated groups ($P>0.05$), while the number was highest in MD group compared to other groups, with significant difference between control versus MD group. *$P<0.05$ and **$P<0.01$ were indicates significant differences.

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rats (P<0.005). Meanwhile, rats that received kolaviron intervention following deprivation had a marked increase in SOD level (Fig. 7).

Histological, histochemical and immunohistochemical observation

Hippocampal morphology of the control and kolaviron-treated rats showed properly delineated dentate gyri with a regular density of granule cells and well-stained nuclei.

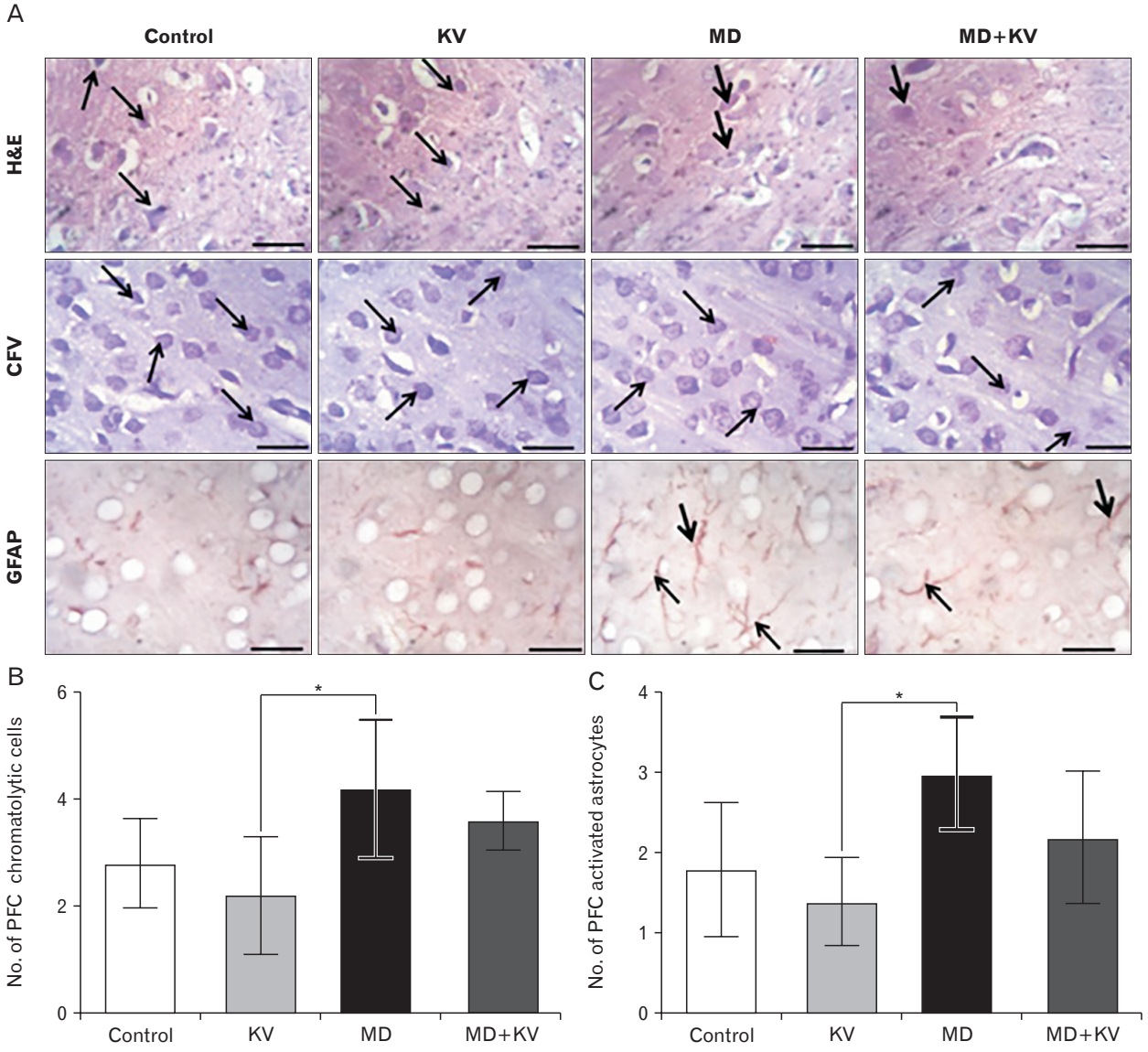

Fig. 9. (A) Representative photomicrographs of the prefrontal cortex showing hematoxylin and eosin (H&E), cresyl fast violet (CFV), and anti-glial fibrillary acidic protein (GFAP) stained sections. The cortex of the control and kolaviron-treated group (KV group) contained deeply stained nuclei of neuronal and non-neuronal cells (H&E, arrows) within their respective neuropils and abundant Nissl substances within the neurons (CFV, arrows), while in maternally deprived, it showed numerous cellular degeneration indicated by poorly stained nuclei (H&E, arrows), as well as severe chromatolysis indicated by poorly stained Nissl substances in the neurons (CFV, arrows). Maternally deprived+kolaviron group (MD+KV group) showed mild chromatolysis compared to maternally deprived group (MD group). Normal astrocyte distribution with no activation around the neuronal cells was revealed in the control and kolaviron-treated prefrontal cortex, with generally low astrocytic cells. The expression of reactive astrocytes was marked in MD group presented with hypertrophied astrocytes (arrows) around neuronal cells with increased astrocyte population as an indicator of astrocyte activation. MD+KV group presented with few hypertrophied astrocytes and lower astrocyte density compared to the MD group. The number of chromatolytic cells (B) and activated astrocytes (C) in the prefrontal cortex (PFC) was least in KV group and highest in MD group with a significant difference between both groups. *Significant difference at P<0.05.

However, the maternally deprived rats showed degenerative changes mainly characterized by poorly stained granule cells in the granular layer of the dentate gyrus. The degenerative changes, however, were reduced in rats administered with kolaviron after MD (Fig. 8). The morphology of neurons within the pyramidal and granular layers of prefrontal cortex was extensively stained, with no apparent architectural alteration in the control and kolaviron group (Fig. 9). Meanwhile, the neurons in maternally deprived prefrontal cortex showed signs of degenerative changes similar to those of hippocampus, but were reduced in rats given kolaviron.

Nissl staining with cresyl fast violet revealed normal Nissl profiles in the hippocampus and prefrontal cortex (Figs. 8A, 9A) of control and kolaviron-treated rats compared to maternally deprived rats, which was characterized by sparse Nissl distribution and chromatolytic cells. Cell counts revealed more chromatolytic cells in MD rats compared to other groups (Figs. 8B, 9B). However, kolaviron treatment markedly improved the distribution and population of Nissl bodies within hippocampal and prefrontal cortical neurons.

The expression of astrocytes appeared normal in the hippocampal and prefrontal cortical sections of control and kolaviron-treated rats. Activation of more astrocytes was seen in MD group with the presence of hypertrophied astrocytes and increased number of astrocytic cells (Figs. 8C, 9C). Kolaviron prevented MD-induced excessive expression of reactive astrocytes in the brain tissues (Figs. 8, 9).

Discussion

Animal model of MD is based on exposure to stress in early postnatal life. It has repeatedly been shown that early perinatal stress can cause various short-term and long-term disturbances in cognitive, emotional, and other behavioral performances [8, 9], as seen in the current study. There was reduction in the body weights of maternally deprived rats. It is suggested that the observed bodyweight reduction in this study was caused by reduced feeding pattern and disruption of energy normal flow. The reduction in body weights of maternally deprived rats was similar to observation of previous authors [36, 37].

Apart from physical growth, MD has been reported to cause behavioral abnormalities [38]. The open field test is one of the most widely used tests in animal models of anxiety testing [28]. Our results revealed that MD significantly reduced exploratory activities and caused anxiety and emotional imbalance. Anxiety-related behaviors in MD animals were also reported by Viveros et al. [39], where they linked these alterations with decrease in concentration of the stress hormone, corticosterone, which was found only in maternally deprived rats. Administration of kolaviron to maternally deprived rats in the current study improved their exploratory drive, emotional balance, and reduced anxiety states. An earlier work by Olajide et al. [40] reported an increase in exploratory activities among rats treated with kolaviron after NaN_3-induced neurodegeneration.

Furthermore, MD led to spontaneous alternation deficits in Y-maze test and significantly increased the escape latency period in the Morris water maze test, which are an indication of memory and cognitive impairment. Neves et al. [41] reported that deficit in memory and cognitive impairment could be related to brain oxidative balance. The imbalance of antioxidant and pro-oxidant markers causes oxidative stress and damage to important regions of the central nervous system related to learning and memory, including the hippocampus [42]. Kolaviron treatment was protective against behavioral deficits induced by MD. Kolaviron is both antioxidative and anti-inflammatory with ability to prevent generation of free radicals and stop inflammatory responses in cells [16, 43]. The improved behavioral outcomes observed in maternally deprived rats after kolaviron intervention could be as a result of the expression of its antioxidant properties in neural cells, thereby disrupting other excitotoxic stimuli that may result in behavioral deficits. The mechanism involved in the protective role of kolaviron against MD-induced behavioral deficits in this study might be due to its ability to prevent dysfunctions in mitochondrial biochemical redox and hence, ROS production.

To ascertain the oxidative status of maternally deprived rats, with or without kolaviron intervention, in this study, the SOD levels in the hippocampus and prefrontal cortex were assessed and noted to be significantly reduced in rats that did not receive an intervention. Previous investigators have similarly demonstrated the free radical scavenging properties of kolaviron using SOD as an enzymatic endogenous marker to assess tissue oxidative status in rodents following neurotoxicity [44, 45].

Redox imbalance contributes immensely to compromise in the integrity and functions of the cell membrane and organelles and could enhance cell death [46]. This effect could be responsible for the adverse morphological alterations seen in the hippocampus and prefrontal cortex of maternally de-

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prived rats. Reduction or disruption in Nissl profile in the hippocampus and prefrontal cortex of maternally deprived rats could affect the protein synthesis mechanism of the neuronal cells and ultimately affect critical cellular processes and neurological functions. Nissl bodies are rough endoplasmic reticulum in the cell bodies of neurons. Endoplasmic reticulum has also been implicated in the generation of ROS, as a redox interplay exists between the trio of mitochondria, endoplasmic reticulum, and peroxisomes in their involvement in ROS production [47]. The introduction of kolaviron, therefore, is essential in restoring the integrity of neuronal somatic rough endoplasmic reticulum (Nissl bodies), to preserve their protein-generating potential for optimal neuronal functions.

Astroglia play important role in the control of brain homeostasis and intrinsic brain defense system [48]. Following an insult to the developing brain, for instance, due to MD, there is a resulting astroglial defense response (reactive astrogliosis), which is essential for both limiting the areas of damage by scar formation and for the post-insult remodeling and recovery of neural function [48]. Although reactive astrogliosis is a nonspecific response, it is highly characteristic and involves various morphological and molecular changes [49]. Astrogliosis has been used as an index for underlying neuronal damage [50]. According to Sun and Jakobs [49], the glial scar produced by reactive astrocytes could impede axon regeneration, further complicating neurological dysfunctions associated with psychiatric disorders modeled by MD. A medicinal plant extract such as kolaviron that could ameliorate these adverse effects should be considered in the management of such clinical conditions. We conclude that early MD adversely affects the hippocampus and prefrontal cortex structurally and functionally. However, kolaviron exhibits protective activity against MD-associated neurological abnormalities characteristic of neuropsychiatric disorders such as schizophrenia, through a mechanism that involves prevention of oxidative stress and ribosomal protein degradation.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

References

1. GBD 2015 Neurological Disorders Collaborator Group. Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol 2017;16:877-97.
2. Kundakovic M, Champagne FA. Early-life experience, epigenetics, and the developing brain. Neuropsychopharmacology 2015;40:141-53.
3. Luna B, Sweeney JA. Studies of brain and cognitive maturation through childhood and adolescence: a strategy for testing neurodevelopmental hypotheses. Schizophr Bull 2001;27:443-55.
4. Aleksić D, Aksić M, Radonjić NV, Jovanović A, Marković B, Petronijević N, Radonjić V, Mališ M, Filipović B. Long-term effects of maternal deprivation on the volume, number and size of neurons in the amygdala and nucleus accumbens of rats. Psychiatr Danub 2016;28:211-9.
5. Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP, Kim DM. The neurobiological consequences of early stress and childhood maltreatment. Neurosci Biobehav Rev 2003;27:33-44.
6. Chocyk A, Dudys D, Przyborowska A, Majcher I, Maćkowiak M, Wędzony K. Maternal separation affects the number, proliferation and apoptosis of glia cells in the substantia nigra and ventral tegmental area of juvenile rats. Neuroscience 2011;173:1-18.
7. Bandeira F, Lent R, Herculano-Houzel S. Changing numbers of neuronal and non-neuronal cells underlie postnatal brain growth in the rat. Proc Natl Acad Sci U S A 2009;106:14108-13.
8. Koehl M, Lemaire V, Vallée M, Abrous N, Piazza PV, Mayo W, Maccari S, Le Moal M. Long term neurodevelopmental and behavioral effects of perinatal life events in rats. Neurotox Res 2001;3:65-83.
9. Viveros MP, Díaz F, Mateos B, Rodríguez N, Chowen JA. Maternal deprivation induces a rapid decline in circulating leptin levels and sexually dimorphic modifications in hypothalamic trophic factors and cell turnover. Horm Behav 2010;57:405-14.
10. Spoletini I, Cherubini A, Banfi G, Rubino IA, Peran P, Caltagi-

rone C, Spalletta G. Hippocampi, thalami, and accumbens microstructural damage in schizophrenia: a volumetry, diffusivity, and neuropsychological study. Schizophr Bull 2011;37:118-30.
11. Bastianetto S, Zheng WH, Quirion R. Neuroprotective abilities of resveratrol and other red wine constituents against nitric oxide-related toxicity in cultured hippocampal neurons. Br J Pharmacol 2000;131:711-20.
12. Maňourová A, Leuner O, Tchoundjeu Z, Van Damme P, Verner V, Pribyl O, Lojka B. Medicinal potential, utilization and domestication status of bitter kola (*Garcinia kola* Heckel) in West and Central Africa. Forests 2019;10:124.
13. Ogu EO, Agu RC. A comparison of some chemical properties of Garcinia kola and hops for assessment of Garcinia brewing value. Bioresour Technol 1995;54:1-4.
14. Ogwu DE. Phytochemicals, vitamins and mineral contents of two Nigeria medicinal plants. Int J Mol Med Adv Sci 2005;1:375-81.
15. Olaleye SB, Onasanwo SA, Ige AO, Wu KK, Cho CH. Anti-inflammatory activities of a kolaviron-inhibition of nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha production in activated macrophage-like cell line. Afr J Med Med Sci 2010;39 Suppl:41-6.
16. Farombi EO, Adedara IA, Ajayi BO, Ayepola OR, Egbeme EE. Kolaviron, a natural antioxidant and anti-inflammatory phytochemical prevents dextran sulphate sodium-induced colitis in rats. Basic Clin Pharmacol Toxicol 2013;113:49-55.
17. Omotoso GO, Olajide OJ, Gbadamosi IT, Rasheed MA, Izuogu CT. Kolaviron protects the prefrontal cortex and hippocampus against histomorphological and neurobehavioral changes in cuprizone model of multiple sclerosis. Malays J Med Sci 2018;25:50-63.
18. Olaleye SB, Farombi EO. Attenuation of indomethacin- and HCl/ethanol-induced oxidative gastric mucosa damage in rats by kolaviron, a natural biflavonoid of Garcinia kola seed. Phytother Res 2006;20:14-20.
19. Farombi EO, Abarikwu SO, Adedara IA, Oyeyemi MO. Curcumin and kolaviron ameliorate di-n-butylphthalate-induced testicular damage in rats. Basic Clin Pharmacol Toxicol 2007;100:43-8.
20. Ayepola OR, Chegou NN, Brooks NL, Oguntibeju OO. Kolaviron, a Garcinia biflavonoid complex ameliorates hyperglycemia-mediated hepatic injury in rats via suppression of inflammatory responses. BMC Complement Altern Med 2013;13:363.
21. Igado OO, Olopade JO, Adesida A, Aina OO, Farombi EO. Morphological and biochemical investigation into the possible neuroprotective effects of kolaviron (Garcinia kola bioflavonoid) on the brains of rats exposed to vanadium. Drug Chem Toxicol 2012;35:371-80.
22. Abarikwu SO, Farombi EO, Kashyap MP, Pant AB. Kolaviron protects apoptotic cell death in PC12 cells exposed to atrazine. Free Radic Res 2011;45:1061-73.
23. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol 2002;62:609-14.
24. Iwu MM. Antihepatoxic constituents of Garcinia kola seeds. Experientia 1985;41:699-700.
25. 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;84:149-55.
26. Omotoso GO, Olajide OJ, Gbadamosi IT, Suleiman FA, Oladimeji JO. Kolaviron ameliorates histomorphological changes associated with cuprizone-induced cerebellar damage. J Anat Sci 2017;8:131-7.
27. Farombi EO, Owoeye O. Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. Int J Environ Res Public Health 2011;8:2533-55.
28. Gould TD, Dao DT, Kovacsics CE. The open field test. In: Gould T, editor. Mood and Anxiety Related Phenotypes in Mice. Vol. 42. Neuromethods. Totowa, NJ: Humana Press; 2009. p.1-20.
29. Monfort P, Erceg S, Piedrafita B, Llansola M, Felipo V. Chronic liver failure in rats impairs glutamatergic synaptic transmission and long-term potentiation in hippocampus and learning ability. Eur J Neurosci 2007;25:2103-11.
30. Wolf A, Bauer B, Abner EL, Ashkenazy-Frolinger T, Hartz AM. A comprehensive behavioral test battery to assess learning and memory in 129S6/Tg2576 mice. PLoS One 2016;11:e0147733.
31. Pearse AG. The role of histochemistry in increasing objectivity in histopathology. Postgrad Med J 1975;51:708-10.
32. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc 2008;2008:pdb.prot4986.
33. Bancroft JD, Stevens A. Theory and practice of histological techniques. 2nd ed. Edinburgh: Churchill Livingstone; 1982.
34. Goldstein M, Watkins S. Immunohistochemistry. Curr Protoc Mol Biol 2008;Chapter 14:Unit 14.6.
35. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal Biochem 1978;90:81-9.
36. Ellenbroek BA, Derks N, Park HJ. Early maternal deprivation retards neurodevelopment in Wistar rats. Stress 2005;8:247-57.
37. Llorente R, Arranz L, Marco EM, Moreno E, Puerto M, Guaza C, De la Fuente M, Viveros MP. Early maternal deprivation and neonatal single administration with a cannabinoid agonist induce long-term sex-dependent psychoimmunoendocrine effects in adolescent rats. Psychoneuroendocrinology 2007;32:636-50.
38. Đorđević J, Mitić M, Lukić I, Adžić M. Maternal deprivation of rat pups reduces body weight and alters behavior in adulthood in a gender-specific manner. Arch Biol Sci 2015;67:131-8.
39. Viveros MP, Llorente R, López-Gallardo M, Suarez J, Bermúdez-Silva F, De la Fuente M, Rodriguez de Fonseca F, Garcia-Segura LM. Sex-dependent alterations in response to maternal deprivation in rats. Psychoneuroendocrinology 2009;34 Suppl 1:S217-26.
40. Olajide OJ, Enaibe BU, Bankole OO, Akinola OB, Laoye BJ, Ogundele OM. Kolaviron was protective against sodium azide (NaN3) induced oxidative stress in the prefrontal cortex. Metab Brain Dis 2016;31:25-35.
41. Neves BH, Menezes J, Souza MA, Mello-Carpes PB. Physical

 https://doi.org/10.5115/acb.19.160

exercise prevents short and long-term deficits on aversive and recognition memory and attenuates brain oxidative damage induced by maternal deprivation. Physiol Behav 2015;152:99-105.
42. Riedel G, Micheau J. Function of the hippocampus in memory formation: desperately seeking resolution. Prog Neuropsychopharmacol Biol Psychiatry 2001;25:835-53.
43. Farombi EO, Akanni OO, Emerole GO. Antioxidant and scavenging activities of flavonoid extract (kolaviron) of Garcinia kola seeds. Pharm Biol 2002;40:107-16.
44. Olajide OJ, Asogwa NT, Moses BO, Oyegbola CB. Multidirectional inhibition of cortico-hippocampal neurodegeneration by kolaviron treatment in rats. Metab Brain Dis 2017;32:1147-61.
45. Omotoso GO, Ukwubile II, Arietarhire L, Sulaimon F, Gbadamosi IT. Kolaviron protects the brain in cuprizone-induced model of experimental multiple sclerosis via enhancement of intrinsic antioxidant mechanisms: possible therapeutic applications? Pathophysiology 2018;25:299-306.
46. Zhang LX, Levine S, Dent G, Zhan Y, Xing G, Okimoto D, Kathleen Gordon M, Post RM, Smith MA. Maternal deprivation increases cell death in the infant rat brain. Brain Res Dev Brain Res 2002;133:1-11.
47. Wanders RJ, Waterham HR, Ferdinandusse S. Metabolic interplay between peroxisomes and other subcellular organelles including mitochondria and the endoplasmic reticulum. Front Cell Dev Biol 2016;3:83.
48. Kettenmann H, Verkhratsky A. Neuroglia: living nerve glue. Fortschr Neurol Psychiatr 2011;79:588-97.
49. Sun D, Jakobs TC. Structural remodeling of astrocytes in the injured CNS. Neuroscientist 2012;18:567-88.
50. Zhang D, Hu X, Qian L, O'Callaghan JP, Hong JS. Astrogliosis in CNS pathologies: is there a role for microglia? Mol Neurobiol 2010;41:232-41.