Psychopharmacology (2020) 237:1027–1040
https://doi.org/10.1007/s00213-019-05432-8

ORIGINAL INVESTIGATION

Kolaviron via anti-inflammatory and redox regulatory mechanisms abates multi-walled carbon nanotubes-induced neurobehavioral deficits in rats

Isaac A. Adedara[1] · Ifeoluwa O. Awogbindin[1] · Olatunde Owoeye[2] · Ikenna C. Maduako[1] · Akinola O. Ajeleti[3] · Solomon E. Owumi[4] · Anita K. Patlolla[5] · Ebenezer O. Farombi[1]

Received: 1 June 2019 / Accepted: 11 December 2019 / Published online: 3 January 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020, corrected publication 2020

Abstract

Exposure to multi-walled carbon nanotubes (MWCNTs) reportedly elicits neurotoxic effects. Kolaviron is a phytochemical with several pharmacological effects namely anti-oxidant, anti-inflammatory, and anti-genotoxic activities. The present study evaluated the neuroprotective mechanism of kolaviron in rats intraperitoneally injected with MWCNTs alone at 1 mg/kg body weight or orally co-administered with kolaviron at 50 and 100 mg/kg body weight for 15 consecutive days. Following exposure, neurobehavioral analysis using video-tracking software during trial in a novel environment indicated that co-administration of both doses of kolaviron significantly ($p < 0.05$) enhanced the locomotor, motor, and exploratory activities namely total distance traveled, maximum speed, total time mobile, mobile episode, path efficiency, body rotation, absolute turn angle, and negative geotaxis when compared with rats exposed to MWCNTs alone. Further, kolaviron markedly abated the decrease in the acetylcholinesterase activity and antioxidant defense system as well as the increase in oxidative stress and inflammatory biomarkers induced by MWCNT exposure in the cerebrum, cerebellum, and mid-brain of rats. The amelioration of MWCNT-induced neuronal degeneration in the brain structures by kolaviron was verified by histological and morphometrical analyses. Taken together, kolaviron abated MWCNT-induced neurotoxicity via anti-inflammatory and redox regulatory mechanisms.

Keywords Multi-walled carbon nanotubes · Kolaviron · Neurotoxicity · Acetylcholinesterase · Oxido-inflammation

UNIVERSITY OF IBADAN LIBRARY

Introduction

Current advances in nanotechnologies have demonstrated that carbon nanotubes (CNTs) are the most investigated nanomaterials. CNTs are often produced as single-walled carbon nanotubes (SWCNTs) with 0.8 to 2 nm diameters or multi-walled carbon nanotubes (MWCNTs) with 10 to 100 nm diameters (Antúnez-Flores et al. 2008). Based on the extraordinary physical, chemical, and electronic properties of CNTs, there are several varieties of existing and evolving applications of CNTs in various fields. For instance, CNTs are widely used in medicine, nanoelectronics, engineering, agriculture, neurobiology, and daily consumable products (Malarkey and Parpura 2007; Kumar et al. 2014; Rode et al. 2018; Kamran et al. 2019). CNTs are used in biosensors and enzyme detection (Jacobs et al. 2010), biomaterials (Lin et al. 2004), drug molecules or vaccine delivery vehicles for therapies or

✉ Ebenezer O. Farombi
olatunde_farombi@yahoo.com

1 Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria

2 Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria

3 Department of Anatomy, College of Medicine, Bowen University, Iwo, Nigeria

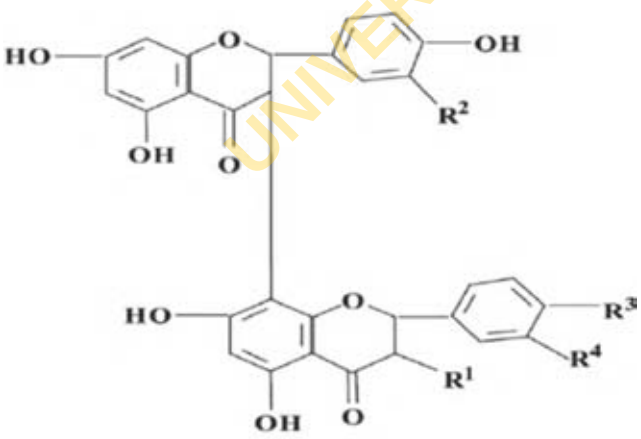
4 Cancer Research and Molecular Biology Laboratory, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria

5 College of Science Engineering and Technology, NIH-RCMI Center for Environmental Health, Jackson State University, Jackson, MS, USA

diagnosis (Bianco et al. 2005), and as components within rechargeable battery electrodes (Han et al. 2012).

The high reactivity and penetration of CNTs have been associated with their dual nature as nanomaterials and fibers (Nel 2013; Wang et al. 2017). Previous studies have demonstrated that low doses of MWCNTs induced hepatotoxicity in mice and reproductive toxicity in rats via induction of oxidative stress (Patlolla et al. 2011; Farombi et al. 2016; Adedara et al. 2018). Moreover, numerous in vitro studies have established that CNTs could elicit neurotoxic effects, including the reduction in the brain cell activity (Belyanskaya et al. 2009) and inhibition of CA1 glutamatergic synaptic transmission in rat hippocampal slices in vitro (Chen et al. 2013; Chen et al. 2014). In vivo studies demonstrated that administration of MWCNTs for 14 consecutive days induced cognitive deficits via increase in the autophagic levels and neuropathological alterations of CA1 neurons (Gao et al. 2015). Hence, potential chemotherapeutic/preventive strategies to mitigate the adverse effects of MWCNTs are warranted.

Natural products are sources of novel drugs and represent model molecules for the discovery and validation of drug targets. Kolaviron (Fig. 1), a biflavonoid isolated from the seed of *Garcinia kola*, is well reported to elicit several pharmacological effects including anti-inflammatory, anti-oxidant, anti-genotoxic activities (Nwankwo et al. 2000; Farombi and Nwaokeafor 2005; Abarikwu 2014; Onasanwo and Rotu 2016). The neuroprotective effects of kolaviron against different disease models including 2-vessel occlusion model of cerebral ischemia/reperfusion injury (Ojo et al. 2019), memory impairment induced by scopolamine, a muscarinic receptor antagonist (Ishola et al. 2017), and the cortico-hippocampal neurodegeneration in Alzheimer's disease models in rats (Olajide et al. 2017) have been previously reported.



	R^1	R^2	R^3	R^4
GB 1	OH	H	OH	H
GB 2	OH	H	OH	OH
Kolaflavanone	OH	H	OMe	OH

Fig. 1 Chemical structure of kolaviron

Recently, kolaviron was reported to abrogate neurotoxicity associated with rotenone model of Parkinson's disease (Farombi et al. 2019).

Hitherto, there is no information on the assessment of locomotor activities and exploratory characteristics associated with MWCNT-induced neurotoxicity as well as on the influence of kolaviron on MWCNT-induced toxicity. Available information in the literature has associated MWCNTs toxicity with oxidative stress, inflammation and apoptosis (Dong and Ma 2016, De Marchi et al. 2018; Abu Gazia and El-Magd 2019). It is hypothesized that kolaviron may interact with the dopaminergic, oxidative stress and inflammatory signaling pathways to ameliorate MWCNT-induced toxicities in rats. Thus, this study was designed to investigate the possible influence of kolaviron on neurotoxicity induced by MWCNTs in adult rats. To understand the role of kolaviron in MWCNT-induced neurotoxicity in rats, an assessment of locomotor activities and exploratory characteristics in experimental rats was initially done using an established neurobehavioral procedure and video-tracking software (Adedara et al. 2017). Subsequently, evaluation of acetylcholinesterase (AChE) activity, antioxidant defense mechanisms, oxidative stress, and inflammatory biomarkers alongside histological and histomorphometrical analyses of the cerebrum, cerebellum, and mid-brain of the experimental rats were performed for the first time.

Materials and methods

Chemicals and reagents

Carboxylated multi-walled carbon nanotubes (MWCNTs) from NanoLab Inc. (Newton, MA) were obtained from Professor Anita K. Patlolla of the Molecular Toxicology Research Laboratory, NIH-RCMI Center for Environmental Health, CSET, Jackson State University, Jackson, Mississippi, USA. All chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Characterization of functionalized MWCNTs

MWCNTs with outer diameter of 15–30 nm and lengths of 15–20 μm (purity > 95%) were produced by NanoLab Inc. (Newton MA, USA) via catalytic chemical vapor deposition procedure. Subsequently, iron-impurities were removed from the MWCNTs by heating under argon (2 l/min) to 2000 °C at the rate of 10 °C/min. The resulting pure MWCNTs [purity > 95% by thermogravimetry analysis (TGA)] were thereafter subjected to a reflux process in sulfuric/nitric acid (3:1) to produce functionalized carboxylated nanotubes of 2–7% COOH by weight. The size and structure of these pure MWCNTs were analyzed using transmission electron

UNIVERSITY OF IBADAN LIBRARY

microscope (TEM). The MWCNTs were directly positioned on a TEM grid and allowed to dry before visualizing the samples with TEM. Moreover, the surface areas were evaluated using the isothermal gas adsorption BET method (Brunauer et al. 1938) and a Micromeritics Flowsorb 2300 (Norcross, USA). Characterization was achieved by dispersing the MWCNTs in 1% tween-80 and sterile saline with the aid of physical mixing and ultrasonication. Finally, purified long MWCNTs with 11.5 nm in diameter, 12 μm in length, and 42 m^2/g specific surface area were obtained.

Isolation of kolaviron

Kolaviron was isolated from *Garcinia kola* seeds using an established protocol (Iwu 1985, Farombi et al. 2019). Succinctly, fresh *Garcinia kola* seeds were sliced, air-dried, and powdered. Subsequently, the powdered seeds were defatted with n-hexane using soxhlet apparatus for 24 h. The defatted dried marc was repacked and extracted with methanol. The methanol extract was thereafter concentrated and diluted twice its volume with distilled water and chloroform to isolate a golden yellow solid called kolaviron. The characterization of kolaviron was achieved by direct comparison of the 1H nuclear magnetic resonance (NMR), 13C NMR, and electron ionization mass spectral results. The purity of the isolated kolaviron was 96%.

Animal model and care

The 50 adult male Wistar rats (10 weeks old, 178 ± 6 g) used for this investigation were obtained from the Faculty of Veterinary Medicine, University of Ibadan, Ibadan. The animals were housed in plastic cages situated in a well-ventilated vivarium, under standard laboratory conditions of a 12 h light/12 h dark photocycle and provided rat chow and water ad libitum. The rats were acclimatized for 1 week prior to the commencement of the experiment. Adequate quantity of wood shavings which cover the whole floor was provided as bedding. Animal care and experimental protocols were performed in line with the approved guidelines set by the University of Ibadan Ethical Committee and the 'Guide for the Care and Use of Laboratory Animals' produced by the National Academy of Science and National Institute of Health.

Experimental design

The study consisted of five groups of ten rats which were treated for 15 consecutive days.

Control: Rats were intraperitoneally injected with saline plus 1% tween-80 alone at 2 mL/kg.

KV alone: Rats were orally administered kolaviron (KV) alone at 100 mg/kg.

MWCNTs alone: Rats were injected with MWCNTs suspension at a dose of 1.0 mg/kg.

MWCNTs + KV 1: Rats were injected with MWCNTs and orally treated with KV at 50 mg/kg.

MWCNTs + KV 2: Rats were injected with MWCNTs and orally treated with KV at 100 mg/kg.

The treatment duration and dose of MWCNTs were chosen from the preliminary range-finding experiments to determine the dose (Adedara et al. 2018) that would keep the animals alive till the exhibition of neurobehavioral deficits. The doses of kolaviron used in the present investigation were selected based on the earlier data from our laboratories (Akinmoladun et al. 2015).

Assessment of behavioral pattern in a novel environment

The behavior of the experimental rats was evaluated 24 h after the last treatment in a novel apparatus (wooden box of 56 cm width × 56 cm length × 20 cm height) according to established procedure (Adedara et al. 2017). Succinctly, the rats from every group were randomly selected, situated in the middle of the apparatus, and allowed to liberally walk around the arena. The behavior of the rats was captured all through the 8-min trial with the aid of a webcam (DNE webcam, Porto Alegre, Brazil) situated directly above the open environment and attached to a laptop computer. The video movies of the rats were thereafter analyzed and the behavioral characteristics automatically computed at a rate of 30 frames per second using appropriate video-tracking software (ANY-maze, Stoelting Co, USA). At the end of each test, the apparatus was cautiously cleaned with cotton wool soaked in 70% ethanol.

Evaluation of neurobehavioral and locomotor parameters

Evaluation of locomotor, motor, and exploratory activities of the rats in the novel environment was performed to demonstrate habituation to novelty stress. Locomotor and motor characteristics were measured using behavioral endpoints namely total distance traveled, maximum speed, total time mobile, mobile episode, path efficiency, body rotation, and absolute turn angle. Assessment of the exploratory activities of the rats in the novel environment was done using representative track plots automatically generated by the video-tracking software (ANY-maze, Stoelting Co, USA). Negative geotaxis test was performed to assess the motor fitness of the experimental rats according to established protocol (Motz and Alberts 2005). Succinctly, the rats from every group were randomly selected, placed downhill in the middle of a rough wooden board inclined at 45° angle, and were monitored for orientation and directional movement against

UNIVERSITY OF IBADAN LIBRARY

gravitation. The time taken to orientate and climb the board with their forelimbs reaching the upper rim was video captured and recorded.

Evaluation of oxidative stress indices

After the assessment of behavioral characteristics, the final body weights of the animals were recorded prior to their sacrifice under light ether anesthesia. The whole brain was immediately removed from the cranium and then carefully separated into mid-brain, cerebellum and cerebrum. The brain structures (mid-brain, cerebellum, and cerebrum) were then homogenized separately in eight volumes of 50 mM Tris–HCl buffer (pH 7.4) and thereafter centrifuged at 12,000*g* for 15 min at 4 °C. The resulting supernatant was used for biochemical analyses. Protein concentration was assayed according to Bradford (1976) using bovine serum albumin as standard.

Acetylcholinesterase (AChE) activity was assayed according to Ellman et al. (1961). Superoxide dismutase (SOD) and catalase (CAT) activities were assayed according to Misra and Fridovich (1972) and Claiborne (1995), respectively. Glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities were assayed according to Rotruck et al. (1973) and Habig et al. (1974), respectively. Moreover, the levels of glutathione (GSH) and lipid peroxidation (LPO) were assayed according to Jollow et al. (1974) and Farombi et al. (2000), respectively. Barring SOD and CAT activities which were measured with the aid of 752S UV-VIS Spectrophotometer (Ningbo, China), all biochemical assays were analyzed using a SpectraMax plate reader (Molecular Devices, CA, USA).

Evaluation of reactive oxygen and nitrogen species level

The level of RONS production in the mid-brain, cerebellum, and cerebrum was assayed using an established procedure which is based on the RONS-dependent oxidation of 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA) to DCF (Adedara et al. 2016a). Succinctly, the reaction mixture consisted of 10 μL of the fresh sample, 150 μL of 0.1 M potassium phosphate buffer (pH 7.4), 35 μL of distilled water, and 5 μL of DCFH-DA (200 μM, final concentration 5 μM) with minimal contact to air. The fluorescence emission of DCF due to DCFHDA oxidation was analyzed for 10 min (30 s intervals) at 488 nm excitation and 525 nm emission wavelengths using a SpectraMax plate reader (Molecular Devices, CA, USA). The rate of DCF generation was expressed in percentage of control value.

Evaluation of pro-inflammatory indices

Myeloperoxidase (MPO) activity was assayed according to Granell et al. (2003) whereas nitric oxide (NO) level was assessed according to established protocol (Green et al. 1982). Succinctly, the reaction mixture consisting of equal volume of sample and Griess reagent was incubated for 15 min and the absorbance taken at 540 nm. The level of NO in the sample was then extrapolated from the standard nitrites curve. In addition, the level of TNF-α was assayed using ELISA Kits (Elabscience Biotechnology Company, Beijing, China) and measured with a SpectraMax plate reader (Molecular Devices, CA, USA) in compliance with the manufacturer's guidebook.

Histology and histomorphometry

Histological examination of the mid-brain, cerebrum, and cerebellum samples was done using 4–5-μm tissue sections stained with hematoxylin and eosin according to established protocol (Bancroft and Gamble 2008). Histomorphometry of the brain sections was performed according to established procedure (Osuagwu et al. 2007). The viability of striatal neurones, cortical neurones, pyramidal neurones of CA3 (PNCA3) and granule cell layer of dentate gyrus (GCDG) were determined by counting whereas the widest diameter of the Purkinje cells (WDPC) of the cerebellum was assessed with the aid of a microscope and a graticule (micrometer embedded in the eye piece objective) at different magnifications. The ten observations recorded for the slides in each group were used to compute the mean values of each group. Viability of the neurones was characterized by the presence of dispersed chromatin, distinct nucleoli, and lack of features of cell death like pyknosis and karyolysis or karyorrhexis at high power.

Statistical analyses

Data were analyzed using one-way analysis of variance (ANOVA) to compare the groups followed by Bonferroni's post hoc test using GRAPHPAD PRISM 5 software (Version 4; GraphPad Software, La Jolla, California, USA). Values of $P < 0.05$ were considered significant.

Results

Kolaviron abrogated locomotor and motor deficits induced by MWCNTs in rats

Figure 2 portrays the data on the locomotor and motor activities of experimental rats in the novel environment. At the end of treatment for 15 consecutive days, the rats administered

UNIVERSITY OF IBADAN LIBRARY

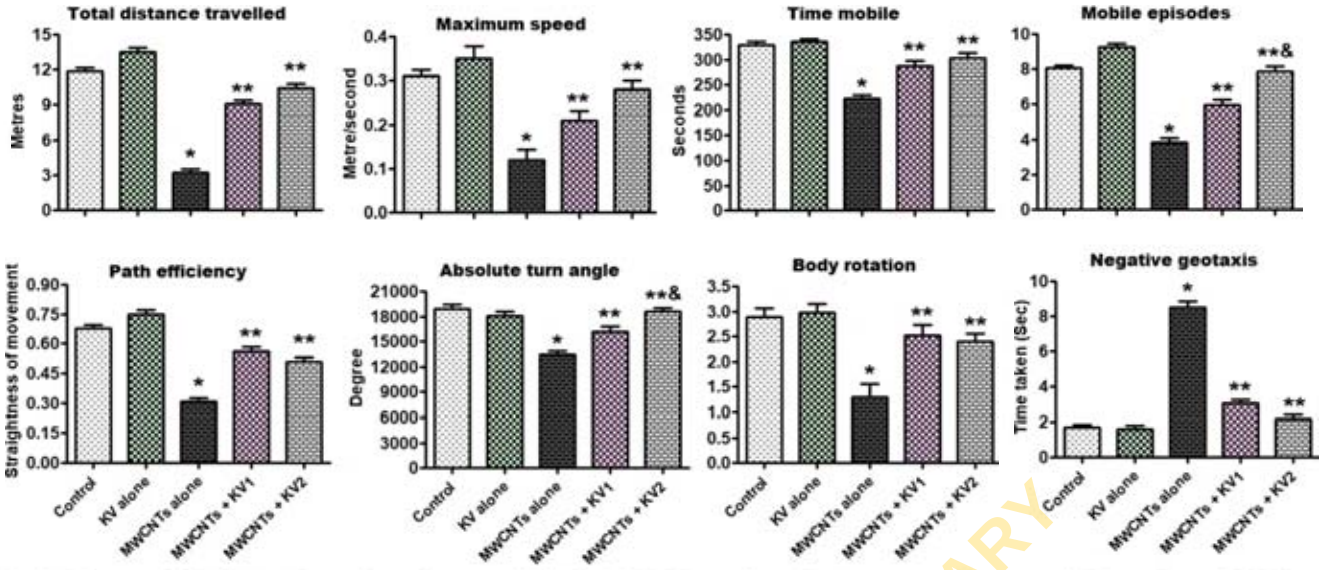


Fig. 2 Influence of kolaviron on locomotion and motor activities in MWCNT-treated rats. Data are expressed as mean ± S.D.; $n = 10$ rats. "*" Values differ significantly from control ($p < 0.05$). "**" Values differ significantly from MWCNTs alone, "&" values differ significantly from MWCNTs + KV1

kolaviron alone exhibited no treatment-related effect on the locomotor and motor activities when compared with control. Conversely, administration of MWCNTs alone to rats resulted in a significant ($p < 0.05$) decrease in the total distance traveled, maximal speed, total time mobile, mobile episodes, ability to maintain a straight path (path efficiency), body rotation, and absolute turn angle in comparison with control. In addition, rats administered MWCNTs alone exhibited a significant increase in the time taken to orientate and climb an inclined board successfully (i.e., increased negative geotaxis). However, rats co-treated with kolaviron at 50 and 100 mg/kg demonstrated significant improvement in the locomotor and motor activities verified by increased total distance traveled, maximum speed, total time mobile, mobile episode, path efficiency, body rotation, and absolute turn angle with concomitant decrease in negative geotaxis when compared with rats exposed to MWCNTs alone.

Kolaviron abated exploratory insufficiencies associated with MWCNTs exposure in rats

Figure 3 portrays the representative track plots of the wandering traces of experimental rats inside the novel apparatus. Rats in the control and kolaviron alone groups demonstrated the normal behavioral characteristics by traveling around the novel apparatus whereas rats treated with MWCNTs alone exhibited exploratory insufficiencies evidenced by marked reduction in the track plots density when compared with control. However, co-treatment with kolaviron at 50 and 100 mg/kg markedly abated MWCNTs-mediated exploratory insufficiencies by increasing the track plot density in the treated rats in comparison with rats exposed to MWCNTs alone.

Kolaviron improved acetylcholinesterase activity and glutathione level in MWCNT-treated rats

Figure 4 portrays the effect of kolaviron co-treatment on the AChE activity and GSH level in MWCNTs-treated rats. Kolaviron administration alone elicited no treatment-related effect on the AChE activity and GSH level in comparison with control. However, administration of MWCNTs alone significantly decreased AChE activity and GSH level in the cerebrum, cerebellum and mid-brain of the treated rats. However, co-treatment with kolaviron at 50 and 100 mg/kg significantly increased the AChE activity and GSH level in the treated rats in comparison with rats exposed to MWCNTs alone.

Kolaviron abrogated MWCNT-mediated increase in RONS and lipid peroxidation in rats

Figure 5 portrays the levels of oxidative stress indices namely RONS and LPO estimated in the cerebrum, cerebellum, and mid-brain of experimental rats. Kolaviron treatment alone caused no treatment-related effect on the levels of RONS and malondialdehyde (an index of LPO) in comparison with control. However, administration of MWCNTs alone elicited marked increase in RONS and LPO levels in cerebrum, cerebellum, and mid-brain of the treated rats when compared with the control group. Co-treatment with kolaviron at 50 and 100 mg/kg significantly abated the levels of RONS and LPO when compared with rats administered MWCNTs alone.

UNIVERSITY OF IBADAN LIBRARY

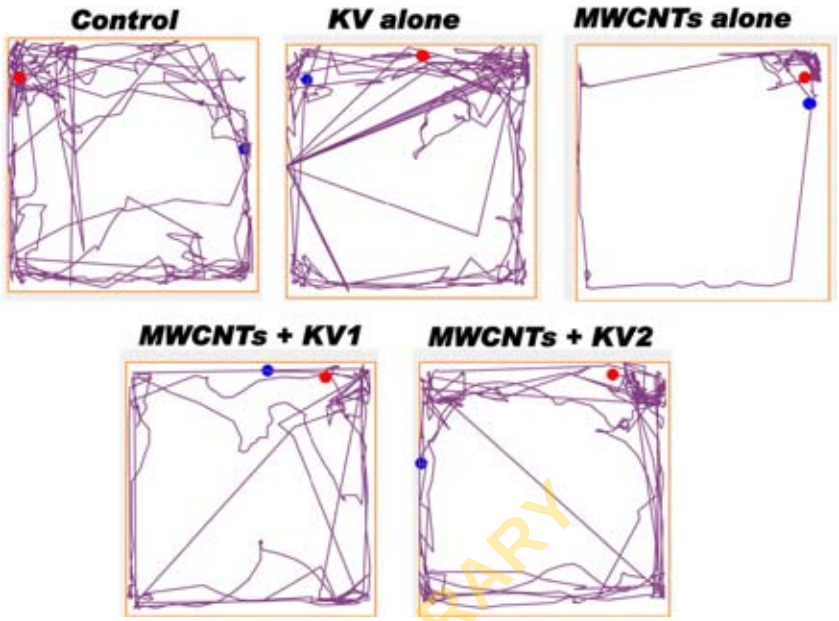


Fig. 3 Influence of kolaviron on exploratory profiles in MWCNT-treated rats represented by track plots during the 8-min trial in a novel apparatus. The data were analyzed using video-tracking software (ANY-maze, Stoelting CO, USA)

Kolaviron augmented antioxidant enzyme activities in MWCNT-treated rats

Figures 6 and 7 depict the activities of antioxidant enzymes namely SOD, CAT, GPx, and GST in the cerebrum, cerebellum, and mid-brain of experimental rats. Administration of kolaviron alone caused no treatment-related effect on the antioxidant enzymes activities in comparison with control. However, exposure to MWCNTs alone significantly decreased SOD, CAT, GPx, and GST activities in the cerebrum, cerebellum, and mid-brain of the treated rats when compared with control. Co-administration of kolaviron at 50 and 100 mg/kg markedly increased the activities of these antioxidant enzymes in the investigated brain structures when compared with rats exposed to MWCNTs alone.

Kolaviron suppressed biomarkers of inflammation in MWCNTs-treated rats

Figures 8 and 9 depict the influence of kolaviron on the pro-inflammatory indices (MPO activity and levels of NO and TNF-α) assessed in cerebrum, cerebellum, and mid-brain of the experimental rats. There were no

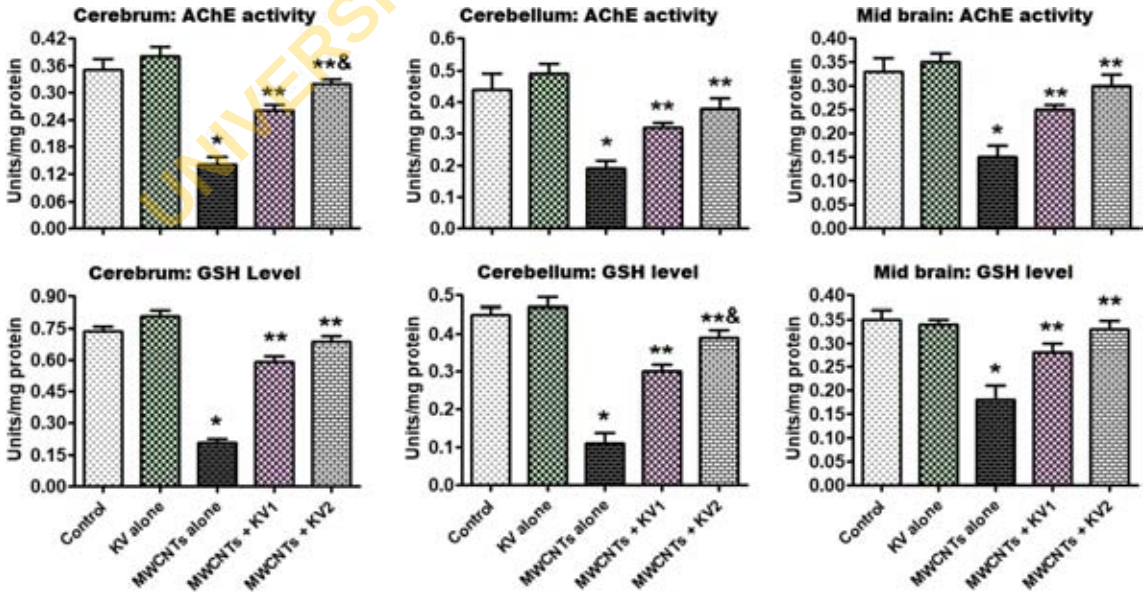


Fig. 4 Influence of kolaviron on AChE activity and GSH level in the cerebrum, cerebellum and mid-brain of MWCNTs-treated rats. Data are expressed as mean ± S.D.; $n = 10$ rats. "*" Values differ significantly from control ($p < 0.05$). "**" Values differ significantly from MWCNTs alone, "&" values differ significantly from MWCNTs + KV1

UNIVERSITY OF IBADAN LIBRARY

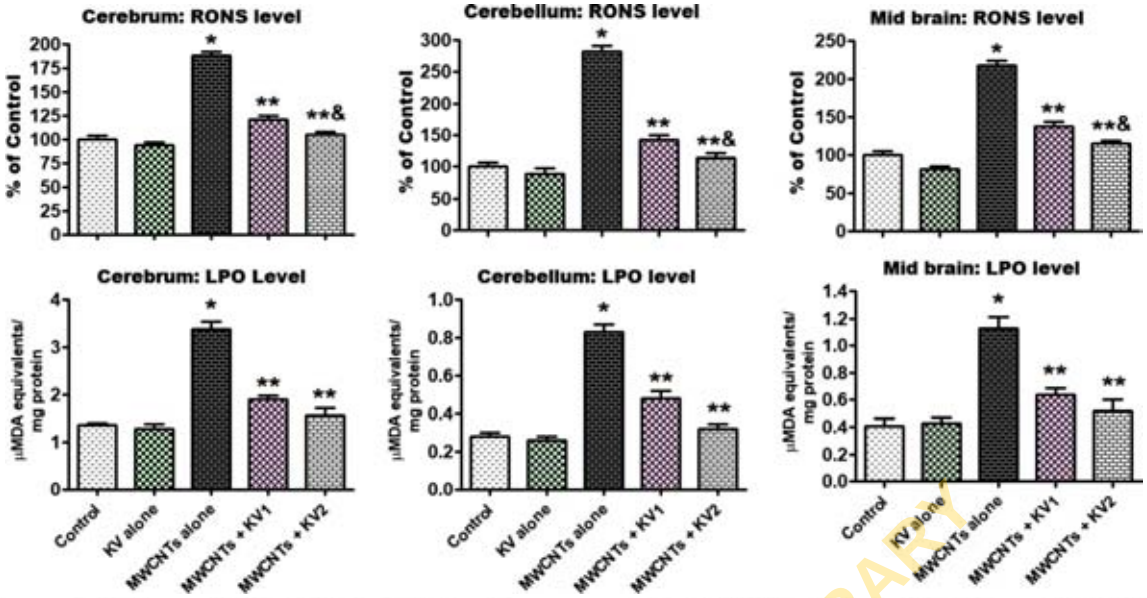


Fig. 5 Influence of kolaviron on RONS and LPO levels in the cerebrum, cerebellum mid-brain of MWCNT-treated rats. Data are expressed as mean ± S.D.; $n = 10$ rats. "*" Values differ significantly from control ($p < 0.05$). "**" Values differ significantly from MWCNTs alone. "&" Values differ significantly from MWCNTs + KV1

treatment-related effects on the pro-inflammatory indices assessed in rats administered kolaviron alone when compared with control. However, rats administered MWCNTs alone showed a marked increase in the MPO activity and levels of NO and TNF-α in cerebrum, cerebellum and mid-brain when compared with the control. Co-treatment with kolaviron at 50 and 100 mg/kg markedly decreased the MPO activity and levels of NO and TNF-α in the investigated brain structures in comparison with rats exposed to MWCNTs alone.

Kolaviron ameliorated neuronal degeneration associated with MWCNT treatment in rats

Figures 10, 11, 12, 13, and 14 portray the representative photomicrographs of the brain structures and the affected neurones namely Purkinje neurones, striatal neurones, cortical neurones, dentate gyrus, and PNCA3 in rats treated with MWCNTs alone or co-treated with kolaviron. The brain structures of control and kolaviron alone groups appeared structurally normal with their neurones. However, the brain of rats administered MWCNTs alone showed marked degeneration

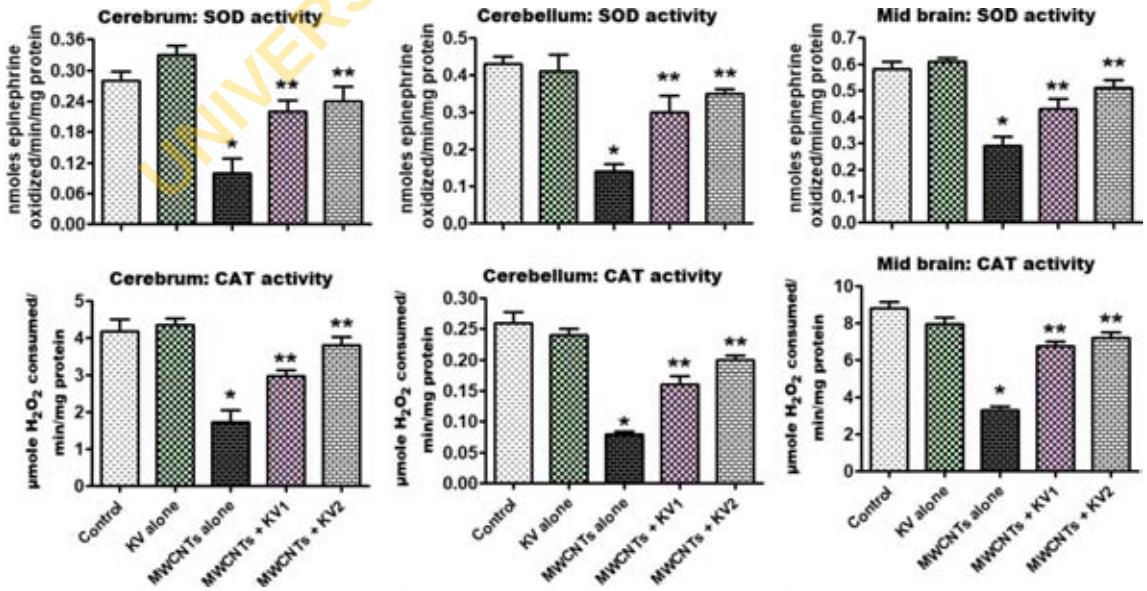


Fig. 6 Influence of kolaviron on SOD and CAT activities in the cerebrum, cerebellum, and mid-brain of MWCNTs-treated rats. Data are expressed as mean ± S.D.; $n = 10$ rats. "*" Values differ significantly from control ($p < 0.05$). "**" Values differ significantly from MWCNTs alone

UNIVERSITY OF IBADAN LIBRARY

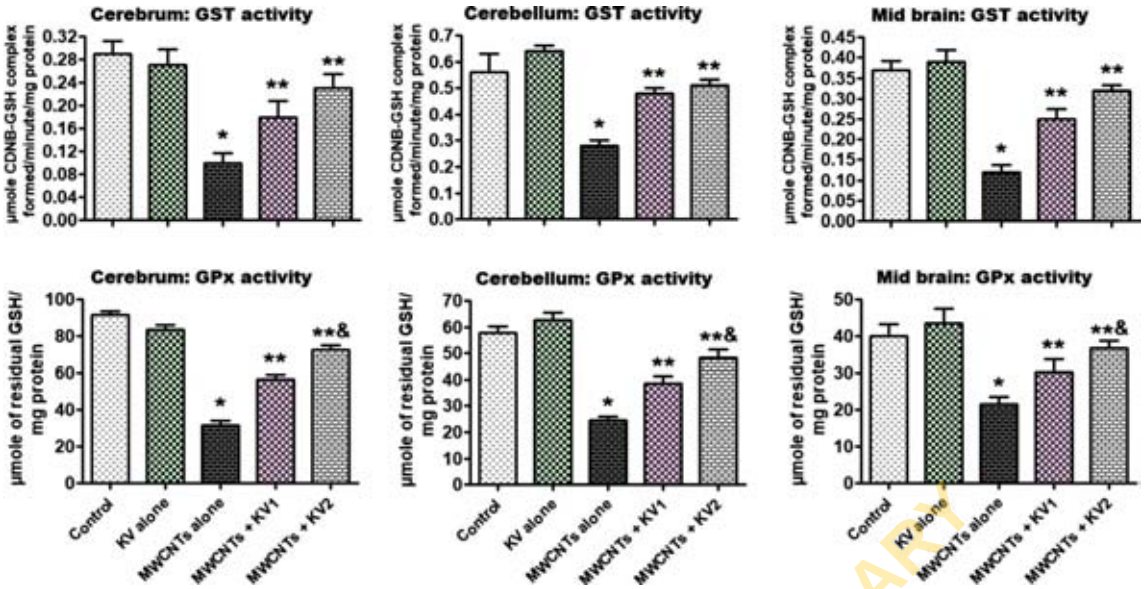


Fig. 7 Influence of kolaviron on GST and GPx activities in the cerebrum, cerebellum, and mid-brain of MWCNT-treated rats. Data are expressed as mean ± S.D.; $n = 10$ rats. "*" Values differ significantly from control ($p < 0.05$). "**" Values differ significantly from MWCNTs alone. "&" Values differ significantly from MWCNTs + KV1

in the striatal neurones, cortical neurones, and PNCA3. Moreover, histomorphometrical analysis showed that administration of MWCNTs alone significantly decreased the densities of viable striatum neurones, cortical neurons, PNCA3, GCDG with concomitant reduction in the WDPC when compared with the control. However, the changes in brain architecture and morphometric parameters were abrogated in rats co-administered MWCNTs and kolaviron when compared with rats treated with MWCNTs alone.

Discussion

There is a mounting concern about the potential threats that the widespread occupational or consumer exposure to MWCNTs may pose to human health (Aschberger et al. 2010; Tsukahara et al. 2014; Zhao et al. 2019). Thus, the prevention or mitigation of adverse effects associated with its widespread application is a major scientific interest so as to safeguard the public health. The present investigation

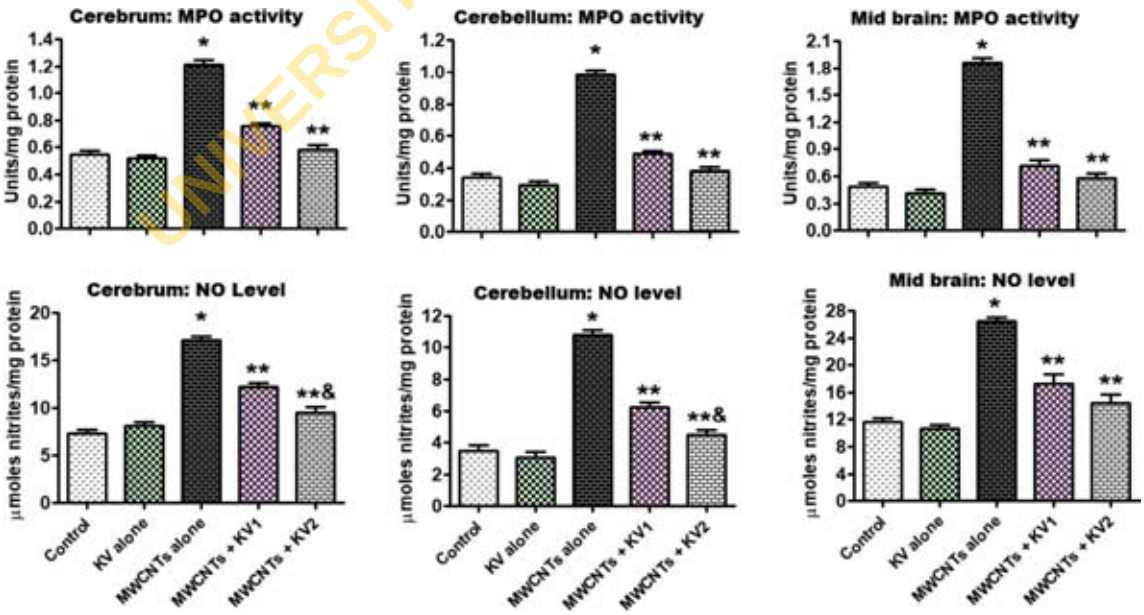


Fig. 8 Influence of kolaviron on MPO activity and NO level in the cerebrum, cerebellum, and mid-brain of MWCNT-treated rats. Data are expressed as mean ± S.D.; $n = 10$ rats. "*" Values differ significantly from control ($p < 0.05$). "**" Values differ significantly from MWCNTs alone. "&" Values differ significantly from MWCNTs + KV1

UNIVERSITY OF IBADAN LIBRARY

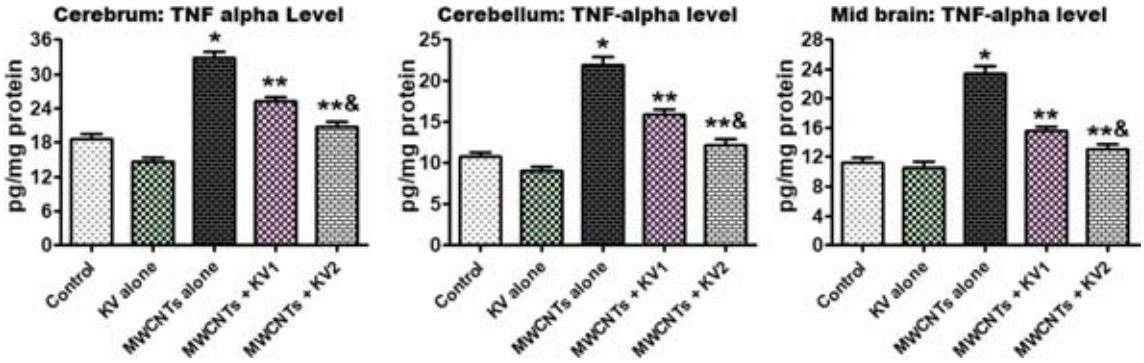


Fig. 9 Influence of kolaviron on TNF-alpha level in the cerebrum, cerebellum, and mid-brain of MWCNT-treated rats. Data are expressed as mean ± S.D.; $n = 10$ rats. "*" Values differ significantly from control ($p < 0.05$). "**" Values differ significantly from MWCNTs alone. "&" Values differ significantly from MWCNTs + KV1

demonstrated that exposure to MWCNTs resulted in neurobehavioral deficits and that kolaviron effectively protected against neurotoxicity associated with MWCNT exposure in rats.

In the present investigation, MWCNT administration elicited locomotor insufficiencies in the treated rats evinced by the reduction in the total distance traveled, maximum speed, total time mobile, mobile episode as well as inability to maintain a straight path. In addition, the marked reduction in the body rotation and absolute turn angle which are fundamental motor coordination factors during bodily movements (Riemann and Lephart 2002; Adedara et al. 2016b) signifies coordination deficiency between the nervous and muscular junctions. However, the marked improvement in these locomotor and motor activities in rats co-treated with kolaviron evidently demonstrated the chemoprotective role of kolaviron in MWCNTs-induced neurobehavioral deficits in rats. Further, exploration is a main approach through which animals obtain information about their spatial environment (Gorny et al. 2002). The decrease in the exploratory competence of rats administered MWCNTs alone was verified by the decrease in density of track plots. However, the improvement in the exploratory competence as shown by the increase in the track plot densities following co-administration of kolaviron at 50 and 100 mg/kg in the present investigation evidently signifies abrogation of MWCNTs-induced disorganization of exploratory and spatial behavior by kolaviron.

To elucidate the neuroprotective mechanisms of kolaviron in MWCNT-treated rats, analyses of AChE activity, oxidative stress indices, antioxidant enzymes activities, biomarkers of inflammation as well as histopathological and histomorphometrical evaluation of the brain structures of rats were performed. It is well known that AChE plays a fundamental role in cholinergic neurotransmission by hydrolyzing

UNIVERSITY OF IBADAN LIBRARY

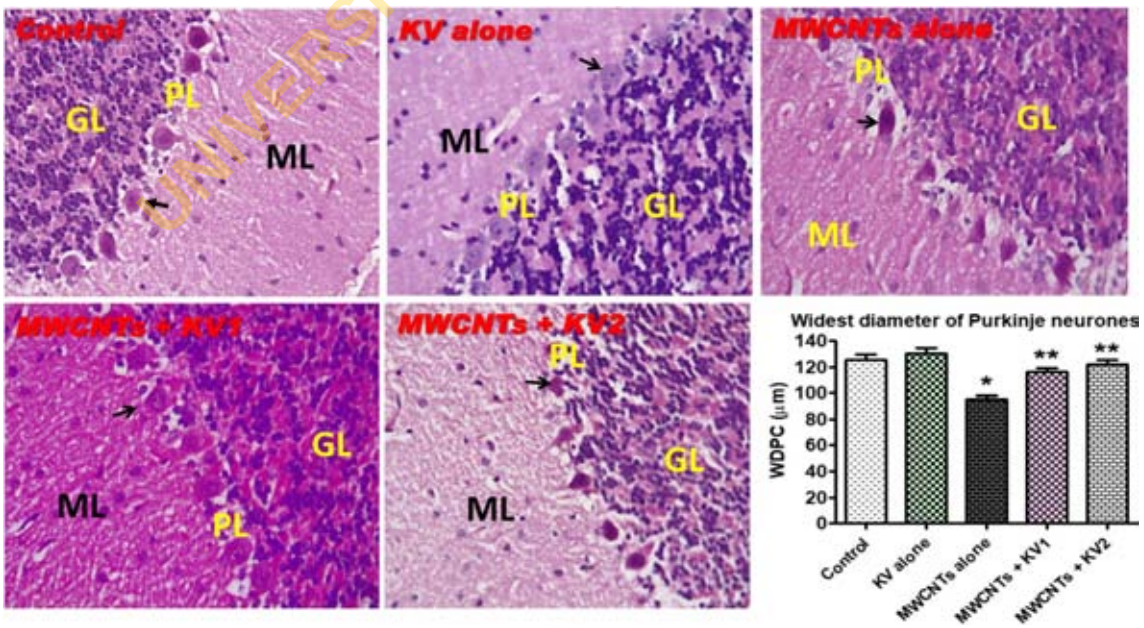


Fig. 10 Representative photomicrographs of cerebellum and histomorphometry showing the widest diameter of the Purkinje cells (WDPC) in treated rats. H&E (× 400)

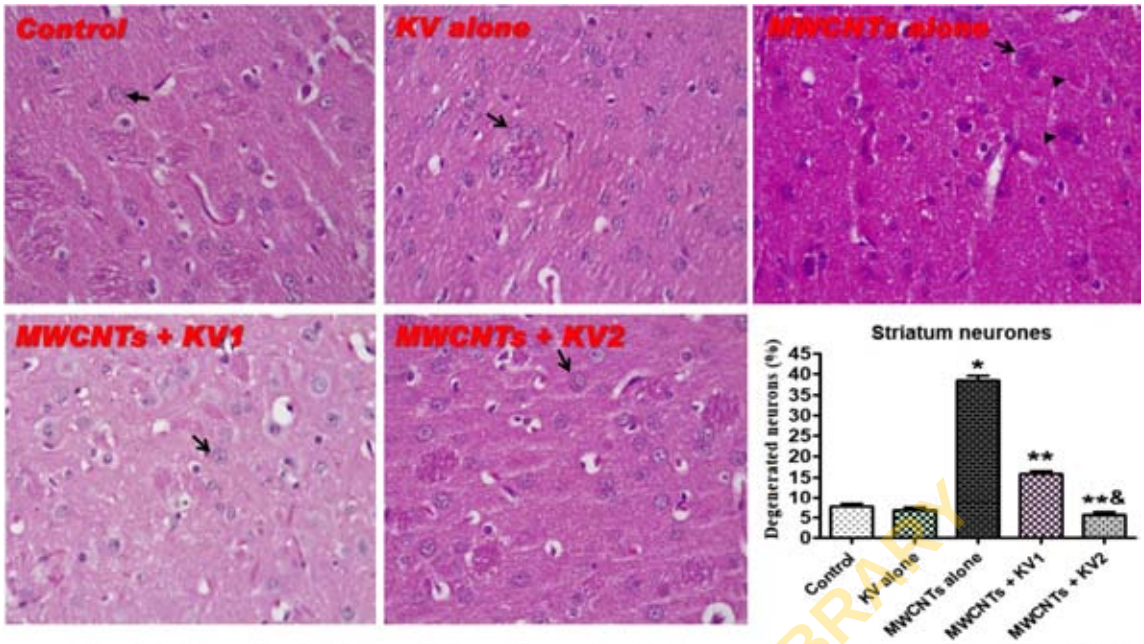


Fig. 11 Representative photomicrographs of striatum and histomorphometry showing degenerated striatum neurones in treated rats. H&E (× 400)

synaptic acetylcholine, a neurotransmitter which regulates locomotion and motor function (Day et al. 1991). The decrease in the AChE activity in rats administered MWCNTs alone in the present study signifies its inhibition to effectively breakdown acetylcholine which consequently reduces the normal neurotransmission in the treated rats. Hence, the MWCNT-mediated decrease in the AChE activity in this study may be correlated with the insufficiencies in locomotor, motor, and exploratory activities exhibited by rats administered MWCNTs alone. However, co-administration of kolaviron enhanced the cholinergic neurotransmission evinced by an increment in the AChE activity and neurobehavioral performance in the treated rats.

The cell is endowed with antioxidant defense system which comprises antioxidant enzymes such as SOD, CAT, GST, and GPx as well as non-enzymatic molecules including GSH. Both enzymatic and non-enzymatic antioxidants are responsible for the detoxification of free radicals including RONS well-known to cause cellular oxidative damage (Poprac et al. 2017; Grochowski et al. 2018). Excessive exposure of

UNIVERSITY OF IBADAN LIBRARY

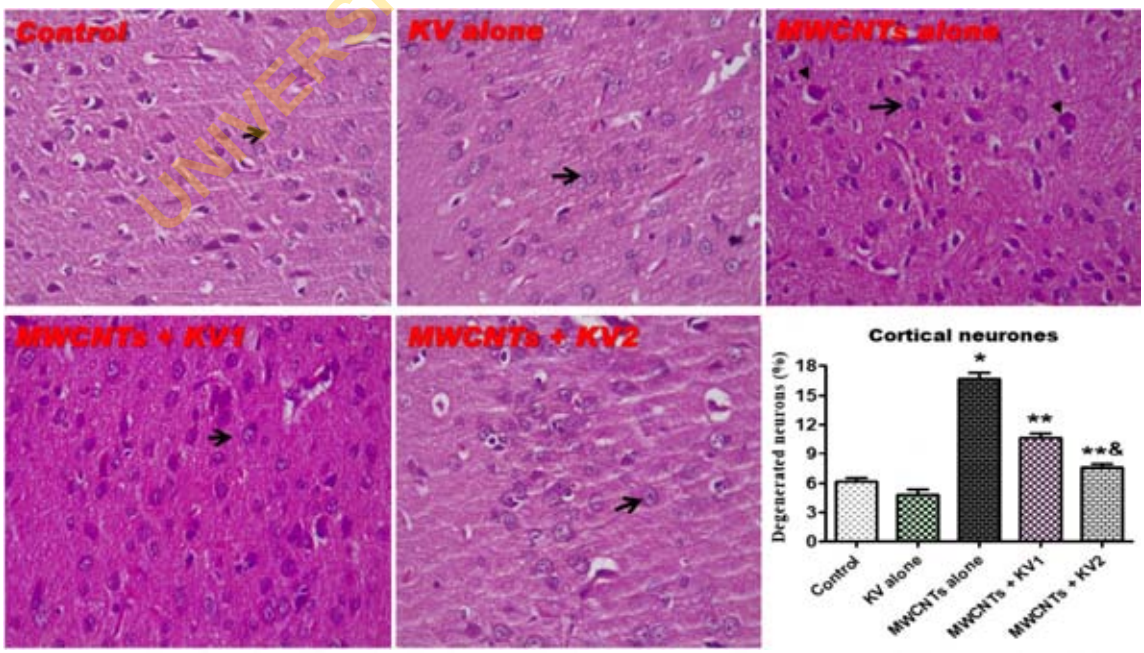


Fig. 12 Representative photomicrographs of frontal cerebral cortex and histomorphometry of degenerated cortical neurones in treated rats

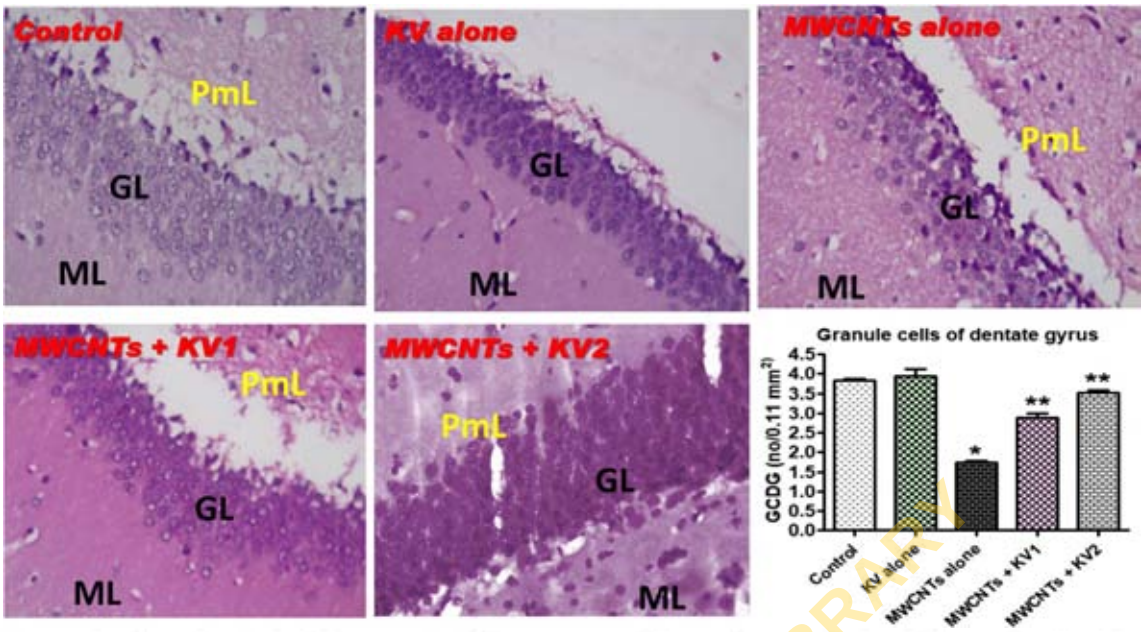


Fig. 13 Representative photomicrographs of dentate gyrus of hippocampus and histomorphometry showing viable granule cells of dentate gyrus (GCDG) in treated rats. Granular layer, GL; molecular layer, ML; polymorphic layer, PmL. H&E × 400

neurotoxic substances reportedly disrupts cellular antioxidant defense mechanism leading to central nervous system dysfunction. The present investigation demonstrated that exposure to MWCNTs alone markedly decreased SOD, CAT, GST, and GPx activities as well as GSH level in the cerebrum, cerebellum and mid-brain when compared with control. This observation signifies inhibition of their functions which may lead to RONS accumulation and induction of oxidative stress in the brain structures of the rats. Glutathione is a thiol group-containing tripeptide which scavenges free radicals and thus preserves the cellular redox status whereas GST is a phase II metabolic enzyme which detoxifies electrophilic compounds by conjugating them with glutathione (Hayes and Strange 2000). In the present investigation, the marked diminution in the GSH level signifies overutilization of GSH whereas the reduction in the GST activity may be related to the decreased availability of GSH, a substrate for the enzyme as well as an inhibition of the GST-mediated detoxification process of free

UNIVERSITY OF IBADAN LIBRARY

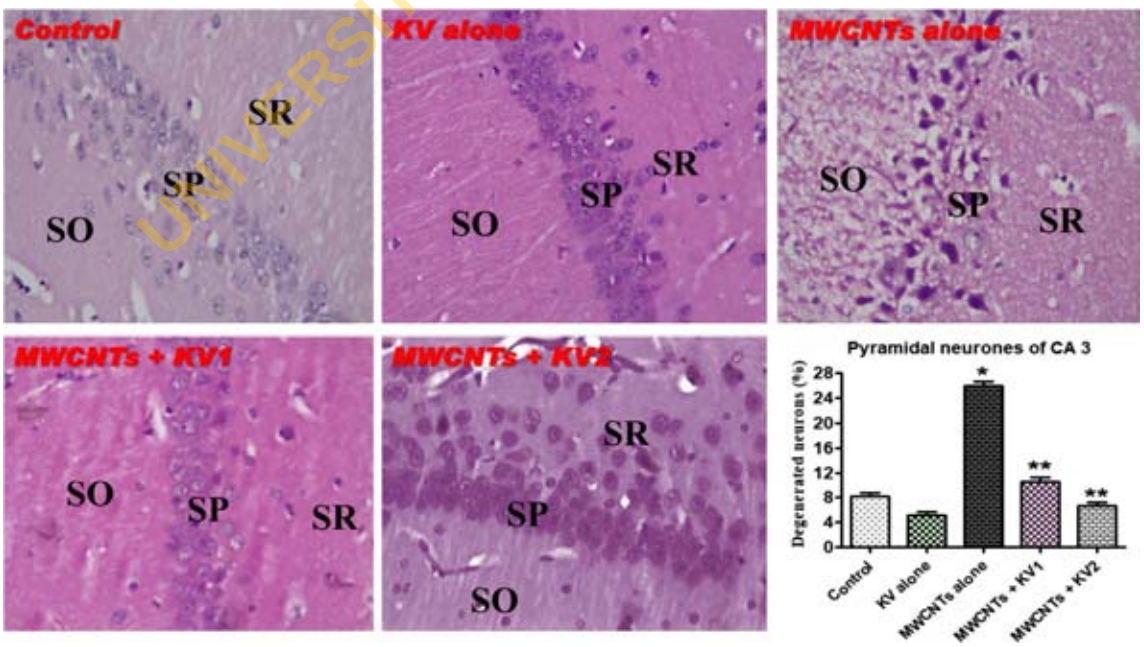


Fig. 14 Representative photomicrographs of cornu ammonis 3 (CA3) of hippocampus showing degenerated pyramidal neurons of CA3 of treated rats. Stratum pyramidalis, SP; stratum oriens, SO; stratum radiatum, SR. H&E × 400

radicals in the MWCNT-treated rats. The MWCNT-mediated reduction in antioxidant enzymes activities and GSH level observed in the present study corroborates previous reports (Reddy et al. 2011; Adedara et al. 2018; Abu Gazia and El-Magd 2019). However, co-administration of kolaviron at 50 and 100 mg/kg abrogated MWCNT-mediated decrease in these antioxidants in the treated rats. The marked improvement in the antioxidant status following administration of kolaviron is related to its antioxidant action in cerebrum, cerebellum and mid-brain of the treated rats.

Moreover, the oxidative destruction of polyunsaturated lipids by a free radical-mediated chain of reactions is termed LPO. The final products of LPO namely MDA and 4-hydroxynonenal reportedly cause deleterious interaction with proteins and nucleic acids (Valko et al. 2007; Shichiri 2014). The decrease in the antioxidant enzyme activities observed in the present investigation is associated with accumulation of RONS and LPO in the brain regions of MWCNT-treated rats, thus signifying induction of cellular oxidative damage in these rats. The involvement of RONS and LPO in MWCNT-mediated health effects has been previously reported (Møller et al. 2014). However, the marked reduction in the RONS and LPO levels in rats co-treated with MWCNTs and kolaviron at 50 and 100 mg/kg evinced the protective role of kolaviron in MWCNT-mediated oxidative damage in cerebrum, cerebellum and mid-brain of the treated rats. As a result, the neuroprotective mechanisms of kolaviron in MWCNT-induced neurotoxicity involve improvement of antioxidant activities of SOD, CAT, GPx, and GST as well as inhibition of RONS and LPO levels in brain of the treated rats. The beneficial effect of kolaviron may be related to its intrinsic antioxidant activity.

Myeloperoxidase is a heme protein which utilizes hydrogen peroxide and chloride anion to produce hypochlorous acid during neutrophil's respiratory burst. It is a well-known index of oxido-inflammatory stress (Aratani 2018; Ndrepepa 2019). Moreover, NO is a signaling molecule well-known to participate in the pathogenesis of inflammation. In addition, excessive production of NO reportedly induces nitrosative stress via peroxynitrite formation which may contribute to cellular damage (Guzik et al. 2003). Tumor necrosis factor (TNF-α) or cachectin is a signaling pro-inflammatory cytokine known to play a fundamental role in the immune system during systemic inflammation and apoptosis (Baud and Karin 2001). In the present study, administration of MWCNTs alone significantly increased MPO activity and the levels of NO and TNF-α in the cerebrum, cerebellum, and mid-brain of the treated rats, thus signifying the involvement of inflammatory response in MWCNT-induced neurotoxicity. However, the marked decrease in the MPO activity and levels of NO and TNF-α in rats co-administered with kolaviron connotes an anti-inflammatory effect of kolaviron against MWCNTs-induced inflammation in the treated rats.

Light microscopic examination showed that administration of MWCNTs alone resulted in marked degeneration of the striatum neurones, cortical neurones and PNCA3 of the treated rats. Moreover, histomophormetrical alterations indicated significant reduction in the densities of viable striatum neurones, cortical neurons, PNCA3, GCDG as well as in the WDPC. The observed histopathological and histomophormetrical changes in the brain of MWCNTs-treated rats may be related to neuronal inflammation and oxidative damage which may cause insufficiency in motor coordination, memory formation, and cognitive functions. Notably, co-treatment with kolaviron effectively abrogated these histological and mophormetrical changes and maintained the brain structures and neurones rather comparable to the control. Hence, this observation supports the biochemical data on the neuroprotective effect of kolaviron on neurotoxicity associated with MWCNT exposure in rats.

Taken together, the novel data from the present investigation demonstrated that kolaviron abated neurobehavioral and biochemical deficits associated with MWCNT exposure in rats. Kolaviron protected against MWCNTs-induced neurotoxicity via several mechanisms involving enhancement of AChE and antioxidant enzymes activities and suppression of oxido-inflammatory stress responses. Moreover, it is worthy of note that neuroprotective effect of kolaviron was dose-dependent with 100 mg/kg body weight more effective than 50 mg/kg body weight. Kolaviron may be a prospective chemotherapeutic agent against neurotoxicity associated with exposure to MWCNTs.

Funding information This research was supported in part by the National Institute of Health (NIH)-NIMHD with the grant number G12MD007581 and TETFUND National Research Fund (NRF) 2015 grant.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Abarikwu SO (2014) Kolaviron, a natural flavonoid from the seeds of Garcinia kola, reduces LPS-induced inflammation in macrophages by combined inhibition of IL-6 secretion, and inflammatory transcription factors, ERK1/2, NF-κB, p38, Akt, p-c-JUN and JNK. Biochim Biophys Acta 1840(7):2373–2381

Abu Gazia M, El-Magd MA (2019) Effect of pristine and functionalized multiwalled carbon nanotubes on rat renal cortex. Acta Histochem 121(2):207–217

Adedara IA, Abolaji AO, Rocha JB, Farombi EO (2016a) Diphenyl diselenide protects against mortality, locomotor deficits and oxidative stress in *Drosophila melanogaster* model of manganese-induced neurotoxicity. Neurochem Res 41:1430–1438

Adedara IA, Rosemberg DB, de Souza D, Farombi EO, Aschner M, Souza DO, Rocha JBT (2016b) Neurobehavioral and biochemical changes in *Nauphoeta cinerea* following dietary exposure to chlorpyrifos. Pestic Biochem Physiol 130:22–30

UNIVERSITY OF IBADAN LIBRARY

Adedara IA, Abolaji AO, Idris UF, Olabiyi BF, Onibiyo EM, Ojuade TD, Farombi EO (2017) Neuroprotective influence of taurine on fluoride-induced biochemical and behavioral deficits in rats. Chem Biol Interact 261:1–10

Adedara IA, Anao OO, Forcados GE, Awogbindin IO, Agbowo A, Ola-Davies OE, Patlolla AK, Tchounwou PB, Farombi EO (2018) Low doses of multi-walled carbon nanotubes elicit hepatotoxicity in rats with markers of oxidative stress and induction of pro-inflammatory cytokines. Biochem Biophys Res Commun 503(4):3167–3173

Akinmoladun AC, Akinrinola BL, Olaleye MT, Farombi EO (2015) Kolaviron, a *Garcinia kola* biflavonoid complex, protects against ischemia/reperfusion injury: pertinent mechanistic insights from biochemical and physical evaluations in rat brain. Neurochem Res 40(4):777–787

Antúnez-Flores W, Valenzuela-Muñiz AM, Amézaga-Madrid P, Alonso-Nuñez G, Verde Y, Martínez-Sánchez R, Miki-Yoshida M (2008) Simplified route to multi-walled carbon nanotube synthesis by aerosol assisted chemical vapor deposition. J Nanosci Nanotechnol 8(12):6451–6455

Aratani Y (2018) Myeloperoxidase: its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys 640:47–52

Aschberger K, Johnston HJ, Stone V, Aitken RJ, Hankin SM, Peters SA, Tran CL, Christensen FM (2010) Review of carbon nanotubes toxicity and exposure–appraisal of human health risk assessment based on open literature. Crit Rev Toxicol 40(9):759–790

Bancroft JD, Gamble M (2008) Theory and practice of histology techniques, 6th edn. Churchill Livingstone Elsevier, Philadelphia (PA), pp 83–134

Baud V, Karin M (2001) Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol 11(9):372–377

Belyanskaya L, Weigel S, Hirsch C, Tobler U, Krug HF, Wick P (2009) Effects of carbon nanotubes on primary neurons and glial cells. Neurotoxicology 30:702–711

Bianco A, Kostarelos K, Partidos CD, Prato M (2005) Biomedical applications of functionalised carbon nanotubes. Chem Commun 7:571–577

Bradford MM (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brunauer S, Emmett PH, Teller E (1938) Adsorption of gases in multimolecular layers. J Am Chem Soc 60:309–319

Chen T, Yang J, Ren G, Yang Z, Zhang T (2013) Multi-walled carbon nanotube increases the excitability of hippocampal CA1 neurons through inhibition of potassium channels in rat's brain slices. Toxicol Lett 217:121–128

Chen T, Yang J, Zhang H, Ren G, Yang Z, Zhang T (2014) Multi-walled carbon nanotube inhibits CA1 glutamatergic synaptic transmission in rat's hippocampal slices. Toxicol Lett 229:423–429

Claiborne A (1995) Catalase activity. In: Greewald AR (ed) Handbook of methods for oxygen radical research. CRC Press, Boca Raton, pp 237–242

Day J, Damsma G, Fibiger HC (1991) Cholinergic activity in the rat hippocampus, cortex and striatum correlates with locomotor activity: an *in vivo* microdialysis study. Pharmacol Biochem Behav 38:723–729

De Marchi L, Neto V, Pretti C, Figueira E, Chiellini F, Morelli A, Soares AMVM, Freitas R (2018) Toxic effects of multi-walled carbon nanotubes on bivalves: comparison between functionalized and non-functionalized nanoparticles. Sci Total Environ 622-623:1532–1542

Dong J, Ma Q (2016) Suppression of basal and carbon nanotube-induced oxidative stress, inflammation and fibrosis in mouse lungs by Nrf2. Nanotoxicology. 10(6):699–709

Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Farombi EO, Nwaokeafor IA (2005) Anti-oxidant mechanisms of kolaviron: studies on serum lipoprotein oxidation, metal chelation and oxidative membrane damage in rats. Clin Exp Pharmacol Physiol 32(8):667–674

Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO (2000) Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron-a *Garcinia kola* seed extract. Food Chem Toxicol 38:535–541

Farombi EO, Adedara IA, Forcados GE, Anao OO, Agbowo A, Patlolla AK (2016) Responses of testis, epididymis, and sperm of pubertal rats exposed to functionalized multiwalled carbon nanotubes. Environ Toxicol 31:543–551

Farombi EO, Awogbindin IO, Farombi TH, Oladele JO, Izomoh ER, Aladelokun OB, Ezekiel IO, Adebambo OI, Abah VO (2019) Neuroprotective role of kolaviron in striatal redo-inflammation associated with rotenone model of Parkinson's disease. Neurotoxicology 73:132–141

Gao J, Zhang X, Yu M, Ren G, Yang Z (2015) Cognitive deficits induced by multi-walled carbon nanotubes *via* the autophagic pathway. Toxicology 337:21–29

Gorny JH, Gorny B, Wallace DG, Whishaw IQ (2002) Fimbria-fornix lesions disrupt the dead reckoning (homing) component of exploratory behavior in mice. Learn Mem 9(6):387–394

Granell S, Gironella M, Bulbena O, Panés J, Mauri M, Sabater L, Aparisi L, Gelpi E, Closa D (2003) Heparin mobilizes xanthine oxidase and induces lung inflammation in acute pancreatitis. Crit Care Med 31:525–530

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982) Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Anal Biochem 126:131–138

Grochowski C, Litak J, Kamieniak P, Maciejewski R (2018) Oxidative stress in cerebral small vessel disease. Role of reactive species. Free Radic Res 52(1):1–13

Guzik TJ, Korbut R, Adamek-Guzik T (2003) Nitric oxide and superoxide in inflammation and immune regulation. J Physiol Pharmacol 54:469–487

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Han YG, Xu J, Li ZG, Ren GG, Yang Z (2012) *In vitro* toxicity of multi-walled carbon nanotubes in C6 rat glioma cells. Neurotoxicology 33:1128–1134

Hayes JD, Strange RC (2000) Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology. 61:154–166

Ishola IO, Adamson FM, Adeyemi OO (2017) Ameliorative effect of kolaviron, a biflavonoid complex from *Garcinia kola* seeds against scopolamine-induced memory impairment in rats: role of antioxidant defense system. Metab Brain Dis 32(1):235–245

Iwu MM (1985) Antihepatoxic constituents of *Garcinia kola* seeds. Experientia 41(5):699–700

Jacobs CB, Peairs MJ, Venton BJ (2010) Review: carbon nanotube based electrochemical sensors for biomolecules. Anal Chim Acta 662:105–127

Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR (1974) Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4 bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11:151–169

Kamran U, Heo YJ, Lee JW, Park SJ (2019) Functionalized carbon materials for electronic devices: a review. Micromachines (Basel) 10(4). https://doi.org/10.3390/mi10040234

Kumar R, Dhanawat M, Kumar S, Singh BN, Pandit JK, Sinha VR (2014) Carbon nanotubes: a potential concept for drug delivery applications. Rec Patents Drug Deliv Formulat 8:12–26

Lin Y, Taylor S, Li H, Shiral Fernando KA, Qu L, Wang W, Gu L, Zhou B, Ping Sun Y (2004) Advances toward bioapplications of carbon nanotubes. J Mater Chem 14:527–541

UNIVERSITY OF IBADAN LIBRARY

Malarkey EB, Parpura V (2007) Applications of carbon nanotubes in neurobiology. Neurodegener Dis 4(4):292–299

Misra HP, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Motz BA, Alberts JR (2005) The validity and utility of geotaxis in young rodents. Neurotoxicol Teratol 27(4):529–533

Ndrepepa G (2019) Myeloperoxidase - a bridge linking inflammation and oxidative stress with cardiovascular disease. Clin Chim Acta 493: 36–51

Nel AE (2013) Implementation of alternative test strategies for the safety assessment of engineered nanomaterials. J Intern Med 274(6):561–577

Nwankwo JO, Tahnteng JG, Emerole GO (2000) Inhibition of aflatoxin B1 genotoxicity in human liver-derived HepG2 cells by kolaviron biflavonoids and molecular mechanisms of action. Eur J Cancer Prev 9(5):351–361

Ojo OB, Amoo ZA, Saliu IO, Olaleye MT, Farombi EO, Akinmoladun AC (2019) Neurotherapeutic potential of kolaviron on neurotransmitter dysregulation, excitotoxicity, mitochondrial electron transport chain dysfunction and redox imbalance in 2-VO brain ischemia/reperfusion injury. Biomed Pharmacother 111:859–872

Olajide OJ, Asogwa NT, Moses BO, Oyegbola CB (2017) Multidirectional inhibition of cortico-hippocampal neurodegeneration by kolaviron treatment in rats. Metab Brain Dis 32(4):1147–1161

Onasanwo SA, Rotu RA (2016) Antinociceptive and anti-inflammatory potentials of kolaviron: mechanisms of action. J Basic Clin Physiol Pharmacol 27(4):363–370

Osuagwu FC, Owoeye O, Avwioro OG, Oluwadara OO, Imosemi IO, Ajani RS, Ogunleye AA, Oladejo OW (2007) Reduction of hippocampal CA 1 neurons in Wistar rats following the administration of phenytoin for seven days. Afr J Med Med Sci 36:103–108

Patlolla AK, Berry A, Tchounwou PB (2011) Study of hepatotoxicity and oxidative stress in male Swiss-Webster mice exposed to functionalized multi-walled carbon nanotubes. Mol Cell Biochem 358:189–199

Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M (2017) Targeting free radicals in oxidative stress-related human diseases. Trends Pharmacol Sci 38(7):592–607

Reddy AR, Rao MV, Krishna DR, Himabindu V, Reddy YN (2011) Evaluation of oxidative stress and anti-oxidant status in rat serum following exposure of carbon nanotubes. Regul Toxicol Pharmacol 59(2):251–257

Riemann BL, Lephart SM (2002) The sensorimotor system, part I: the physiologic basis of functional joint stability. J Athl Train 37:71–79

Rode A, Sharma S, Mishra DK (2018) Carbon nanotubes: classification, method of preparation and pharmaceutical application. Curr Drug Deliv 15(5):620–629

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588–590

Shichiri M (2014) The role of lipid peroxidation in neurological disorders. J Clin Biochem Nutr 54(3):151–160

Tsukahara T, Matsuda Y, Haniu H (2014) The role of autophagy as a mechanism of toxicity induced by multi-walled carbon nanotubes in human lung cells. Int J Mol Sci 16(1):40–48

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39(1):44–84

Wang J, Schlagenhauf L, Setyan A (2017) Transformation of the released asbestos, carbon fibers and carbon nanotubes from composite materials and the changes of their potential health impacts. J Nanobiotechnol 15(1):15. https://doi.org/10.1186/s12951-017-0248-7

Zhao X, Chang S, Long J, Li J, Li X, Cao Y (2019) The toxicity of multi-walled carbon nanotubes (MWCNTs) to human endothelial cells: the influence of diameters of MWCNTs. Food Chem Toxicol 126: 169–177

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

UNIVERSITY OF IBADAN LIBRARY