Responses of Testis, Epididymis, and Sperm of Pubertal Rats Exposed to Functionalized Multiwalled Carbon Nanotubes

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ABSTRACT: The present study investigated the response of testes, epididymides and sperm in pubertal Wistar rats following exposure to 0, 0.25, 0.5, 0.75, and 1.0 mg kg^{-1} functionalized multi-walled carbon nanotubes (f-MWCNTs) for 5 days. The results showed that administration of (f-MWCNTs) significantly increased the activities of superoxide dismutase, catalase, and glutathione peroxidase in a dose-dependent manner in both testes and sperm compared with control group. Moreover, the significant decrease in the activity of glutathione-*S*-transferase and glutathione level was accompanied with significant elevation in the levels of hydrogen peroxide and malondialdehyde in both testes and sperm of (f-MWCNTs)-treated rats. The spermiogram of (f-MWCNTs)-treated rats indicated significant decrease in epididymal sperm number, sperm progressive motility, testicular sperm number and daily sperm production with elevated sperm abnormalities when compared with the control. Exposure to (f-MWCNTs) decreased plasma testosterone level and produced marked morphological changes including decreased geminal epithelium, edema, congestion, reduced spermatogenic cells and focal areas of tubular degeneration in the testes. The lumen of the epididymides contained reduced sperm cells and there was mild to severe hyperplasia epithelial cells lining the duct of the epididymis. Collectively, pubertal exposure of male rats to (f-MWCNTs) elicited oxidative stress response resulting in marked testicular and epididymides dysfunction. © 2014 Wiley Periodicals, Inc. Environ Toxicol 31: 543–551, 2016.

Keywords: functionalized multiwalled carbon nanotubes; testicular toxicity; oxidative stress; nanotoxicology; rats

INTRODUCTION

Carbon nanotubes (CNTs) have drawn massive industrial interest owing to their distinct properties which make them suitable for many applications in medicine, engineering and daily consumable products (Bianco et al., 2005; Becheri et al., 2008). Although nanotechnology offers enormous potential societal benefits, there are also potential risks associated with these novel therapeutic strategies (Bonner, 2011). To realize the safety of nanotechnology in medicine, a concerted multidisciplinary approach from engineering and toxicology would be required (Bonner, 2011). The toxicity of nanoproducts with regard to human and environment remains an area of intense research focus (Bonner et al., 2013).

Carbon nanotubes exist in two forms namely the single-walled (SWCNTs) and the multi-walled (MWCNTs). Different investigations assessing CNTs toxicities have been carried

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out using mouse, earthworm, rat and plant models (Miralles et al., 2012; Hougaard et al., 2013; Hu et al., 2014). Numerous studies have demonstrated the toxic effects of CNTs in the liver, lungs and kidney using different routes of administration including inhalation, intra-tracheal instillation and intra-peritoneal (Bonner 2010; Jain et al., 2011; van Berlo et al., 2014). The MWCNTs have received considerable attention due to their similarities to harmful asbestos fibers (Bonner, 2011, van Berlo et al., 2014). Despite extensive studies of MWCNTs toxicity, relatively little information is available concerning the reproductive effects in animals.

Owing to the increasing rate of applications in medicine and the widespread availability of MWCNTs in the environment, its safety on the mammalian reproductive system should not be assumed. A report on the effects of lung exposure to carbon nanotubes on female fertility and pregnancy in mice showed that instillation of a single dose of MWCNT caused pathological changes in lungs and livers, resulted in a slight delay in the delivery of a first litter without interfering with the behavior and sperm quality in male offspring (Hougaard et al., 2013). The mammalian testes are responsible for the steroidogenesis and good quality sperm production essential for reproductive success (Adedara et al., 2013). Carbon nanotubes were reported to generate reactive oxygen species (ROS) in liver, kidney and lung of experimental animals (Jain et al., 2011). The testis is vulnerable to ROS generated from a variety of therapeutic agents and environmental contaminants. Hence, the present study investigated the response of male reproductive system to MWCNTs by assessing the spermatogenic indices, testosterone level, biomarkers of oxidative stress and histology using a dose response exposure paradigm in pubertal Wistar rats.

MATERIALS AND METHODS

Chemicals

Multi-walled carbon nanotubes (MWCNTs) was a gift from Professor Anita K. Patlolla of the Molecular Toxicology Research Laboratory, NIH-RCMI Center for Environmental Health, CSET, Jackson State University, Jackson, MS. Epinephrine, hydrogen peroxide, 5',5'-dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), 1-chloro-2,4-dinitrobenzene (CDNB), tricholoroacetic acid (TCA), reduced glutathione (GSH) and bovine serum albumin (BSA) were obtained from Sigma Chemical (St Louis, MO). All other reagents were of analytic grade and were purchased from the British Drug Houses (Poole, Dorset, UK).

Multiwalled CNTs Characteristics

Multi-walled carbon nanotubes (MWCNTs) were synthesized by NanoLab Inc. (Newton, MA) using catalytic chemical vapor deposition (Patlolla et al., 2011). Subsequently, MWCNTs were heated under argon (2 L min^{-1}) to 2000°C at the rate of 10°C min^{-1} to extract catalyst (Fe-impurities). Functionalization of the MWCNTs (purity > 95%) surfaces was achieved by subjecting the MWCNTs to a reflux process in sulfuric/nitric acid (3:1). The resulting carboxylated nanotubes after functionalization have 2–7% COOH by weight. MWCNTs were dispersed in 1% tween-80 and sterile saline with the help of physical mixing and ultrasonication. At the end of functionalization, the diameter and length of the longer CNTs (60 min of sonication) were 11.5 nm and 12 μm, respectively (Fig. 1).

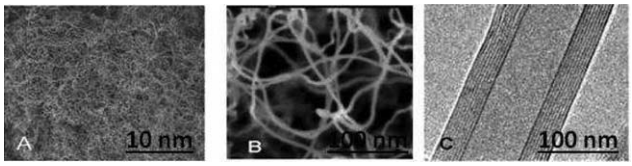

Fig. 1. Transmission electron microscope (TEM) photographs of functionalized carbon nanotubes: A. low magnification, B: high magnification C: Inside multiwall nature of the carbon nanotube (10 nm inner diameter, 9 concentric walls, and a clear inner channel).

Animal Model and Experimental Design

Fifty healthy pubertal male Wistar rats (8-weeks old; 165 ± 8 g) used in the present study were obtained from the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. They were kept in plastic cages placed in a well-ventilated rat house, provided with rat pellets and water *ad libitum*, and subjected to photoperiod of 12-h light/12-h dark. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institute of Health. The experiment was performed according to the guidelines and approval of institutional animal ethics committee. The animals were randomly assigned to five groups of ten animals each.

The choice of rats for the present study is because the male Wistar rat reaches the pubertal age when the plasma testosterone increases and spermatogenesis starts. At this stage, the testicular weight and sperm production per gram of testis continue to increase till adult age (Robb et al., 1987; Zanato et al., 1994). (f-MWCNTs) suspension (hydrodynamic diameter of ≈1 μm) was administered intraperitoneally to animals at the doses of 0.25, 0.5, 0.75, and 1.0 mg/kg/day for 5 days. Corresponding group of rats received saline plus 1% tween-80 in the same manner as in the treatment groups and served as control. Twenty-four hours following the last treatment, the blood from each group was drawn from retro-orbital venous plexus for testosterone determination before they were sacrificed by cervical dislocation. The testes and epididymides were quickly excised, weighed and kept frozen. The body weights of rats were recorded before exposure to various treatments and again before they were sacrificed.

Determination of Sperm Motility and Count

The progressive motility of the sperm from the control and (f-MWCNTs) -treated rats was evaluated according to the method of Zemjanis (1970). Briefly, cauda epididymis was cut with surgical blades and the sperm released onto a sterile clean glass slide. The sperm was subsequently diluted with 2.9% sodium citrate dehydrate solution and carefully mixed to assess the sperm progressive motility with the aid of a microscope within 2–4 min of their isolation and data expressed as percentages. Epididymal sperm count was obtained by mincing the caudal epididymis in distilled water and filtering through a nylon mesh. The sperm were counted by haemocytometer using the improved Neubauer (Deep 1/10 m; LABART, Munich, Germany) chamber according to Pant and Srivastava (2003).

Sperm Morphologic Abnormalities and Percentage Viability Assay

Sperm morphology and viability were evaluated according to the method of Wells and Awa (1970). Briefly, a portion of the sperm suspension placed on a glass slide was smeared out with another slide and stained with Wells and Awa's stain (0.2 g eosin along with 0.6 g fast green were dissolved in distilled water and ethanol in a 2:1 ratio) for morphologic examination. The sperm viability was determined using a stain containing 1% eosin and 5% nigrosine in 3% sodium citrate dehydrate solution. A total of 400 sperm from each rat were used for morphologic examination.

Daily Sperm Production and Testicular Sperm Number

Daily sperm production was determined using five frozen left testes from control and (f-MWCNTs) -treated rats according to Blazak et al. (1993). Briefly, the testis was decapsulated and homogenized in ice-cold physiologic saline containing 0.01% Triton X-100. Subsequently, aliquot of the resulting homogenate was transferred to a glass vial and stored on ice. Sample aliquots were then placed on the Neubauer haemocytometer and counted twice at 100× magnification under a microscope to determine the number of sperm heads (step 19 spermatid head). These values were used to obtain the total number of spermatids per testis, and this number was then divided by the testes weight to yield spermatids per gram of testes. The daily sperm production was calculated by dividing the number of spermatids at stage 19 with 6.1, which is the number of days of the seminiferous cycle in which these spermatids are present in the seminiferous epithelium.

Evaluation of Plasma Testosterone Concentrations

The plasma concentration of testosterone was assayed using the commercial enzyme immunoassay kit specific for rats according to the manufacturer's instructions [testosterone (EIA-5179)] from DRG Diagnostics. The sensitivity of the testosterone assay was 0.06 ng mL^{-1} and with negligible cross-reactivity with other androgen derivatives like androstenedione, methyl testosterone and 5α-dihydrotestosterone. The intra-assay coefficients of variation for the testosterone assay were 6.3%.

Collection of Epididymal Sperm

The sperm from the control and (f-MWCNTs)-treated rats were obtained according to Adedara and Farombi (2012). Briefly, sperm were collected immediately after each rat was sacrificed. The right cauda epididymis was placed in cold phosphate buffer saline solution and cut with surgical blades into pieces. The solution was pipetted several times to obtain the sperm suspension which was subsequently filtered through a nylon mesh. The sperm suspension was then homogenized at 4°C with a glass Teflon homogenizer for 10 s and centrifuged at 2000 × *g* for 10 min to obtain the supernatant which was used for biochemical assays.

Biochemical Assays

The right testes were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride. The resulting homogenate was centrifuged at 10,000 × *g* for 15 min at 4°C and the supernatant was thereafter collected for biochemical assays. Protein concentration was determined by the method of Lowry et al. (1951). The estimation of superoxide dismutase (SOD) activity was done according to the method described by Misra and Fridovich (1972). Catalase (CAT) activity was assayed using hydrogen peroxide as a substrate according to the method of Clairborne (1995). Glutathione-Stransferase (GST) was assayed by the method of Habig et al. (1974). Glutathione peroxidase (GPx) activity was determined according to the method of Rotruck et al. (1973). Reduced GSH was determined at 412 nm using the method described by Jollow et al. (1974). Hydrogen peroxide generation was assessed by the method of Wolff (1994). Lipid peroxidation was quantified as MDA according to the method described by Farombi et al. (2000) and expressed as μmole MDA/mg protein.

Histopathology of Testes and Epididymis

Biopsies of the testes and epididymis were carefully removed and fixed with Bouin's solution. After dehydration procedures, the samples were blocked in paraffin. Sections of 4–5 μm were prepared by a microtome and stained with hematoxylin and eosin (H & E). All slides were coded before examination with light microscope and photographed using a digital camera by investigators who were blinded to control and (f-MWCNTs)-treated groups.

TABLE I. Effects of MWCNTs on body weight gain, absolute and relative weights of testes and epididymis in rats

	Control	0.25 mg kg^{-1}	0.5 mg kg^{-1}	0.75 mg kg^{-1}	1.0 mg kg^{-1}
BWG	6.42 ± 0.13	5.52 ± 0.15	6.11 ± 0.11	5.28 ± 0.18	5.61 ± 0.13
Testes	2.13 ± 0.26	2.15 ± 0.29	2.14 ± 0.23	2.12 ± 0.22	2.11 ± 0.16
RTW	1.25 ± 0.13	1.28 ± 0.17	1.24 ± 0.16	1.21 ± 0.15	1.24 ± 0.07
Epididymis	0.16 ± 0.07	0.18 ± 0.02	0.17 ± 0.02	0.19 ± 0.05	0.15 ± 0.04
REW	0.08 ± 0.04	0.08 ± 0.01	0.07 ± 0.02	0.09 ± 0.03	0.07 ± 0.02

BWG, Body weight gain; RTW, Relative testes weight; REW, Relative epididymides weight. The data are expressed as mean ± SD for ten rats per group.

Statistical Analysis

Statistical analyses were carried out using one-way analysis of variance, followed by Bonferroni's test to identify significantly different groups (SPSS for Windows, version 17). Values of $p < 0.05$ were considered significant.

RESULTS

Effect of (f-MWCNTs) on Body Weight Gain and Organ Weights in Rats

The body weight gain, absolute and relative weights of the organs of control and (f-MWCNTs)-treated rats are presented in Table I. Acute exposure to (f-MWCNTs) produced no effect on the body weight gain as well as on the absolute and relative weights of testes and epididymis when compared with the control.

Effect of MWCNTs on Antioxidant Systems in Testes and Sperm Rats

The activities of antioxidant enzymes, GSH, H_2O_2 and MDA levels in the testes and sperm of rats during acute exposure to (f-MWCNTs) are presented in Figures 2–4. Exposure to (f-MWCNTs) for 5 days resulted in a significant increase in the activities of SOD, CAT, and GPx but decreased the activity of GST and GSH level in the testes and sperm when compared with control group. Moreover, (f-MWCNTs) significantly increased the levels of H_2O_2 and MDA in the testes and sperm of the experimental rats above the control. There were no treatment-related effects on antioxidant system in both testes and sperm at 0.25 mg kg^{-1} (f-MWCNTs).

Effects of MWCNTs on Sperm Functional Characteristics and Testosterone Level in Rats

Effects of (f-MWCNTs) on sperm functional characteristics after the treatment period are shown in Table II. The animals treated with purified (f-MWCNTs) demonstrated significant decrease in epididymal sperm number (ESN), sperm progressive motility but increased sperm abnormality significantly when compared with the control. The viability of the sperm remains unaffected. The major abnormalities seen in sperm of (f-MWCNTs)-treated rats were tailless heads, curved mid-pieces and bent tails whereas rudimentary tails occurred less frequently. There was a significant decrease

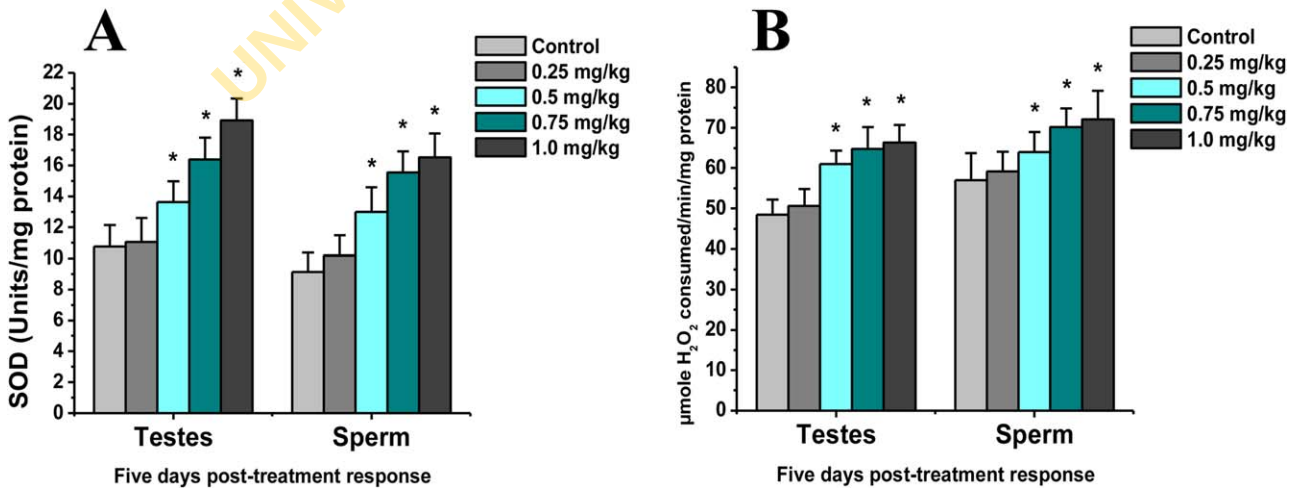

Fig. 2. Effect of (f-MWCNTs) on SOD and CAT activities in testes and sperm of rats after 5 days. Each bar represents mean ± SD of 10 rats. Values with asterisks were significantly different from control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

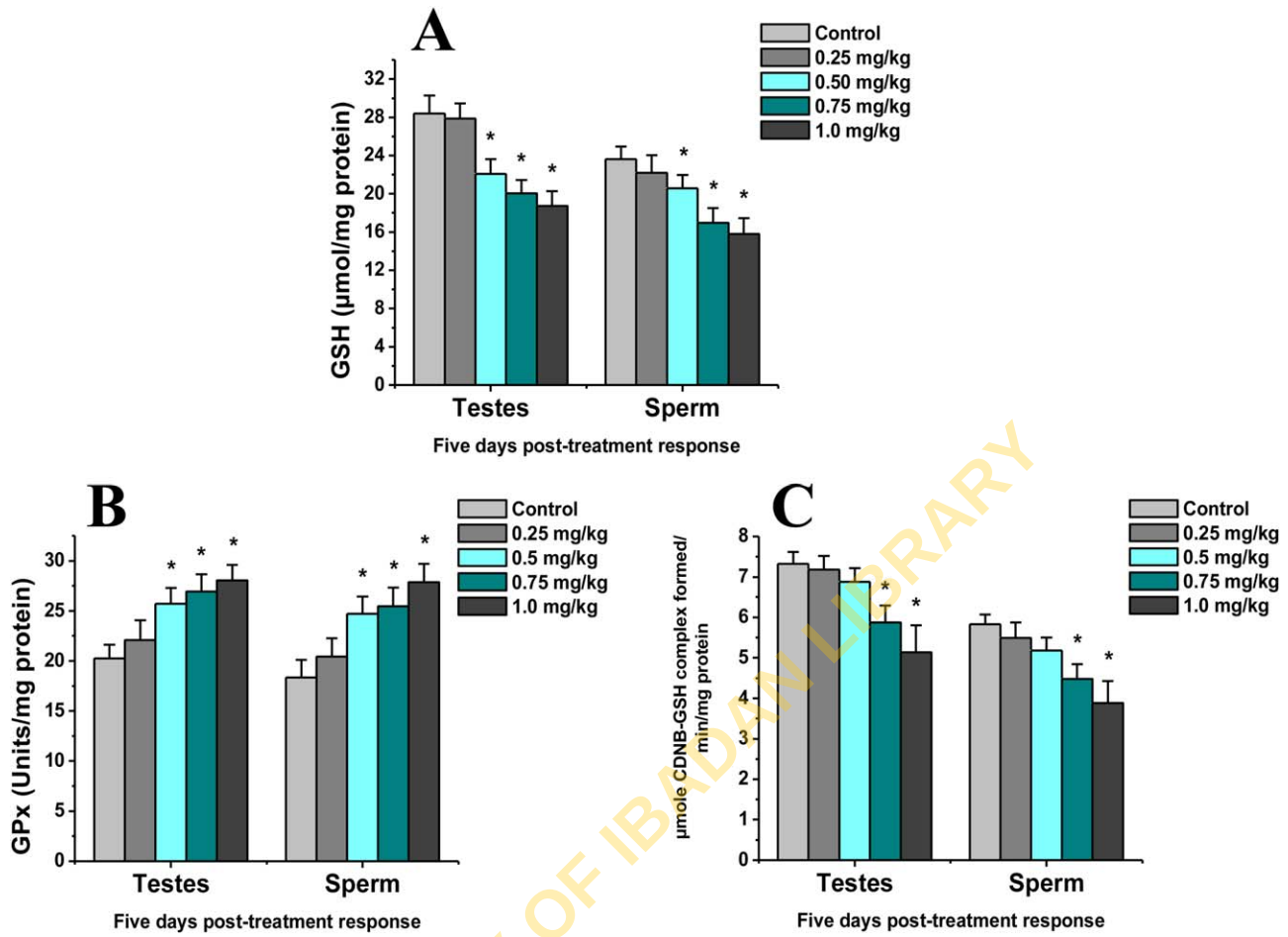

Fig. 3. Effect of (f-MWCNTs) on GSH level and activities of GPx and GST in testes and sperm of rats after 5 days. Each bar represents mean ± SD of 10 rats. Values with asterisks were significantly different from control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

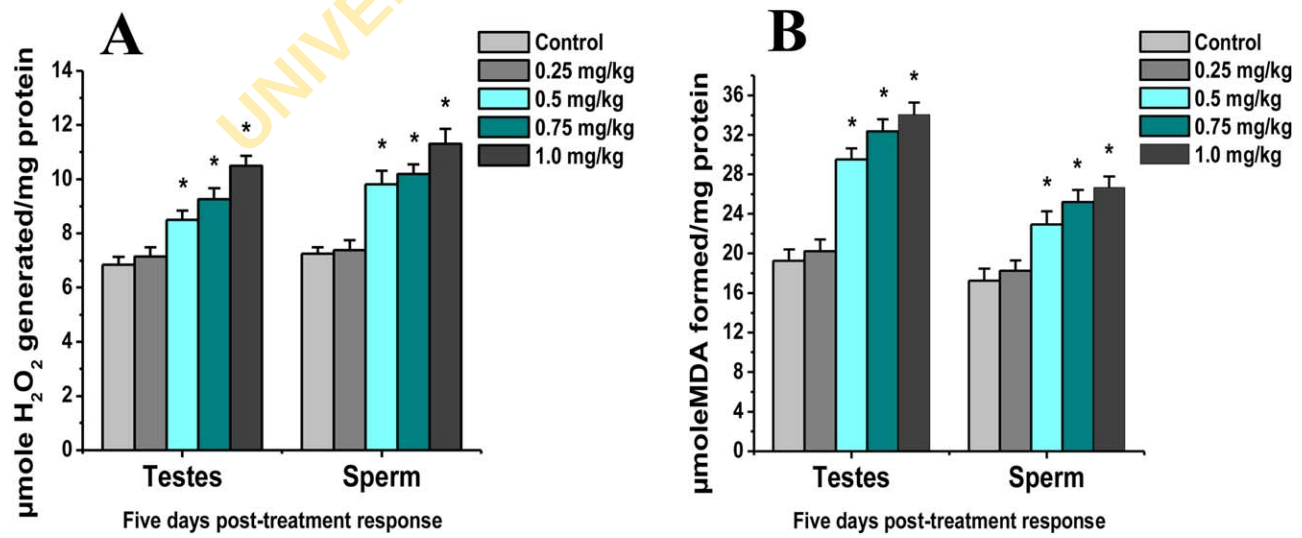

Fig. 4. Effect of (f-MWCNTs) on H_2O_2 and LPO levels in testes and sperm of rats after 5 days. Each bar represents mean ± SD of 10 rats. Values with asterisks were significantly different from control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II. Effects of MWCNTs on sperm functional parameters and plasma testosterone level in rats

	Control	0.25 mg kg^{-1}	0.5 mg kg^{-1}	0.75 mg kg^{-1}	1.0 mg kg^{-1}
ESN (%)	95.74 ± 5.06	90.75 ± 8.92	59.76 ± 9.61*	58.81 ± 8.04*	56.50 ± 8.34*
Motility (%)	92.20 ± 4.74	87.46 ± 8.94*	68.32 ± 8.37*	67.72 ± 8.37*	62.18 ± 8.37*
Viability (%)	96.80 ± 3.64	94.16 ± 2.88	96.21 ± 2.64	96.28 ± 3.64	95.06 ± 3.70
Abnormalities (%)	10.82 ± 0.74	11.02 ± 0.80	12.42 ± 0.68*	13.80 ± 0.57*	14.97 ± 0.86*
TSN (10^6 /g testes)	28.46 ± 2.14	27.55 ± 2.12	25.58 ± 3.06	23.85 ± 2.67*	20.51 ± 3.26*
DSP (10^6 /g testes)	4.11 ± 0.49	4.20 ± 0.54	3.98 ± 0.50	3.34 ± 0.41*	3.28 ± 0.41*
Testosterone (ng mL^{-1})	3.70 ± 0.84	3.50 ± 0.52	2.80 ± 0.27*	2.20 ± 0.27*	2.50 ± 0.27*

The data are expressed as mean ± SD for 10 rats per group. *$p < 0.05$ against control.

observed in the daily sperm production and testicular sperm number in rats treated with 0.75 and 1.0 mg kg^{-1} (f-MWCNTs) when compared with the control. The circulatory concentrations of testosterone in (f-MWCNTs)-treated rats were significantly decreased when compared with the control. The treatment-related effect of 0.25 mg kg^{-1} (f-MWCNTs) was observed on sperm progressive motility alone.

Histopathological Alterations in Testes and Epididymis of (f-MWCNTs)-treated Rats

Figures 5 and 6 show the representative photomicrographs of testes and epididymis of the control and the (f-MWCNTs)-treated rats. Control and 0.25 mg kg^{-1} (f-MWCNTs) -treated testes showed normal seminiferous tubules, the seminiferous epithelium consists of spermatogonia, spermatocytes, spermatids, spermatozoa sertoli cells while the interstitia have the leydig cells. There is active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated sperm cells. The Sertoli cells are also seen to be undergoing mitosis (A and B). The 0.50 mg kg^{-1} (f-MWCNTs)-treated testes showed decreased geminal epithelium with few terminally differentiated sperm cells (C). The 0.75 mg kg^{-1} (f-MWCNTs)-treated testes showed mild interstitial congestion, oedema and few strata of maturation along the epithelium (D). The 1.0 mg kg^{-1} (f-MWCNTs)-treated testes showed focal area of tubular degeneration with few sperm cells (E).

Control and 0.25 mg kg^{-1} (f-MWCNTs)-treated epididymis showed normal epithelial cells lining the duct of epididymis. The lumen contains adequate number of sperm

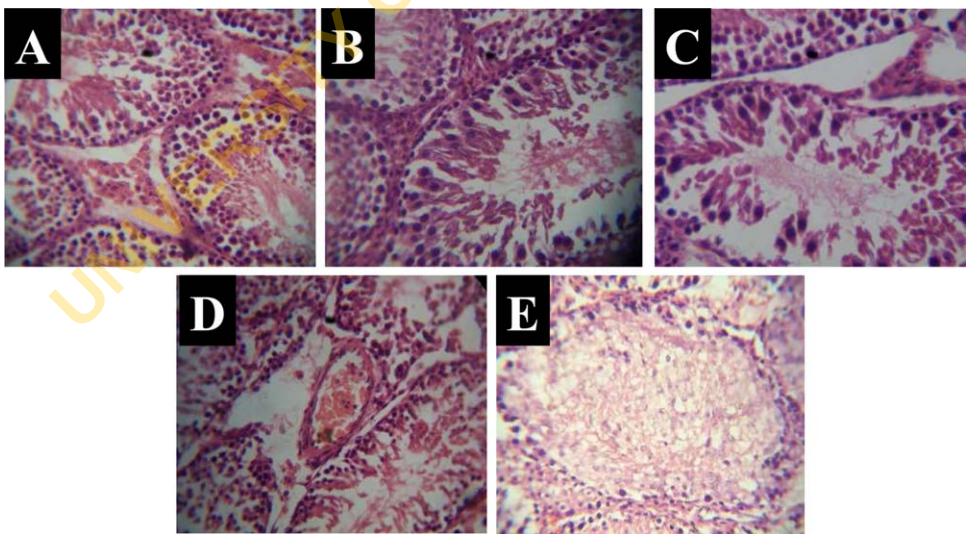

Fig. 5. Histopathology guide of testes from control and (f-MWCNTs) -treated rats. Control and 0.25 mg kg^{-1} (f-MWCNTs) -treated rats showing normal seminiferous tubules, the seminiferous epithelium consists of spermatogonia, spermatocytes, spermatids, spermatozoa sertoli cells while the interstitia have the leydig cells. There is active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated sperm cells. The Sertoli cells are also seen to be undergoing mitosis (A and B). Testes from 0.50 mg kg^{-1} (f-MWCNTs) -treated rats showing decreased geminal epithelium with few terminally differentiated sperm cells (C). Testes from 0.75 mg kg^{-1} (f-MWCNTs) -treated rats showing mild interstitial congestion, oedema and few strata of maturation along the epithelium (D). Testes from 1.0 mg kg^{-1} (f-MWCNTs) -treated rats showing focal area of tubular degeneration with few sperm cells (E). ×160. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

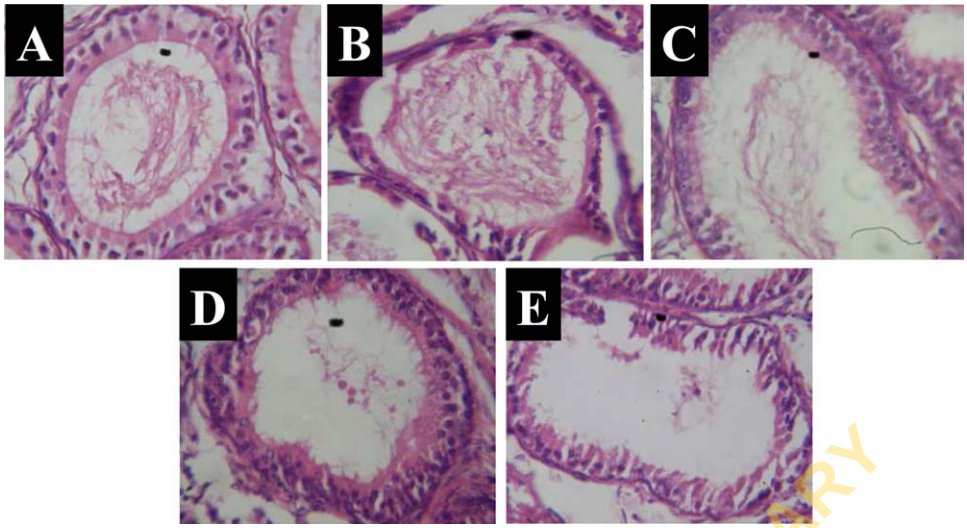

Fig. 6. Histopathology guide of epididymides from control and (f-MWCNTs) -treated rats. Control and 0.25 mg kg^{-1} (f-MWCNTs) -treated rats showing normal epithelial cells lining the duct of epididymis. The lumen contains adequate number of sperm (A and B). Epididymis from 0.50 mg kg^{-1} (f-MWCNTs) -treated rats showing normal epithelial cells lining the duct of epididymis with the lumen containing few sperm (C). Epididymis from 0.75 mg kg^{-1} (f-MWCNTs) -treated showing mild hyperplasia of epithelial cells lining the duct of epididymis with the lumen containing scanty sperm (D). Epididymis from 1.0 mg kg^{-1} (f-MWCNTs) -treated showing severe hyperplasia of epithelial cells lining the duct of epididymis with the lumen containing very scanty sperm (E). ×160. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(A and B). The 0.50 mg kg^{-1} (f-MWCNTs)-treated epididymis showed normal epithelial cells lining the duct of epididymis with the lumen containing few sperm (C). The 0.75 mg kg^{-1} (f-MWCNTs)-treated epididymis showed mild hyperplasia of epithelial cells lining the duct of epididymis with the lumen containing scanty sperm (D). The 1.0 mg kg^{-1} (f-MWCNTs) -treated epididymis showed severe hyperplasia of epithelial cells lining the duct of epididymis with the lumen containing very scanty sperm (E).

DISCUSSION

Nanomaterials may affect reproduction and development via direct and indirect means (Hougaard et al., 2011; Hougaard and Campagnolo, 2012). Certain nanoparticles reportedly can penetrate human tissues through digestive system and accumulate in reproductive system including testis (Borm and Kreyling, 2004). Nanomaterials is known to affect tissue or disrupt with physiological processes by generation of ROS (Patlolla et al., 2011; Awasthi et al., 2013). The antioxidant defenses comprising the enzymatic and non-enzymatic are particularly essential because they are responsible for the direct removal of free radicals, thus providing protection for biological tissues including testes (Adedara and Farombi, 2010).

The present study demonstrated that pubertal exposure of rats to (f-MWCNTs) had adverse effects on the antioxidant status of the testes and sperm. The first mechanisms of antioxidant defense against the toxic effects of ROS in the tissues are carried out by SOD and CAT. The conversion of superoxide radicals to H_2O_2 is executed by SOD whereas the CAT subsequently decomposes H_2O_2 into water and oxygen. The increased SOD activity observed in the (f-MWCNTs) -treated rats possibly indicates enzyme stimulation due to high levels of the superoxide anion radical in the testes and sperm of the rats. Moreover, the increase in CAT and GPx activities suggests an adaptive mechanism to reduce the toxic effects of elevated levels of H_2O_2 in the testes and sperm of (f-MWCNTs) -treated rats. An increase in CAT activity has been correlated with the overproduction of H_2O_2 under stress conditions (Islam et al., 2014). The GSH level and GST activity decreased significantly in the testes and sperm of (f-MWCNTs) -treated rats in the present study. The diminution in the GSH level suggests overutilization in the detoxification process so as to cope with oxidative stress. The decrease in GST activity may result from decrease substrate GSH or inhibition by increased free radicals in the testes and sperm of (f-MWCNTs) -treated rats. The damages due to oxidative stress in testes and sperm by (f-MWCNTs) was evidenced by the elevated levels of H_2O_2 and MDA in the experimental rats.

The present investigation demonstrated that exposure to (f-MWCNTs) produced marked morphological changes in the testes and epididymides of the experimental rats. The testicular alterations include decreased geminal epithelium, edema, congestion, reduced spermatogenic cells and focal areas of tubular degeneration. Similarly, (f-MWCNTs) -treated rats showed mild to severe hyperplasia epithelial cells lining the duct of epididymis with the lumen containing very

scanty sperm. The degree of degeneration in these tissues is (f-MWCNTs) concentration dependent. The morphological changes in the testes and epididymides in the present study may be associated with the induction of lipid peroxidation, a chemical mechanism capable of disrupting the structure and function of the organ (Adedara and Farombi, 2012). Leydig cells are predominantly responsible for the biosynthesis and secretion of testosterone which supports sperm production and regulates the male phenotype (Hancock et al., 2009; Sherrill et al., 2010). The reduction in the plasma concentration of testosterone may result from changes in testes histology of the (f-MWCNTs) -treated rats. Low levels of testosterone adversely affect spermatogenesis and can lead to Sertoli cells dysfunction (Yoshida et al. 2009).

The body weight gain, absolute and relative weights of the testes and epididymides were not affected in the (f-MWCNTs) -treated rats. The lack of effect on the body weight gain indicates that the general metabolic functions are in the normal range in the experimental rats. However, with exemption of sperm viability, the sperm functional parameters namely, epididymal sperm number (ESN), sperm progressive motility, sperm abnormalities, testicular sperm number (TSN) and daily sperm production (DSP) were adversely affected in the (f-MWCNTs) -treated rats. The reduction in the TSN and DSP evidently implicates (f-MWCNTs) exposure in interfering with spermatogenesis in the experimental rats. The decrease in the ESN, sperm progressive motility and the elevated sperm abnormalities could be the outcome of altered and hostile internal environment of the epididymis due to ROS in the (f-MWCNTs) -treated rats. The frequent occurrence of curved mid-pieces and bent tails of the sperm in (f-MWCNTs) -treated rats could decrease sperm progressive motility because the mid-piece houses the mitochondria for energy (ATP) production while the sperm tail encompasses the structural components directly involved in motility (Paoli et al., 2011). Our data on sperm parameters are in contrast with the previous report on sperm quality in offspring from female mice exposed to carbon nanotubes via the lung (Hougaard et al., 2013). The differences in the data are related to the animal models, route of administration and experimental design.

In conclusion, our data showed that pubertal exposure of male rats to (f-MWCNTs) elicited oxidative stress response in the testes and sperm. To our knowledge, this is the first report describing the toxic effects of (f-MWCNTs) on the testes, epididymides and sperm in pubertal rats. The toxic effects of (f-MWCNTs) is associated with disruption of antioxidant enzyme activities, a subsequent increase in lipid peroxidation and marked testicular and epididymides dysfunction in the experimental rats. The increased response of testes, epididymides and sperm in pubertal Wistar rats to functionalized CNTs which are considered better suited for biological applications, may well be because they are better dispersed in aqueous solution and therefore reach higher concentrations of free CNTs at similar weight per volume values. Significantly, we find that the physical form of carbon has a major impact on toxicity, the molecular structure and topology is essential for the carbonaceous nanomaterials to cause adverse effects.

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