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RESEARCH ARTICLE

Kolaviron suppresses dysfunctional reproductive axis associated with multi-walled carbon nanotubes exposure in male rats

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Abstract

Reproductive toxicity associated with excessive exposure to multi-walled carbon nanotubes (MWCNTs), which are commonly used in medicine as valuable drug delivery systems, is well documented. Kolaviron, a bioflavonoid isolated from *Garcinia kola* seeds, elicits numerous health beneficial effects related to its anti-inflammatory, anti-genotoxic activities, anti-apoptotic, and antioxidant properties. However, information on the role of kolaviron in MWCNTs-induced reproductive toxicity is not available in the literature. Herein, we assessed the protective effects of kolaviron on MWCNTs-induced dysfunctional reproductive axis in rats following exposure to MWCNTs (1 mg/kg) and concurrent treatment with kolaviron (50 or 100 mg/kg body weight) for 15 successive days. Results showed that MWCNTs-induced dysfunctional reproductive axis as evidenced by deficits in pituitary and testicular hormones, marker enzymes of testicular function, and sperm functional characteristics were abrogated in rats co-administered with kolaviron. Moreover, co-administration of kolaviron-abated MWCNTs-induced inhibition of antioxidant enzyme activities increases in oxidative stress and inflammatory indices. This is evidenced by diminished levels of tumor necrosis factor-alpha, nitric oxide, lipid peroxidation, reactive oxygen, and nitrogen species as well as reduced activity of myeloperoxidase in testes, epididymis, and hypothalamus of the rats. Biochemical data on the chemoprotection of MWCNTs-induced reproductive toxicity were corroborated by histological findings. Taken together, kolaviron suppressed dysfunctional reproductive axis associated with MWCNTs exposure via abrogation of oxidative stress and inflammation in male rats.

Keywords Multi-walled carbon nanotubes · Reproductive axis · Kolaviron, · Oxido-inflammation · Rats

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Introduction

Carbon nanotubes (CNTs) are unique allotropes of carbon with remarkable chemical, physical, and electronic characteristics which make them suitable for numerous applications in different fields (Negri et al. 2020; Patel et al. 2020). Specifically, CNTs are important in vaccine or drug delivery for diagnosis or treatments (Klumpp et al. 2006) and as essential constituents in rechargeable battery electrodes (Han et al. 2012). Moreover, CNTs are essential in the removal of several environmental contaminants due to their hollow, layered structure and large surface area (Ding et al. 2019; Chauhan et al. 2019; Ganzoury et al. 2020). Multi-walled carbon nanotubes (MWCNTs) are important CNT widely used in nanoelectronics, medicine, agriculture, engineering, and consumer products (Kumar et al. 2014; Rode et al. 2018; Kamran et al. 2019). Indeed, MWCNTs are effective delivery systems for several drugs including carbamazepine, doxorubicin, and

dorzolamide (Ncibi and Sillanpää 2017). However, there is a growing scientific interest on the use MWCNTs in recent years because of the health concerns associated with undue human and animal exposure (Kobayashi et al. 2017; Fukushima et al. 2018). For instance, MWCNTs administration elicits adverse effects on the brain, liver, and reproduction in zebrafish and rats (Li et al. 2015; Adedara et al. 2018, 2020).

Earlier investigators reported that nanomaterials affect reproduction and development through direct and indirect means (Ema et al. 2016a). Reproductive toxicity of some nanoparticles has been associated with their ability to enter human tissues via digestive system and bioaccumulate in the reproductive system, particularly the testis (Wang et al. 2018). Moreover, carbon nanotubes including MWCNTs are well known to generate reactive oxygen species (ROS) in experimental animals (Patlolla et al. 2011; Ghanbari et al. 2017; Rasras et al. 2019). The testis which is responsible for steroidogenesis and spermatogenesis which are required for reproductive success is vulnerable to increased ROS (Guerriero et al. 2014). Previous in vitro studies using mice spermatocyte cell line (GC-2spd) demonstrated that MWCNTs accumulated in the mitochondria leading to mitochondrial DNA damage in spermatocyte and decrease in the expression of mitochondria-related genes and cellular ATP level (Xu et al. 2016). Indeed, exposure to low doses of MWCNTs reportedly elicited oxidative stress response leading to testicular and epididymal dysfunction in pubertal rats (Farombi et al. 2016). Hence, there is a need to search for suitable remedies for toxicities associated with MWCNTs exposure.

Kolaviron is a bioflavonoid isolated from the seeds of *Garcinia kola Heckel* commonly used in African Traditional Medicine as a therapy for throat infections, liver disorders, and as an aphrodisiac and fertility-enhancing substance (Ralebona et al. 2012; Farombi et al. 2013; Sewani-Rusike et al. 2016). Previous in vitro and in vivo studies revealed that kolaviron elicits numerous health beneficial effects related to its anti-inflammatory, anti-genotoxic, anti-apoptotic and antioxidant properties (Nwankwo et al. 2000; Farombi and Nwaokeafor 2005; Farombi et al. 2013). Kolaviron reportedly protected against testicular toxicity associated with therapeutic drugs (Olayinka and Ore 2014; Kehinde et al. 2016), exposure to environmental pollutants (Adedara et al. 2013; Adedara et al. 2015), and diabetes-mediated reproductive dysfunction in rats (Adaramoye and Lawal 2014). However, scientific information on the influence of kolaviron on the reproductive dysfunction associated with MWCNT exposure is currently unavailable.

In view of the increasing rate of MWCNT applications and their widespread presence in the environment, the present study was designed to elucidate the role of kolaviron administration in MWCNT-induced reproductive dysfunction by assessing the indices of oxidative stress, inflammation, endocrine function, and spermatogenesis in male Wistar rats.

Materials and methods

Chemicals and reagents

Carboxylated multi-walled carbon nanotubes (MWCNTs) are a product of NanoLab Inc. (Newton, MA). All other reagents and chemicals were bought from Sigma Chemical Co. (St. Louis, MO, USA).

Functionalized MWCNT characterization

The MWCNTs (purity > 95%) were synthesized in the NanoLab Inc. (Newton MA, USA) through catalytic chemical vapor deposition process. The outer diameter and lengths were 15–30 nm and 15–20 μm, respectively. Moreover, the MWCNTs were purified from iron-impurities by heating under argon (2 l/min) to 2000 °C at the rate of 10 °C/min. The pure MWCNTs (purity > 95% by thermogravimetry analysis (TGA)) obtained were then exposed to a reflux process in sulfuric/nitric acid (3:1) to generate functionalized carboxylated nanotubes of 2–7% COOH by weight. The structure and size of these pure MWCNTs were evaluated by means of transmission electron microscope (TEM). The MWCNTs were directly situated on a TEM grid and allowed to dry prior to examining the samples with TEM. Further, the surface areas were assessed with the aid of an isothermal gas adsorption BET method (Brunauer et al. 1938) and a Micromeritics Flowsorb 2300 (Norcross, USA). Characterization was done by dissolving the MWCNTs in 1% Tween-80 and sterile saline using physical mixing and ultrasonication. Eventually, purified long MWCNTs with 42 m^2/g specific surface area, 12 μm in length, and 11.5 nm in diameter were produced.

Isolation of kolaviron

To isolate kolaviron from *Garcinia kola* seed, we followed an established procedure (Iwu 1985, Farombi et al. 2019). Concisely, fresh *Garcinia kola* seeds were cut into pieces, air dried, and pulverized before defatting in a Soxhlet apparatus with *n*-hexane. The defatted dried marc was further extracted with methanol using Soxhlet apparatus. Following this, the extract was concentrated and partitioned with distilled water and chloroform to obtain a golden yellow isolate known as kolaviron. Kolaviron was characterized with direct comparison of the 1H nuclear magnetic resonance (NMR), ^{13}C NMR, and electron ionization mass spectral results. The isolated kolaviron was 95% pure.

Care of experimental animals

Fifty post pubertal male Wistar rats (11 weeks old) weighing between 180 and 185 g were used for the present investigation. They were obtained from the Faculty of Veterinary

Medicine, University of Ibadan. The animals were accommodated in well-ventilated plastic cages under a 12-h light/12-h dark photocycle in a vivarium, where they were provided water and rat victuals ad libitum. Moreover, sufficient quantity of wood shavings was provided as bedding in the cages. The rats were allowed to acclimatize for 1 week before the start of the treatment. The care of animals and experimental modus operandi were carried out in accordance with the approved guidelines of the University of Ibadan Ethical Committee and the "Guide for the Care and Use of Laboratory Animals" of the National Institute of Health.

Experimental design

The animals were randomly distributed to five groups of ten rats each and treated once daily for 15 consecutive days as follows:

Control: The animals received saline plus 1% Tween-80 at 2 mL/kg body weight via intraperitoneal (i.p.) route.

KV alone: The animals were administered kolaviron (KV) orally at 100 mg/kg body weight.

MWCNTs alone: The animals were treated (i.p.) with 1.0 mg/kg pure MWCNTs.

MWCNTs + KV 1: The animals were treated (i.p.) with 1.0 mg/kg pure MWCNTs and administered KV (orally) at 50 mg/kg body weight.

MWCNTs + KV 2: The animals were treated (i.p.) with 1.0 mg/kg pure MWCNTs and administered KV (orally) at 100 mg/kg body weight.

Previous investigators have administered MWCNTs via intravenous injection, intraperitoneal injection, pharyngeal aspiration, oral gavage, and intratracheal instillation (reviewed by Ema et al. 2016b). The current investigation adopted intraperitoneal injection because it delivers a greater drug concentration with a minor systemic toxicity in comparison with other routes of administration (Shimada et al. 2005; Ema et al. 2016b). A dose of 1 mg/kg pure MWCNTs was selected because it is an environmentally relevant concentration which reportedly caused significant oxidative stress and histological lesions in the different organs whereas 50 and 100 mg/kg of kolaviron were selected based on their effective pharmacological data from preliminary studies and earlier published studies from our laboratory (Akinmoladun et al. 2015; Farombi et al. 2016; Adedara et al. 2018; Adedara et al. 2020). The final body weights of the animals were recorded 24 h following the last treatment. The blood was collected from retro-orbital venous plexus into plain tubes and allowed to clot before centrifugation at 3000×*g* for 10 min to get the serum for the hormonal assays. The animals were euthanized with light ether anesthesia. Following this, the testes, hypothalamus, and epididymis were instantly and cautiously excised, weighed, and prepared for histological and biochemical analyses.

Sperm analysis

Evaluation of the sperm progressive motility was done in line with the established method (Zemjanis 1970). We estimated epididymal sperm count with standard procedure of WHO (1999). Morphological aberrations and viability of sperm cells were evaluated as described in the previous studies (Wells and Awa 1970; Adedara et al. 2019). We assessed the testicular sperm number and daily sperm production in the left testes by following the protocol of Blazak et al. (1993).

Assay of pituitary and testicular hormones

ELISA kits specific for rats were used to analyze serum levels of pituitary (follicle stimulating hormone, luteininzing hormone and prolactin) and testicular (testosterone) hormones. Specifically, FSH (E-EL-R0391), LH (E-EL-R0026), prolactin (E-EL-R0052), and testosterone (E-EL-R0033) were assayed according to the instructions from the manufacturer (Elabscience Biotechnology Company, Beijing, China). The sensitivities of FSH and prolactin were 0.23 ng and 0.11 ng, while LH and testosterone were 0.44 ng and 0.47 ng, respectively. While the intra-assay coefficients of variations for FSH, LH, prolactin, and testosterone were 2.9%, 2.5%, 2.2%, and 3.0%, respectively.

Preparation of homogenates for biochemical analysis

The excised tissues—testes, epididymis and hypothalamus—were homogenized in Tris–HCl buffer (50 mM; pH 7.4) and centrifuged at 12,000×*g* for 15 min. The supernatants were used for biochemical assays. Protein concentration of the testes, epididymis, and hypothalamus was assessed in line with Bradford (1976).

Assessment of testicular function indices

Biomarkers of testicular functions were assayed in the supernatant of the testes. Acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were assessed according to the established methods (Malymy and Horecker 1966; Vanha-Perttula and Nikkanen 1973). Glucose-6-phosphate dehydrogenase (G6PD) activity was evaluated as described previously (Salihu et al. 2017) using glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate as substrates. Lactate dehydrogenase-X (LDH-X) was assayed in line with established technique (Vassault 1983) based on the principle of interconversion of lactate and pyruvate.

Measurement of antioxidant, oxidative stress, and pro-inflammatory markers

Activities of antioxidant enzymes in the testes, epididymis, and hypothalamus were assayed according to established methods. Succinctly, superoxide dismutase (SOD) activity

was evaluated as described by Misra and Fridovich (1972), catalase (CAT) by Claiborne (1995), glutathione peroxidase (GPx) by Rotruck et al. (1973), and glutathione-S-transferase (GST) by Habig et al. (1974). Glutathione (GSH) level was assayed according to standard method (Jollow et al. 1974). In addition, oxidative stress indices specifically lipid peroxidation (LPO) and reactive oxygen and nitrogen species (RONS) levels were measured as described in Farombi et al. (2000) and Adedara et al. (2016), respectively. Myeloperoxidase (MPO) activity and nitric oxide (NO) level were evaluated by following the procedures of Granell et al. (2003) and Green et al. (1982), respectively. The tissue level of tumor necrosis factor-alpha (TNF-α) was measured using ELISA Kits (Elabscience Biotechnology Company, Beijing, China) as specified in the manufacturer's manual. Apart from SOD and CAT activities which were analyzed using 752S UV-VIS Spectrophotometer (Ningbo, China), all the other biochemical analyses were done using a SpectraMax plate reader (Molecular Devices, CA, USA).

Histological examination

Histological examination of the testes, epididymis, and hypothalamus was performed according to Bancroft and Gamble (2008). Concisely, Bouin's fixed tissues were dehydrated, embedded in paraffin, and sectioned (5 μm) with a microtome. Further, the sections were stained with hematoxylin and eosin on slides. Examination under a light microscope (Leica DM 500, Germany) with an attached digital camera (Leica ICC50 E, Germany) was done by two pathologists blinded to the study.

Statistical analyses

The data from the present study were analyzed with one-way analysis of variance (ANOVA) and Bonferroni's post hoc test. Values of p less than 0.05 were considered statically significant.

Results

Kolaviron abrogated MWCNTs-mediated deficits in reproductive hormones and impaired testicular function in rats

The impact of kolaviron on the levels of FSH, LH, and testosterone in serum and activities of testicular function enzymes in MWCNT-treated rats is depicted in Figs. 1 and 2. In comparison with the control rats, administration of MWCNTs alone significantly ($p < 0.05$) diminished serum levels of FSH, LH, prolactin, and testosterone as well as activities of G6PD, ACP, ALP, and LDH in the testes of the rats. However, co-administration of MWCNTs with kolaviron at both 50 and 100 mg/kg markedly abrogated MWCNTs-induced deficits in the levels of circulating hormones and activities enzymatic indices of testicular function in the treated rats. Specifically, MWCNTs administration decreased FSH, LH, prolactin, and testosterone levels by 40%, 45%, 45%, and 49%, respectively. However, FSH level was increased by 28% and 40%, LH level by 49% and 80%, prolactin by 39% and 59%, and testosterone level by 58% and 78% following kolaviron treatment at 50 and 100 mg/kg, respectively. Similarly, MWCNTs exposure decreased G6PD, ACP, ALP, and LDH activities by 77%, 53%, 40%, and 56%, respectively. On the other hand, G6PD activity was increased by 179% and 262%, ACP activity by 64% and 81%, ALP activity by 34% and 51%, and LDH activity by 63% and 87% following kolaviron treatment at 50 and 100 mg/kg, respectively.

Kolaviron abated MWCNTs-induced reductions in the sperm functional indices in rats

The effect of kolaviron on sperm number and daily sperm production in the testes of MWCNTs-treated rats is depicted in Fig. 3. Rats administered MWCNTs alone exhibited significant diminutions in the testicular sperm number and daily

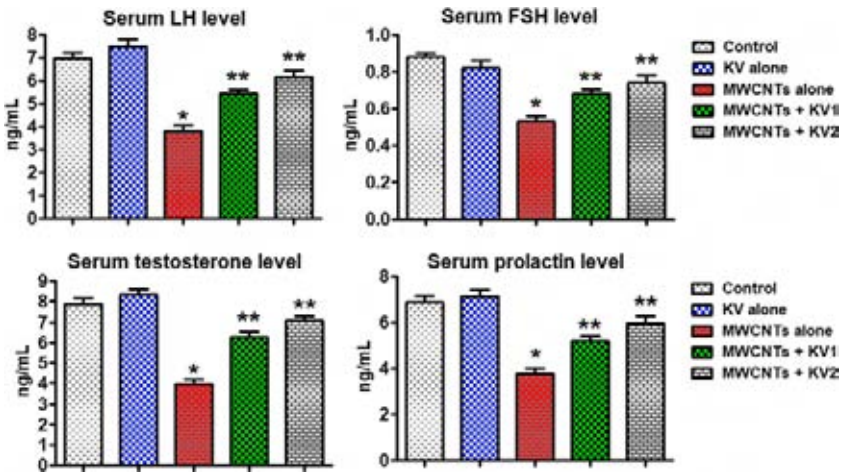

Fig. 1 Effect of kolaviron on serum hormonal concentrations in MWCNTs-exposed rats. MWCNTs denote multi-walled carbon nanotubes at 1 mg/kg; KV1 denotes kolaviron at 50 mg/kg; KV2 denotes kolaviron at 100 mg/kg. The data are expressed as mean ± S.D. for 10 rats per group. *Values differ significantly from control ($p < 0.05$). **Values differ significantly from MWCNTs alone ($p < 0.05$)

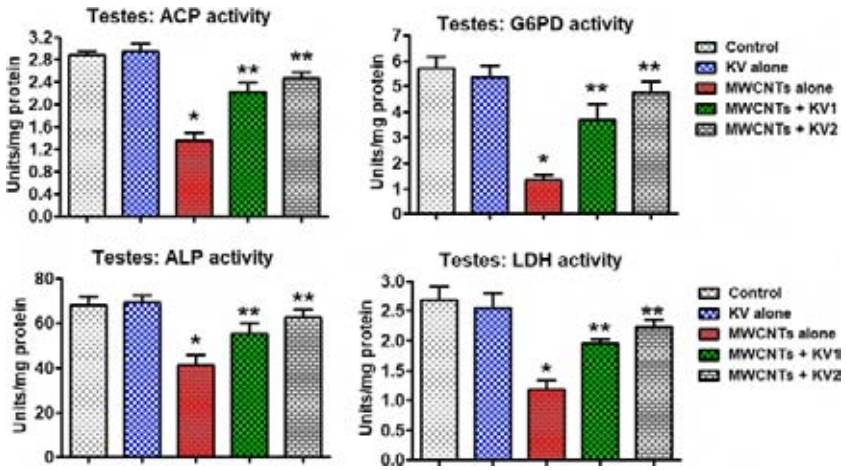

Fig. 2 Effect of kolaviron on the marker enzymes of testicular function in MWCNTs-exposed rats. MWCNTs denote Multi-walled carbon nanotubes at 1 mg/kg; KV1 denotes kolaviron at 50 mg/kg; KV2 denotes kolaviron at 100 mg/kg. The data are expressed as mean ± S.D. for 10 rats per group. *Values differ significantly from control ($p < 0.05$). **Values differ significantly from MWCNTs alone ($p < 0.05$)

sperm production in the treated rats when compared with the control. Similarly, we observed significant reduction in sperm count and motility in the epididymis with concomitant increase in sperm morphological aberrations in rats administered MWCNTs alone. However, kolaviron co-treatment abolished MWCNTs-mediated deficits in sperm parameters, daily sperm production, and testicular sperm number. Sperm viability was not significantly affected in all the treatment groups.

Kolaviron improved the redox status in the testes, epididymis, and hypothalamus of MWCNTs-treated rats

The effects of kolaviron on antioxidant status and indices of oxidative stress in the testes, epididymis, and hypothalamus of MWCNT-treated rats are depicted in Figs. 4, 5, 6, and 7. Compared with the control, activities of antioxidant enzymes, namely, CAT, SOD, GPx, and GST, along with GSH level were markedly decreased whereas levels of RONS and MDA were significantly elevated in these tissues of rats administered MWCNTs alone. Conversely, concurrent treatment of MWCNTs with 50 and 100 mg/kg kolaviron significantly enhanced the activities of these antioxidant enzymes while the levels of RONS and MDA in the testes, epididymis, and hypothalamus of the treated rats were significantly reduced when compared with MWCNTs alone–treated rats.

Kolaviron diminished inflammatory indices in the testes, epididymis, and hypothalamus of MWCNTs-treated rats

The impacts of kolaviron treatment on markers of inflammation in MWCNTs-treated rats are shown in Figs. 8 and 9. Administration of MWCNTs significantly elevated the activity of MPO and levels of TNF-α and NO in the testes, epididymis, and hypothalamus of the treated rats, when compared

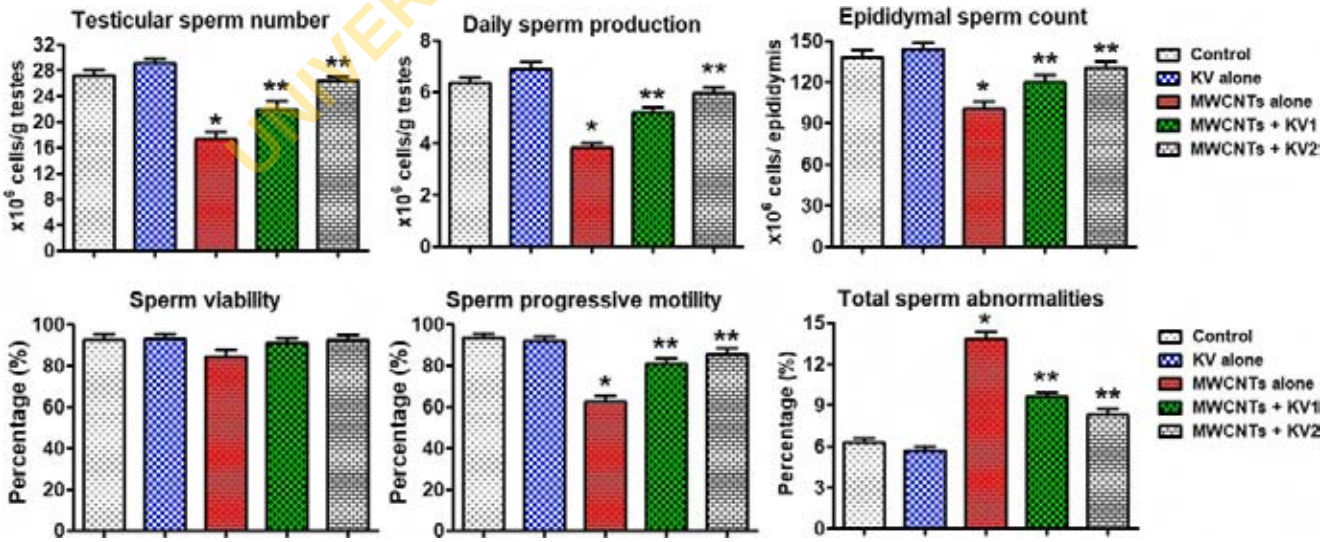

Fig. 3 Effect of kolaviron on the spermiogram in MWCNTs-exposed rats. MWCNTs denote multi-walled carbon nanotubes at 1 mg/kg; KV1 denotes kolaviron at 50 mg/kg; KV2 denotes kolaviron at 100 mg/kg. The data are expressed as mean ± S.D. for 10 rats per group. *Values differ significantly from control ($p < 0.05$). **Values differ significantly from MWCNTs alone ($p < 0.05$)

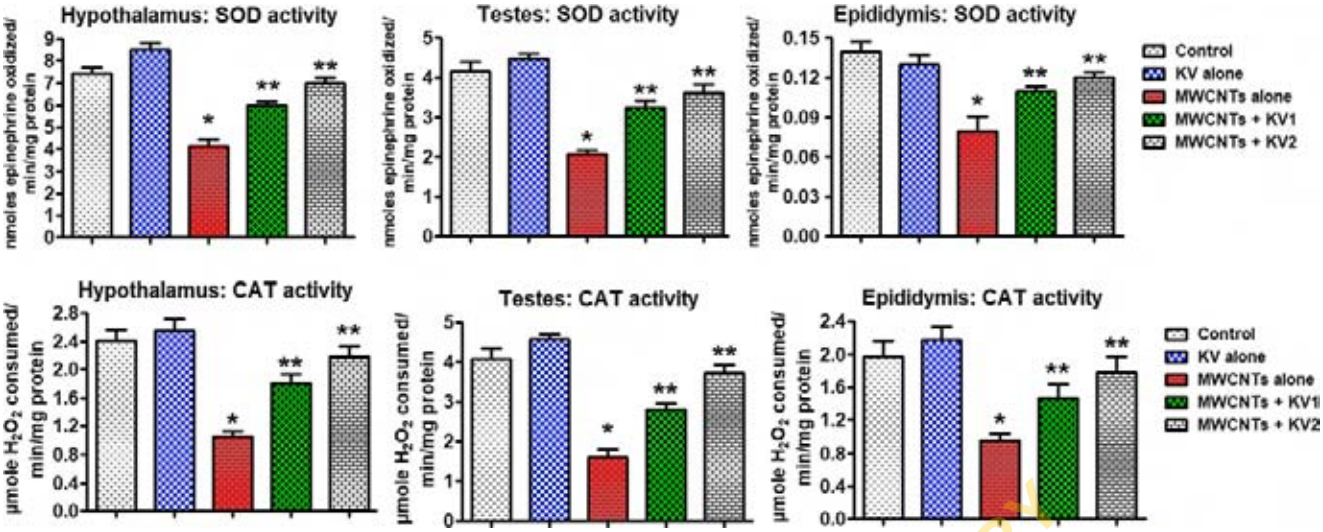

Fig. 4 Effect of kolaviron on SOD and CAT activities in the testes, epididymis, and hypothalamus of rats. MWCNTs denote multi-walled carbon nanotubes at 1 mg/kg; KV1 denotes kolaviron at 50 mg/kg; KV2 denotes kolaviron at 100 mg/kg. The data are expressed as mean ± S.D. for 10 rats per group. *Values differ significantly from control ($p < 0.05$). **Values differ significantly from MWCNTs alone ($p < 0.05$)

with the control. However, treatments of MWCNTs-challenged rats with 50 and 100 mg/kg kolaviron caused noticeable reduction in the activity of MPO and levels of NO and TNF-α, compared with rats treated with MWCNTs alone.

Kolaviron-abrogated MWCNTs-associated histological alterations in rats

The histological features of the tissues from representative animals in the experimental groups are depicted in Fig. 10. Rats from control and kolaviron alone showed the usual architectures of the testes, epididymis, and hypothalamus. Rats administered MWCNTs alone exhibited severe neuronal degeneration in the hypothalamus, testicular vacuolization with focal area of necrosis in the seminiferous tubules while epididymal lesion was characterized by scanty sperm cells and diminished epididymal lining. However, the testes, epididymis, and hypothalamus of rats co-administered with kolaviron exhibited histological features comparable to control.

Discussion

The male reproductive axis comprises of the hypothalamus, pituitary gland, and testes which are collectively called hypothalamic-pituitary-gonadal axis (HPG axis). The HPG

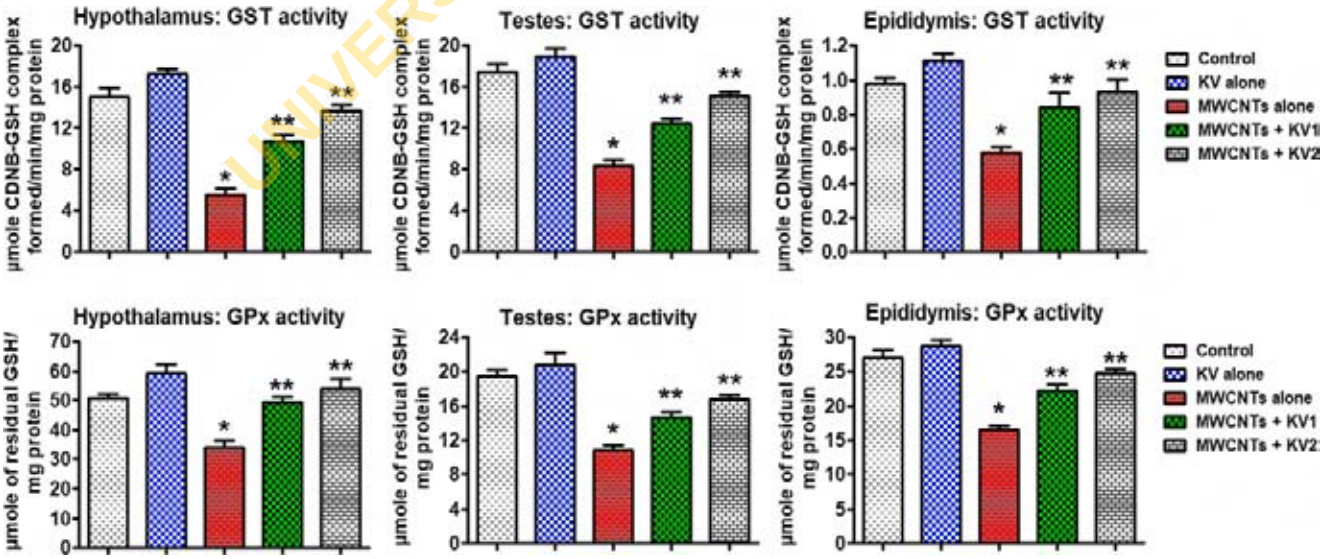

Fig. 5 Effect of kolaviron on GST and GPx activities in the testes, epididymis, and hypothalamus of rats. MWCNTs denote multi-walled carbon nanotubes at 1 mg/kg; KV1 denotes kolaviron at 50 mg/kg; KV2 denotes kolaviron at 100 mg/kg. The data are expressed as mean ± S.D. for 10 rats per group. *Values differ significantly from control ($p < 0.05$). **Values differ significantly from MWCNTs alone ($p < 0.05$)

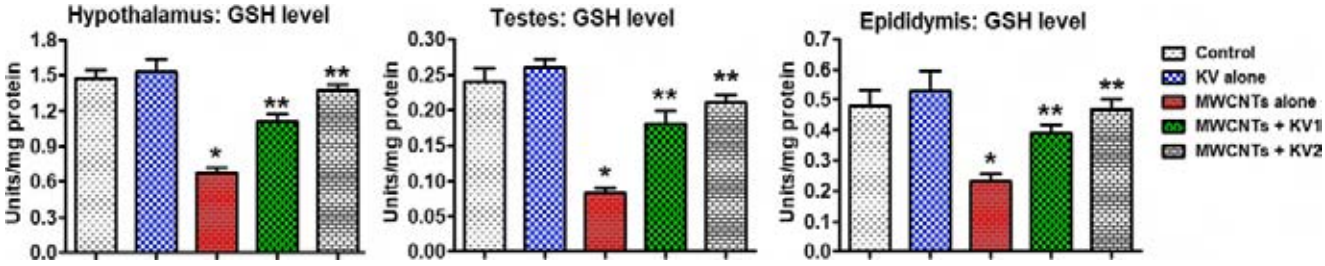

Fig. 6 Effect of kolaviron on GSH level in the testes, epididymis, and hypothalamus of rats. MWCNTs denote multi-walled carbon nanotubes at 1 mg/kg; KV1 denotes kolaviron at 50 mg/kg; KV2 denotes kolaviron at 100 mg/kg. The data are expressed as mean ± S.D. for 10 rats per group. *Values differ significantly from control ($p < 0.05$). **Values differ significantly from MWCNTs alone ($p < 0.05$)

axis as a distinctive system is particularly important for normal reproductive function (Fischer et al. 2019). The mammalian HPG axis regulates the production of reproductive hormones and spermatogenesis. Ordinarily, LH, FSH, and prolactin from the pituitary gland act on the testicular cells namely Leydig, Sertoli, and germ cells to initiate testosterone and sperm production in the testes (Clavijo and Hsiao 2018). The current study focused on the reproductive axis because there is a global worry about the increasing rate of reproductive health decline in both humans and animals. For the first time, the current study evidenced the protective potential of kolaviron against the noxious effects of MWCNTs on male reproductive axis in rats.

The diminution in the serum LH and FSH levels in the present investigation connotes the detrimental effect of MWCNTs on the pituitary function which may impair the action of LH on the Leydig cell and FSH on the Sertoli cell to produce testosterone and sperm respectively. The decrease in serum testosterone level may be associated with the direct inhibitory effect of MWCNTs on the steroidogenic function of the Leydig cells and/or reduction in the circulatory LH level in the treated animals. Further, prolactin reportedly increases the sensitivity of LH receptors in Leydig cells to circulatory LH which consequently leads to testosterone secretion and spermatogenesis (Morris and Saxena 1980; Chowdhury et al. 1983; Adedara et al. 2014). Thus, the diminution in serum prolactin level in rats exposed to MWCNTs may connote its direct inhibitory action on the pituitary gland which produces prolactin. The reduced prolactin level might result in reduced LH receptors and less sensitivity to circulatory LH, which consequently may impair testosterone synthesis in MWCNT-exposed animals. The re-establishment of serum FSH, LH, prolactin, and testosterone concentrations in rats co-treated with MWCNTs and kolaviron at 50 or 100 mg/kg obviously revealed the protective influence of kolaviron on reproductive endocrine deficits induced by MWCNTs in the experimental animals.

The crucial biochemical changes that occur in the testes during spermatogenesis have been linked to the activities of specific enzymes including ALP, ACP, G6PD, GGT, and LDH. These enzymes are well-known markers of testicular function which are often used in the assessment of spermatogonial stem cell survival following toxicant exposure (Salihu et al. 2017). Thus, the reduction in the testicular G6PD and LDH activities following exposure to MWCNTs alone may

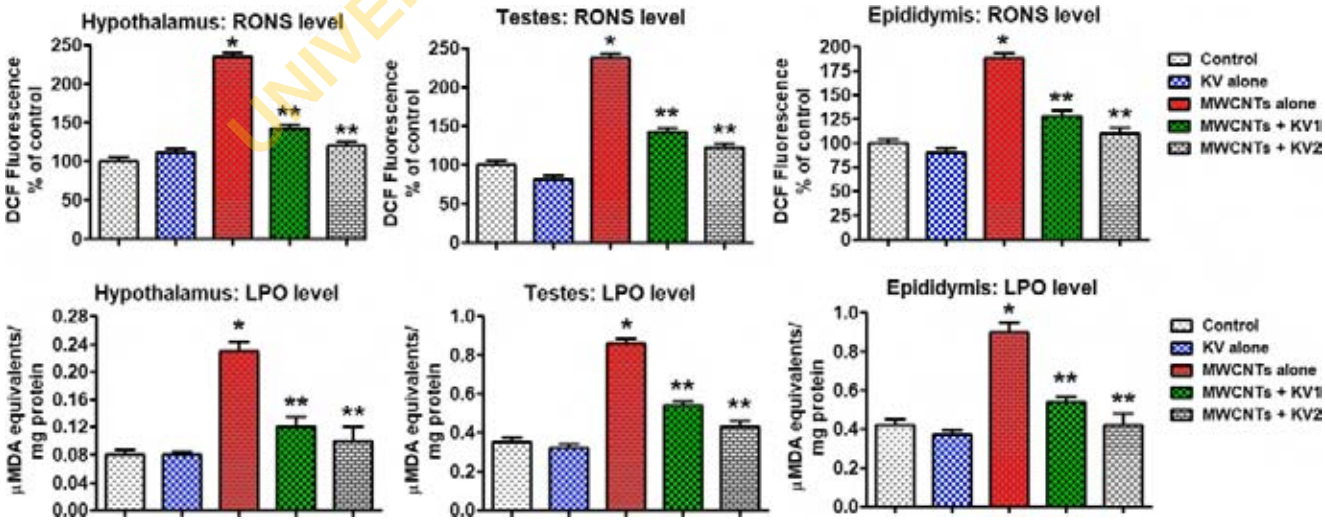

Fig. 7 Effect of kolaviron on RONS and LPO levels in the testes, epididymis, and hypothalamus of rats. MWCNTs denote multi-walled carbon nanotubes at 1 mg/kg; KV1 denotes kolaviron at 50 mg/kg; KV2 denotes kolaviron at 100 mg/kg. The data are expressed as mean ± S.D. for 10 rats per group. *Values differ significantly from control ($p < 0.05$). **Values differ significantly from MWCNTs alone ($p < 0.05$)

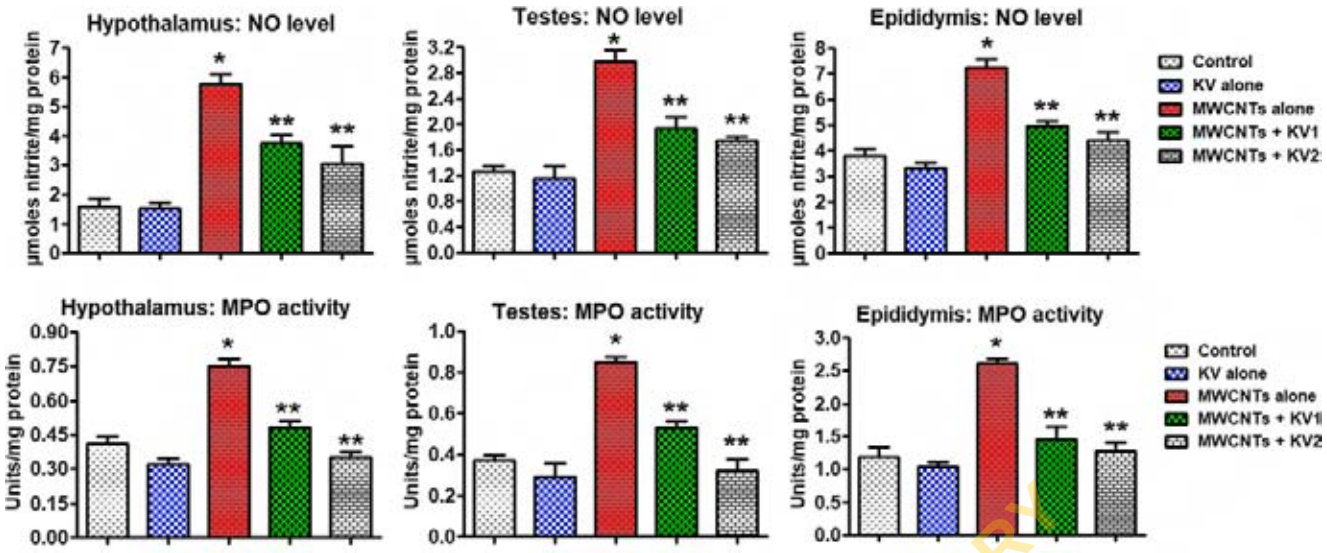

Fig. 8 Effect of kolaviron on MPO activity and NO level in the testes, epididymis, and hypothalamus of rats. MWCNTs denote multi-walled carbon nanotubes at 1 mg/kg; KV1 denotes kolaviron at 50 mg/kg; KV2 denotes kolaviron at 100 mg/kg. The data are expressed as mean ± S.D. for 10 rats per group. *Values differ significantly from control ($p < 0.05$). **Values differ significantly from MWCNTs alone ($p < 0.05$)

impair the synthetic pathway of nicotinamide adenine dinucleotide phosphate and metabolic pathway of lactate in the spermatogenic cells respectively. In addition, the decrease in the testicular activities of ACP and ALP in MWCNTs-treated rats may impair their roles in the supply of molecules vital for steroidogenesis and spermatogenesis between Sertoli and germ cells (Peruquetti et al. 2010). The re-establishment of these indices of testicular function in rats co-treated with MWCNTs and kolaviron at 50 or 100 mg/kg connotes its protective effect against MWCNTs-induced germ cell toxicity.

Further, the marked reduction in the sperm count and motility with concurrent increase in sperm morphological aberrations connotes toxic effects of MWCNTs on the epididymis, which stores, concentrates, transports, and protects the sperm produced by the testes. The reduction in daily production and total number of testicular sperm in rats exposed to MWCNTs alone corroborates the noxious impact of MWCNTs on the testicular function of spermatogenesis. Elevated sperm morphological aberrations are related to the decreased sperm motility which may cause infertility due to the inability of the sperm to reach the fertilization site and to penetrate zonal pellucida. However, the improvement in the spermatogenic and sperm characteristics in rats co-treated with MWCNTs and kolaviron evidenced its protective influence on testicular and epididymal toxicity in MWCNTs-exposed rats.

To elucidate the biochemical mechanisms underlining the protective effects of kolaviron on MWCNTs-induced male reproductive dysfunction, we assayed key antioxidant enzymes and biomarkers of redo-inflammatory stress in the testes, epididymis, and hypothalamus of the animals. The antioxidant defense system protects against ROS namely superoxide anion, peroxides, and hydroxyl radical which have been associated with reproductive dysfunction (Baskaran et al. 2020). In this study, MWCNTs alone–treated rats exhibited marked decrease in the activities of CAT, SOD, and GSH-dependent enzymes (GST and GPx) as well as in GSH level. This connotes deficiency in the antioxidant function to maintain redox status and subdue oxidative stress in the investigated tissues. The increase in the RONS and LPO levels in the

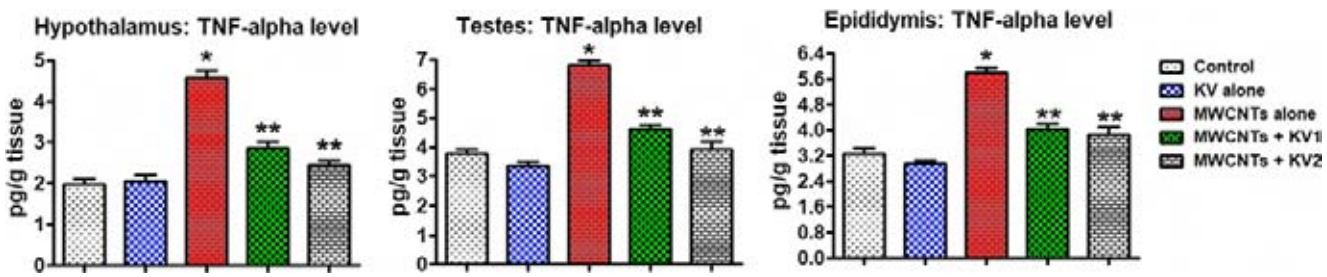

Fig. 9 Effect of kolaviron on TNF-α level in the testes, epididymis, and hypothalamus of rats. MWCNTs denote multi-walled carbon nanotubes at 1 mg/kg; KV1 denotes kolaviron at 50 mg/kg; KV2 denotes kolaviron at 100 mg/kg. The data are expressed as mean ± S.D. for 10 rats per group. *Values differ significantly from control ($p < 0.05$). **Values differ significantly from MWCNTs alone ($p < 0.05$)

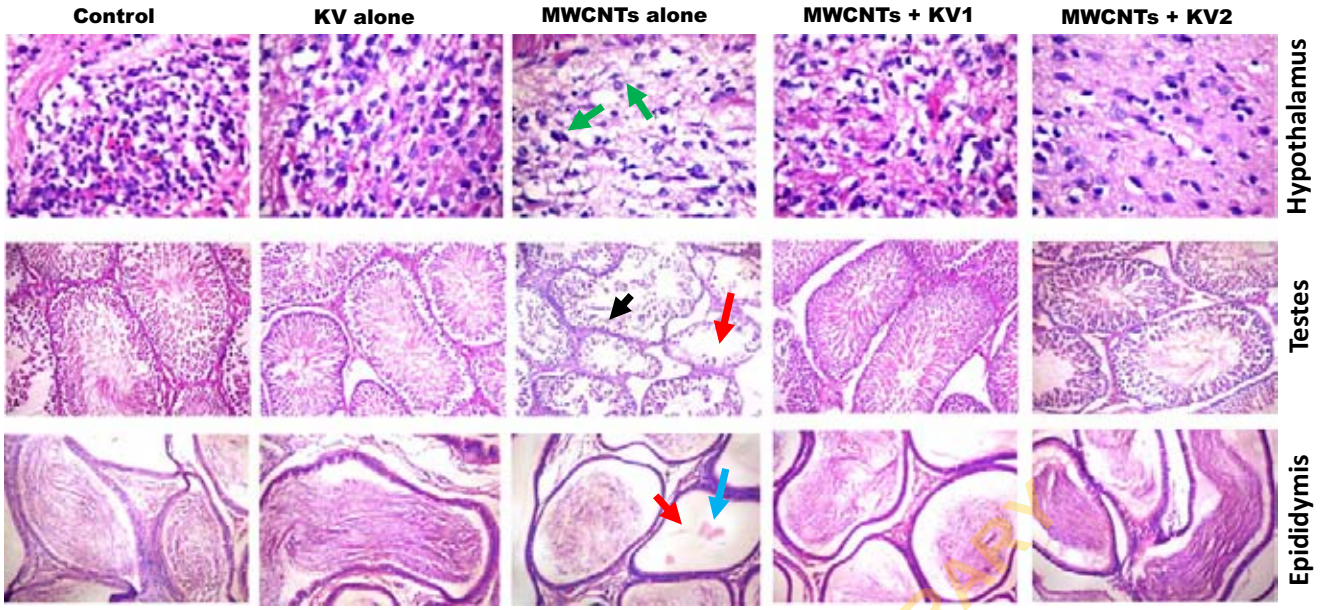

Fig. 10 Representative images of the hypothalamus, testes, and epididymis of experimental animals. Histology of the control and kolaviron alone–treated rats is normal. MWCNTs alone–exposed rats showing severe neuronal degeneration characterized by necrosis (green arrows) in the hypothalamus, the seminiferous tubules of the testes showing severe atrophy (black arrow) and vacuolation (red arrow) whereas the epididymis exhibited diminished epididymal lining (red arrow) with very few sperm cells in the lumen (blue arrow). The testes, epididymis, and hypothalamus of rats co-treated with MWCNTs and kolaviron at 50 and 100 mg/kg showed normal histoarchitecture comparable with the control. Mag × 400

tissues of rats exposed to MWCNTs alone in the present investigation demonstrated that MWCNTs elicited RONS production which surpassed the antioxidant capacity. This resulted in oxidative damage in the testes, epididymis, and hypothalamus in the treated animals. However, the reduced levels of RONS and MDA with concurrent increase in the antioxidant status of MWCNTs-exposed rats co-treated with kolaviron suggest the antioxidant and anti-lipid peroxidative effects of kolaviron in moderating oxidative damage in the animals.

TNF-α is well known to regulate cytokine production during inflammatory response that involves activation of inducible nitric oxide (iNOS) synthase and production of NO in the cell. Elevated cellular NO level causes nitrosative stress which modifies cellular lipids, proteins, and nucleic acids following depletion of antioxidant defense systems. Besides, activation of MPO results in the production of hypochlorous acid and other ROS with damaging effect on tissues (Anatoliotakis et al. 2013). Hence, increase in the testicular, epididymal, and hypothalamic activity of MPO and levels of TNF-α and NO in MWCNTs-exposed rats suggest nitrosative stress and inflammation in the animals. Hence, the marked reduction in these parameters in rats co-administered with MWCNTs and kolaviron indicates the anti-inflammatory mechanism of kolaviron in abating MWCNTs-induced reproductive toxicity.

The biochemical data on the protective effects of kolaviron on MWCNTs-associated reproductive toxicity were well supported by the histopathological findings. The testes, epididymis, and hypothalamus of rats administered MWCNTs per se exhibited treatment-related histopathological alterations which may be associated with the diminished antioxidative capacity and oxido-inflammatory damage in these tissues. Incessant LPO reportedly alters the structures and functions of the reproductive tissues (Adedara et al. 2015). However, the improvement in the histological architecture of these tissues when rats were co-administered with MWCNTs and kolaviron demonstrates the ameliorative potential of kolaviron.

In conclusion, excessive exposure to MWCNTs occasioned dysfunctional hypothalamic-pituitary-gonadal axis in male rats. Kolaviron inhibits MWCNTs-mediated reproductive toxicity in rats by mechanisms relating to subdual of RONS, LPO, and inflammation along with restoration of redox status, endocrine function, and sperm production.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

References

Adaramoye OA, Lawal SO (2014) Effect of kolaviron, a biflavonoid complex from *Garcinia kola* seeds, on the antioxidant, hormonal and spermatogenic indices of diabetic male rats. Andrologia 46:878–886

Adedara IA, Vaithinathan S, Jubendradass R, Mathur PP, Farombi EO (2013) Kolaviron prevents carbendazim-induced steroidogenic dysfunction and apoptosis in testes of rats. Environ Toxicol Pharmacol 35:444–453

Adedara IA, Ebokaiwe AP, Mathur PP, Farombi EO (2014) Nigerian bonny light crude oil induces endocrine disruption in male rats. Drug Chem Toxicol 37:198–203

Adedara IA, Owoeye O, Aiyegbusi MA, Dagunduro JO, Daramola YM, Farombi EO (2015) Kolaviron protects against benzo[a]pyrene-induced functional alterations along the brain-pituitary-gonadal axis in male rats. Environ Toxicol Pharmacol 40:459–470

Adedara IA, Abolaji AO, Rocha JBT, Farombi EO (2016) 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, 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

Adedara IA, Okpara ES, Busari EO, Omole O, Owumi SE, Farombi EO (2019) Dietary protocatechuic acid abrogates male reproductive dysfunction in streptozotocin-induced diabetic rats via suppression of oxidative damage, inflammation and caspase-3 activity. Eur J Pharmacol 849:30–42

Adedara IA, Awogbindin IO, Owoeye O, Maduako IC, Ajeleti AO, Owumi SE, Patlolla AK, Farombi EO (2020) Kolaviron via anti-inflammatory and redox regulatory mechanisms abates multi-walled carbon nanotubes-induced neurobehavioral deficits in rats. Psychopharmacology 237:1027–1040

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 40:777–787

Anatoliotakis N, Deftereos S, Bouras G, Giannopoulos G, Tsounis D, Angelidis C, Kaoukis A, Stefanadis C (2013) Myeloperoxidase: expressing inflammation and oxidative stress in cardiovascular disease. Curr Top Med Chem 13:115–1138

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

Baskaran S, Finelli R, Agarwal A, Henkel R (2020) Reactive oxygen species in male reproduction: a boon or a bane? Andrologia: e13577. https://doi.org/10.1111/and.13577

Blazak WF, Trienen KA, Juniewicz PE (1993) Application of testicular sperm head counts in the assessment of male reproductive toxicity. In: Chapin RE, Heindel J. (eds), Methods in Toxiciology, vol 3A., Male reproductive toxicology Academic Press, San Diego, pp 86–94

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

Chauhan A, Sillu D, Agnihotri S (2019) Removal of pharmaceutical contaminants in wastewater using nanomaterials: a comprehensive review. Curr Drug Metab 20:483–505

Chowdhury M, Kabir SN, Pal AK, Pakrashi A (1983) Modulation of luteinizing hormone receptors: effect of an inhibitor of prolactin secretion, p-coumaric acid. J Endocrinol 98:307–311

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

Clavijo RI, Hsiao W (2018) Update on male reproductive endocrinology. Transl Androl Urol 7(Suppl 3):S367–S372

Ding T, Li W, Li J (2019) Influence of multi-walled carbon nanotubes on the toxicity and removal of carbamazepine in diatom *Navicula sp*. Sci Total Environ 697:134104. https://doi.org/10.1016/j.scitotenv.2019.134104

Ema M, Gamo M, Honda K (2016a) Developmental toxicity of engineered nanomaterials in rodents. Toxicol Appl Pharmacol 299:47–52

Ema M, Hougaard KS, Kishimoto A, Honda K (2016b) Reproductive and developmental toxicity of carbon-based nanomaterials: a literature review. Nanotoxicology 10:391–412

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 32:667–674

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, Oyenihi AB, Ekakitie E, Kehinde S (2013) Hepatic, testicular and spermatozoa antioxidant status in rats chronically treated with *Garcinia kola* seed. J Ethnopharmacol 146:536–542

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

Fischer S, Ehlert U, Amiel CR (2019) Hormones of the hypothalamic-pituitary-gonadal (HPG) axis in male depressive disorders - a systematic review and meta-analysis. Front Neuroendocrinol 55:100792. https://doi.org/10.1016/j.yfrne.2019.100792

Fukushima S, Kasai T, Umeda Y, Ohnishi M, Sasaki T, Matsumoto M (2018) Carcinogenicity of multi-walled carbon nanotubes: challenging issue on hazard assessment. J Occup Health 60:10–30

Ganzoury MA, Chidiac C, Kurtz J, de Lannoy CF (2020) CNT-sorbents for heavy metals: electrochemical regeneration and closed-loop recycling. J Hazard Mater 393:122432. https://doi.org/10.1016/j.jhazmat.2020.122432

Ghanbari F, Nasarzadeh P, Seydi E, Ghasemi A, Taghi Joghataei M, Ashtari K, Akbari M (2017) Mitochondrial oxidative stress and dysfunction induced by single- and multiwall carbon nanotubes: a comparative study. J Biomed Mater Res A 105:2047–2055

Granell S, Gironella M, Bulbena O, Panés J, Mauri M, Sabater L, Aparisi L, Gelpí 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 [15 N] nitrate in biological fluids. Anal Biochem 126:131–138

Guerriero G, Trocchia S, Abdel-Gawad FK, Ciarcia G (2014) Roles of reactive oxygen species in the spermatogenesis regulation. Front Endocrinol (Lausanne) 5:56. https://doi.org/10.3389/fendo.2014.00056

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 multiwalled carbon nanotubes in C6 rat glioma cells. Neurotoxicology 33:1128–1134

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

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

Kehinde A, Adefisan A, Adebayo O, Adaramoye O (2016) Biflavonoid fraction from Garcinia kola seed ameliorates hormonal imbalance and testicular oxidative damage by anti-tuberculosis drugs in Wistar rats. J Basic Clin Physiol Pharmacol 27:393–401

Klumpp C, Kostarelos K, Prato M, Bianco A (2006) Functionalized carbon nanotubes as emerging nanovectors for the delivery of therapeutics. Biochim Biophys Acta 1758:404–412

Kobayashi N, Izumi H, Morimoto Y (2017) Review of toxicity studies of carbon nanotubes. J Occup Health 59:394–407

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

Li J, Ying GG, Jones KC, Martin FL (2015) Real-world carbon nanoparticle exposures induce brain and gonadal alterations in zebrafish (*Danio rerio*) as determined by biospectroscopy techniques. Analyst. 140:2687–2695

Malymy M, Horecker BL (1966) Alkaline phosphatase. In: Methods in Enzymology Volume IX. Academy Press, New York, pp 639–642

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

Morris PL, Saxena BB (1980) Dose- and age-dependent effects of prolactin (PRL) on luteinizing hormone- and PRL-binding sites in rat leydig cell homogenates. Endocrinology 107:1639–1645

Ncibi MC, Sillanpää M (2017) Optimizing the removal of pharmaceutical drugs carbamazepine and dorzolamide from aqueous solutions using mesoporous activated carbons and multi-walled carbon nanotubes. J Mol Liq 238:379–388

Negri V, Pacheco-Torres J, Calle D, López-Larrubia P (2020) Carbon nanotubes in biomedicine. Top Curr Chem (Cham) 378:15. https://doi.org/10.1007/s41061-019-0278-8

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 9:351–361

Olayinka ET, Ore A (2014) Kolaviron and L-ascorbic acid attenuate chlorambucil-induced testicular oxidative stress in rats. J Toxicol 2014:587015–587019. https://doi.org/10.1155/2014/587015

Patel DK, Kim HB, Dutta SD, Ganguly K, Lim KT (2020) Carbon nanotubes-based nanomaterials and their agricultural and biotechnological applications. Materials (Basel) 13(7):E1679. https://doi.org/10.3390/ma13071679

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(1–2): 189–199

Peruquetti RL, Taboga SR, Azeredo-Oliveira MT (2010) Expression of acid phosphatase in the seminiferous epithelium of vertebrates. Genet Mol Res 9:620–628

Ralebona N, Sewani-Rusike CR, Nkeh-Chungag BN (2012) Effects of ethanolic extract of *Garcinia kola* on sexual behavior and sperm parameters in male Wistar rats. Afr J Pharm Pharmacol 6:1077–1082

Rasras S, Kalantari H, Rezaei M, Dehghani MA, Zeidooni L, Alikarami K, Dehghani F, Alboghobeish S (2019) Single-walled and multiwalled carbon nanotubes induce oxidative stress in isolated rat brain mitochondria. Toxicol Ind Health 35:497–506

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

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

Salihu M, Ajayi BO, Adedara IA, Farombi EO (2017) 6-Gingerol-rich fraction prevents disruption of histomorphometry and marker enzymes of testicular function in carbendazim-treated rats. Andrologia 49(10). https://doi.org/10.1111/and.12782

Sewani-Rusike CR, Ralebona N, Nkeh-Chungag BN (2016) Dose- and time-dependent effects of Garcinia kola seed extract on sexual behaviour and reproductive parameters in male Wistar rats. Andrologia. 48:300–307

Shimada T, Nomura M, Yokogawa K, Endo Y, Sasaki T, Miyamoto K, Yonemura Y (2005) Pharmacokinetic advantage of intraperitoneal injection of docetaxel in the treatment for peritoneal dissemination of cancer in mice. J Pharm Pharmacol 57:177–181

Vanha-Perttula T, Nikkanen V (1973) Acid phosphatases of the rat testis in experimental conditions. Acta Endocrinol 72:376–390

Vassault A (1983) Lactate dehydrogenase. UV-method with pyruvate and NADH. In: Bergmeyer HU (ed) Methods of enzymatic analysis (3rd ed). Volume III. Plenum, New York, pp 118–125

Wang R, Song B, Wu J, Zhang Y, Chen A, Shao L (2018) Potential adverse effects of nanoparticles on the reproductive system. Int J Nanomedicine 13:8487–8506

Wells ME, Awa OA (1970) New technique for assessing acrosomal characteristics of spermatozoa. J Diary Sci 53:227–232

World health organization (1999) Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th edn, vol 76. Cambridge University press, New York, pp 4–33

Xu C, Liu Q, Liu H, Zhang C, Shao W, Gu A (2016) Toxicological assessment of multi-walled carbon nanotubes in vitro: potential mitochondria effects on male reproductive cells. Oncotarget. 7:39270–39278

Zemjanis R (1970) Collection and evaluation of semen. In: Zemjanis R (ed) Diagnostic and therapeutic technique in animal reproduction, 2nd edn. William and Wilkins company, Waverly Press, Inc, Baltimore, pp 139–153