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ORIGINAL ARTICLE

Ameliorative effects of Garcinia hydroxybiflavanonol 1 (GB1) isolated from *Garcinia kola* seeds on cadmium chloride ($CdCl_2$) induced reproductive toxicity in the testis of the male Wistar rats

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Abstract

A high association between cadmium, a common environmental pollutant, and infertility in males has been established. Studies have established that antioxidants can enhance fertility either directly or indirectly. Garcinia hydroxybiflavanonol (GB1) extracts from bitter kola is known for its natural antioxidant properties. This study is aimed at investigating the ameliorative effects of administration of (GB1) on the reproductive health of cadmium chloride ($CdCl_2$)–intoxicated male Wistar rats. Extraction, fractionation and isolation of Garcinia hydroxybiflavanonol (GB 1) were done from seeds of *Garcinia kola*. Thirty-six male adult Wistar rats weighing 170–190 g were acclimatized for 2 weeks and randomly divided into three groups. Group A were given distilled water as control, group B 2.5 mg/kg b.w. of $CdCl_2$ and group C 2.5 mg/kg b.w. of $CdCl_2$ and 2 mg/kg b.w. of GB1 dissolved in Tween20. The rats were orally dosed daily for 90 days. Every 30 days, 4 animals from each group were euthanized and blood samples collected for testosterone, GnRH, LH and FSH assay. Sperm qualities were determined using the cauda epididymis. The neutral-buffered formalin-fixed testes were processed for histology. The result showed significantly higher levels of testosterone, GnRH, FSH, LH and sperm quality in the $CdCl_2$ + GB1–treated rats than in the $CdCl_2$-only-treated rats. Histopathology showed progressive distortion, disorganisation, erosion and vacuolation of the seminiferous epithelium in $CdCl_2$-only-treated rats which were not observed in the other groups. These findings indicate a protective effect of the GB1 extract on the testes of Wistar rats.

Keywords Cadmium · Testis · Garcinia hydroxybiflavanonol · Oxidative stress · Spermatozoa

Introduction

Globally, infertility has become a problem with the prevalence rate of 10.5% and is believed to be the second most prevalent health care issue in sub-Saharan Africa (Chinnoch 1996). Spermatogenesis as a process is an extremely active complex replicative process that generates approximately 1000 sperm a second (Aitken and Roman 2008). This complex series of spermatogenesis can be interfered by toxic chemicals, heavy metals, heat, radiation, deficiencies of hormones and immunodeficiency (Akunna et al. 2012, 2014; Khanna et al. 2016). Heavy metals such as lead, cadmium and uranium disrupt spermatogenesis by triggering oxidative stress through induction of lipid peroxidation, depletion of ROS scavengers and disruption of testicular antioxidant enzyme activity (Santos et al. 2004; Marchlewicz et al. 2007; Aitken and Roman 2008; Nna et al. 2017).

Our environment is highly polluted by toxic metals, especially lead and cadmium, and the effect of exposure to cadmium is of great concern in present day Nigeria (Anetor 2002). The increase in solid minerals and petroleum exploratory activities, usage, the attendant pollution and improper management of industrial and municipal refuse had contributed to cadmium pollution and introduction of this highly potential

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reproductive toxicant into the environment. Bioaccumulation of cadmium, a ubiquitous non-degradable environmental pollutant that enters the food chain, is an issue of severe global concern. The United Nations Environment Program listed cadmium along with other heavy metals, in her International Register of Potentially Toxic Chemicals (IRPTC 1995). Its environmental accumulation is due to its increased industrial usage in mining, electroplating, dyeing and paints, as well as its occurrence in pesticides and agricultural fertilizers (Newairy et al. 2007; Renugadevi and Prabu 2009). Most of the cadmium pollution can be traced to exposure wastes from mining activities, smelting and electroplating and intensive use of consumer products containing cadmium. It is mainly taken in by the body through the air, food and drinking water, in the absence of adverse habits such as tobacco and environmental exposure.

Cadmium is a highly toxic metal that can disrupt a number of biological systems, usually at doses that are much lower than most toxic metals (Jarup et al. 1998). Many long-term and short-term studies have demonstrated a wide spectrum of deleterious effects of cadmium exposure on the male reproductive system (Hew et al. 1993a; Lafuente et al. 2000; Aoyagi et al. 2002; Akinloye et al. 2006; Nair et al. 2015; Reddy et al. 2016; Uwagie-Ero et al. 2018). Akinloye et al. (2006) had established a high association between presence of cadmium in seminal vesicular plasma and decreased sperm quality in infertile couples in Nigeria. It has also been reported to be carcinogenic (Koyama et al. 2002) and causes histopathological damages to the male reproductive organs (Massanyi et al. 2007).

Many studies have shown that antioxidants can enhance fertility either directly or indirectly and that most plants rich in antioxidants have the tendency to increase sperm count and motility and enhance sperm viability and morphology (Oluyemi et al. 2007; Adesanya et al. 2007; Alabi et al. 2017). This had led to the administration of antioxidants to infertile males to improve their sperm quality (Aitken and Roman 2008). The biflavonoid extracts from bitter kola are known natural antioxidants and have been shown to improve negative effects of oxidative stress in lipids, proteins and DNA (Farombi et al. 2017). Garcinia hydroxybiflavanonol 1 (GB1) is a component of the biflavonoids with strong analgesic, anti-inflammatory and antipyretic effects (Madubunyi 2010; Nwaehujor et al. 2013, 2015). To the best of our knowledge, the antioxidant effects of GB1 extract from the kolaviron complex of *Garcinia kola* on reproductive functions have not been ascertained. Thus, more research work should be encouraged to ascertain the antioxidant effects of GB1 on the testis and reproductive potential of infertile males exposed to oxidative chemicals. The aim of the present study is to investigate the ameliorative effects of administration of GB1 on the reproductive health of cadmium chloride–intoxicated male Wistar rats.

Materials and methods

Garcinia kola seeds were purchased from the local market and characterised (Iwu et al. 1990). Extraction, fractionation of crude extract and isolation of Garcinia hydroxybiflavanonol-1 (GB1) were done as described by Nwaehujor et al. (2015). The dried *Garcinia kola* seeds were reduced to coarse powder and defatted with 3 L of n-hexane in a Soxhlet apparatus (Büchi, Switzerland) for 48 h. The n-hexane was distilled off to give a yellowish-brown oily sample. The fat-free sample was then extracted with 80% methanol for 72 h. The methanol was distilled off to give a methanol extract (brown sticky gum) which was then suspended in distilled water and subjected to liquid-liquid partitioning with ethyl acetate (EA) to give off the EA fraction. The pure compound of GB1 was isolated from the EA fraction using column and thin-layer chromatography, lyophilized and stored in the fridge at 4 °C until used for the experiments (Asuzu and Nwaehujor 2013).

Thirty-six (36) adult male Wistar rats weighing 170–190 g were obtained from the animal house of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria. They were acclimatized for 14 days and were allowed ad libitum access to feed and water. Experimental animals were kept in accordance with the guidelines for animal care as contained in the animal ethics handbook of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria.

The rats were randomly assigned to 1 of 3 groups ($n = 12$) as follows: A: control, B: $CdCl_2$-only group and C: $CdCl_2$ + GB1 group. Group A rats were orally administered distilled water only, group B rats received $CdCl_2$ (2.5 mg/kg b.w. in drinking water) and group C rats were treated with GB1 dissolved in Tween20 (2 mg/kg b.w. daily) and $CdCl_2$ (2.5 mg/kg b.w. day) in drinking water. Cadmium chloride ($CdCl_2$) was dissolved in the drinking water at a dose of 2.5 mg/kg and GB1 was dissolved in 0.5% Tween20. GB1 was administered per os by oral gavage for 90 days. The doses used for the study were chosen from previous studies by El-Demerdash et al. (2004) and Alkhedaide et al. (2016) for cadmium chloride and Nwaehujor et al. (2015) for GB1. The chosen dose for cadmium chloride was shown to cause significant oxidative stress in various tissues of the body (El-Demerdash et al. 2004; Alkhedaide et al. 2016) while that of GB1 showed significant antioxidant effect (Nwaehujor et al. 2015). After every 30 days, 4 animals from each group were weighed and humanely euthanized under chloroform anaesthesia. The experiment lasted for 90 days.

Following euthanasia, blood samples were collected from the medial canthus of the eye using a capillary tube. Whole blood was collected into test tubes without anticoagulant and allowed to clot in a slanting position. Sera were harvested with disposable pipettes, transferred into microtubes and stored at −20 °C for hormonal analysis (Tietz 1995).

- Testosterone assay was accomplished by the microplate enzyme-linked immunosorbent assay (ELISA) technique using Testosterone AccuBind ELISA Test Kit (Monobind; Lake Forest, CA, USA).
- Follicle-stimulating hormone (FSH) assay was accomplished by ELISA technique using FSH AccuBind ELISA Test Kit (Monobind; Lake Forest, CA, USA).
- Luteinising hormone (LH) assay was accomplished by ELISA technique using AccuBind ELISA Test Kit (Monobind; Lake Forest, CA, USA).
- Gonadotropin releasing hormone was accomplished by ELISA technique using AccuBind ELISA Test Kit (Monobind; Lake Forest, CA, USA).

Upon euthanasia, the left testes were immediately exteriorized through a mid-caudoventral abdominal incision with sterile scalpel blade. Sperm cells were then collected from the cauda epididymis (Oyeyemi et al. 2011). This was done by removing the cauda epididymis from the right testes and blotting with filter paper. The cauda epididymis was immersed in 5 mL formal saline in a graduated test tube, and the volume of fluid displaced was taken as the volume of the epididymis. The volume of the epididymis and the cauda epididymis was poured into a ceramic mortar and homogenized into a suspension from which the sperm count was carried out using the improved Neubauer haemocytometer under the microscope (WHO 1999).

A small drop of sperm suspension was collected with fluid from the cauda epididymis via a scalpel and dropped onto a slide. The diluents (buffered 2.9% sodium citrate solution) kept at 37 °C was added to the sperm suspension until the desired dilution was obtained. Sperm motility was assessed by the method described by WHO (1999). The motility of the epididymal sperm was evaluated microscopically 2–4 min of their isolation from cauda epididymis and later expressed as percentages.

Sperm viability or live/dead ratio was assessed by adding 2 drops of warm eosin-nigrosin stain to the semen on a pre-warmed slide, a uniform smear was made and dried in air and the stained slide was immediately examined under the microscope using ×400 magnification. The live sperm cells were unstained while the dead sperm absorbed the stain. The stained and unstained sperm were counted and the percentage calculated (Oyeyemi et al. 2011).

Testicular histopathology

The left testes of each rat were fixed in Bouin's fluid for 24 h, re-fixed in 70% ethanol, then passed through ascending series of ethanol, cleared in xylene and embedded in paraffin wax. The tissues were sectioned at 5 μm thickness on a rotary microtome. The tissues were mounted on clean glass slides and stained with haematoxylin and eosin. All sections were examined under a light microscope at ×100 and ×400 magnifications. Photomicrographs of the lesions were captured for observation and documentation of histopathological lesions.

Data analysis

The mean and standard error of the mean were calculated for the semen characteristics and hormonal assay and were presented in percentages. One-way ANOVA (analysis of variance) and Duncan multiple range test was done using Statistical Package for the Social Sciences (SPSS) v20 for Windows to establish any significant differences. Values of $p < 0.05$ were considered significant.

Results

Body and testicular weight

The average body weights of the rats treated with $CdCl_2$ only, $CdCl_2$ + GB1 and the control rats at the end of the first month of the study were 178.90 ± 14.12 kg, 180.33 ± 13.14 kg and 185.47 ± 10.26 kg respectively. The analysis of variance indicated that there were no significant ($p > 0.05$) differences in the mean weights of the rats treated with only $CdCl_2$, $CdCl_2$ + GB1 and control groups at the end of the first month of treatment (Fig. 1).

At the end of the second month of treatment, the mean body weight of the $CdCl_2$-treated rats was significantly ($p < 0.05$) lower than that of the $CdCl_2$ + GB1–treated rats and control rats. The $CdCl_2$ + GB1–treated group showed no significant difference ($p > 0.05$) in their mean body weight when compared to that of the control group (Fig. 1). At the end of the third month of treatment, the mean body weights of the control rats and $CdCl_2$ + GB1–treated rats did not also show significant differences ($p > 0.05$). However, their mean body weights were significantly ($p < 0.05$) higher than the $CdCl_2$-only-treated group (Fig. 1).

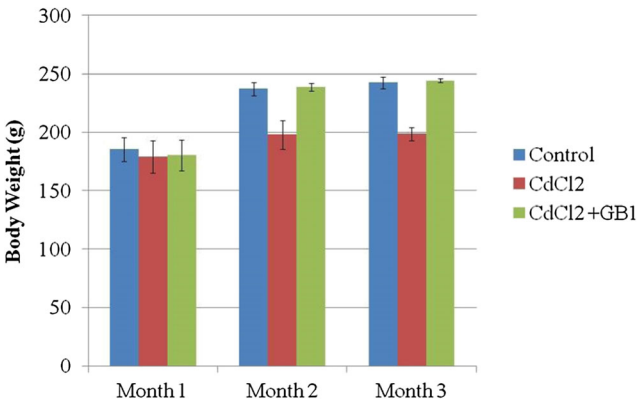

Fig. 1 Effects of $CdCl_2$ and GB1 on the body weights of $CdCl_2$-induced toxicity in Wister rats

The mean testicular weights of $CdCl_2$-only-treated rats at the end of the first month was 1.57 ± 0.07 kg while the mean testicular weights of the $CdCl_2$ + GB–treated rats and control rats were 1.74 ± 0.12 kg and 2.06 ± 0.03 kg respectively. Analysis of variance indicated that the mean testicular weight of the $CdCl_2$ + GB1–treated group at the end of month 1 was significantly ($p < 0.05$) higher than that of $CdCl_2$-only-treated rats but was however significantly ($p < 0.05$) lower than the control rats (Fig. 2). At the end of the second month of treatment, the mean testicular weight of the $CdCl_2$-only-treated rats was significantly ($p < 0.05$) lower than the mean testicular weight of the $CdCl_2$ + GB1–treated rats and control rats. Equally, the mean testicular weight of the $CdCl_2$ + GB1–treated group was significantly ($p < 0.05$) lower than the mean testicular weight of the control group (Fig. 2). At the end of the third month of treatment, the mean testicular weight of the $CdCl_2$-only-treated rats was significantly ($p < 0.05$) lower than that of the $CdCl_2$ + GB1–treated rats which in turn was also significantly ($p < 0.05$) lower than that of the control rats (Fig. 2).

Hormonal assay

Hormone assay during the 3 months of treatment revealed that the mean values of the testosterone, gonadotropin releasing hormone (GnRH) and follicle stimulating hormones (FSH) in the $CdCl_2$-only-treated groups were significantly lower than the values obtained for $CdCl_2$ + GB1–treated and control groups.

Equally, the mean testosterone, GnRH and FSH hormone values obtained for the $CdCl_2$ + GB1–treated group was significantly ($p < 0.05$) lower than those for the control group but significantly ($p < 0.05$) higher than the mean values obtained for the $CdCl_2$-only-treated group (Figs. 3, 4 and 5).

At the end of the first month of treatment, the values of luteinising hormone (LH) obtained for the control, $CdCl_2$ + GB1– and $CdCl_2$-only-treated groups showed no significant ($p > 0.05$) differences between the groups. However, LH values obtained for the $CdCl_2$-only-treated group at the end of the second and third months were significantly lower than the values of LH for the $CdCl_2$ + GB1 and control groups obtained at the end of the same months. The LH values obtained for the $CdCl_2$ + GB1 and control groups at the end of the second and third months were not significantly ($p > 0.05$) different between the two groups (Fig. 6).

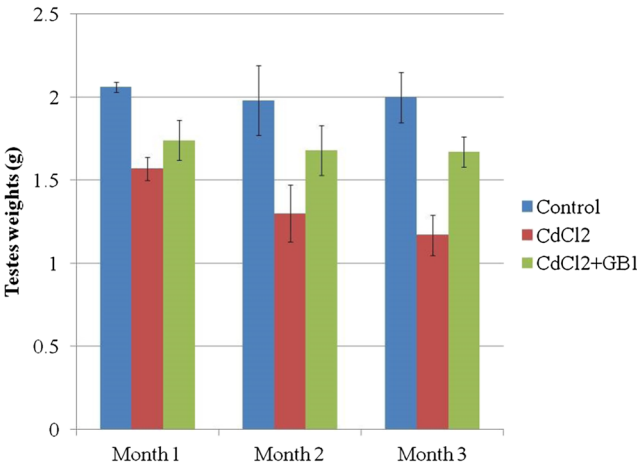

Fig. 2 Effect of GB1 on the testicular weight of $CdCl_2$-induced toxicity in Wister rats

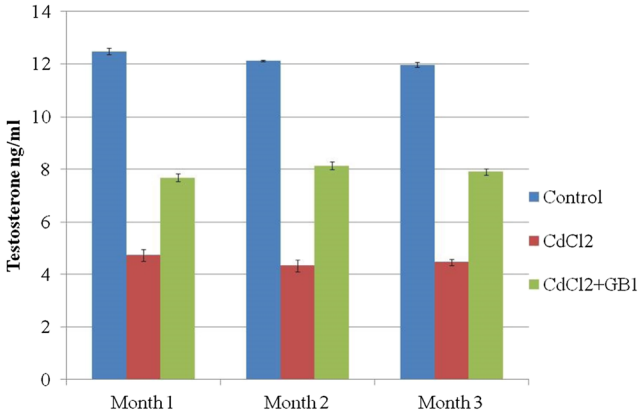

Fig. 3 Effect of GB1 on testosterone concentration in $CdCl_2$-induced toxicity in Wister rats

Sperm quality

The total sperm count (Fig. 7), percentage sperm viability (Fig. 8) and percentage sperm progressive motility (Fig. 9) values obtained at the end of each month of study were significantly ($p < 0.05$) higher in the $CdCl_2$ + GB1–treated group than in the $CdCl_2$-only-treated group. However, these values obtained for the $CdCl_2$ + GB1–treated group were significantly ($p < 0.05$) lower than the values obtained for the control group.

The percentage non-progressive/sluggish sperm motility (Fig. 10), percentage immotile sperm (Fig. 11) and percentage headless sperm (Fig. 12) at the end of the 3 months of the

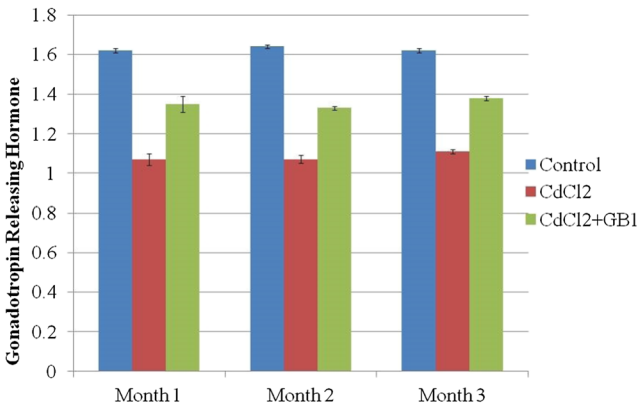

Fig. 4 Effect of GB1 on GnRH concentration in $CdCl_2$-induced toxicity in Wister rats

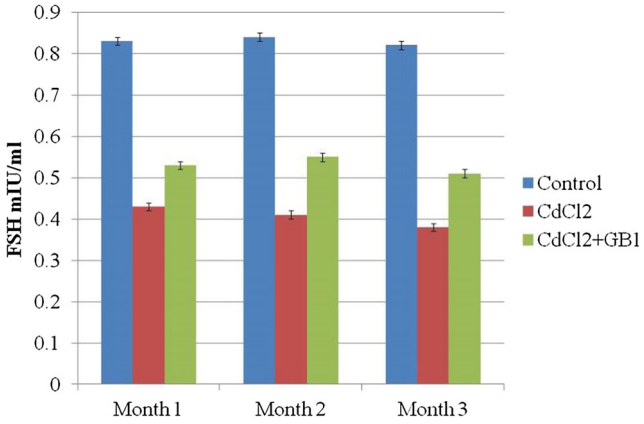

Fig. 5 Effect of GB1 on FSH concentration in $CdCl_2$-induced toxicity in Wister rats

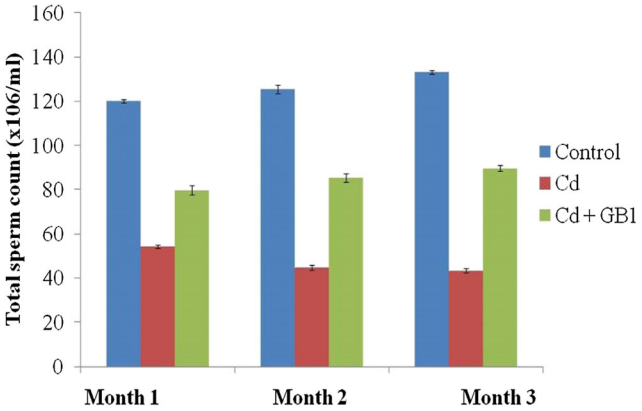

Fig. 7 Effect of GB1 on the total sperm count in $CdCl_2$-induced toxicity in Wister rats

study were significantly ($p < 0.05$) higher in the $CdCl_2$-only-treated group than in the $CdCl_2$ + GB1–treated group. The percentage non-progressive/sluggish sperm motility, percentage immotile sperm and percentage headless sperm were significantly ($p < 0.05$) lower in the control group than in the $CdCl_2$ + GB1–treated group.

Testicular histopathology

Histologically, the testes of the control, $CdCl_2$-only- and $Cdcl_2$ + GB1–treated Wistar rats at the end of the first month of study exhibited few visible lesions within the groups (Fig. 13). The $CdCl_2$-only-treated rats showed more disorganisation of the interstitial connective tissue when compared with $CdCl_2$ + GB1–treated and control rats. The seminiferous epithelium in all three groups at the end of the first month of study showed similar histology with all the spermatogenic cells and Sertoli cells observed.

At the end of the second month of study, the seminiferous tubules of the $CdCl_2$-only-treated rats exhibited progressive erosion of the germinal epithelium (Fig. 14a) when compared with the $CdCl_2$ + GB1–treated rats (Figs. 14b). The structural integrity of the seminiferous epithelium in the $CdCl_2$-only-treated rats was severely compromised with disruption of the epithelium and disorganisation of spermatogenic cells (Fig. 14c). The different spermatogenic cells were not easily identifiable. However, there was no visible disruption of the seminiferous epithelium or disorganisation of the spermatogenic cells observed in the $CdCl_2$ + GB1–treated rats (Fig. 14d).

At the end of the third month of the study, the testis of the $CdCl_2$-only-treated rats showed few normal seminiferous tubules and numerous necrotised seminiferous tubules (Fig. 15a) while the $CdCl_2$ + GB–1 treated groups showed normal seminiferous tubules (Fig. 15b). The necrotised seminiferous tubules of the $CdCl_2$-only-treated groups were lined by degenerated seminiferous epithelium containing few spermatogenic cells and numerous vacuoles (Fig. 15c). In some cases, the epithelium was completely eroded leaving only the basement membrane of the seminiferous epithelium and vacuoles. Spermatogenesis appeared impeded in the $CdCl_2$-only-treated rats as numerous vacuoles were observed in the luminal compartment. However, the testis of the $CdCl_2$ + GB1–treated rats showed averagely normal seminiferous tubules lined by well-organised seminiferous epithelium and

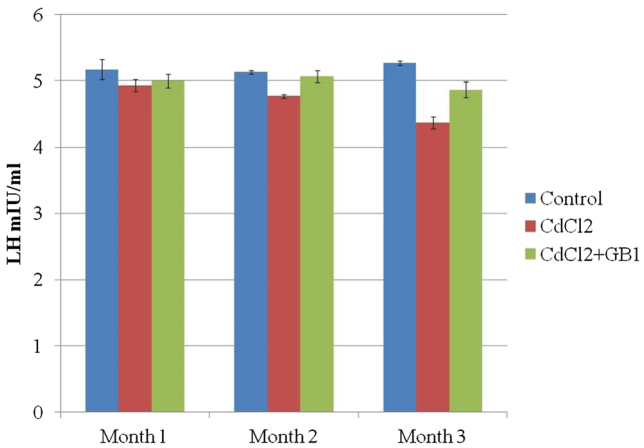

Fig. 6 Effect of GB1 on LH concentration in $CdCl_2$-induced toxicity in Wister rats

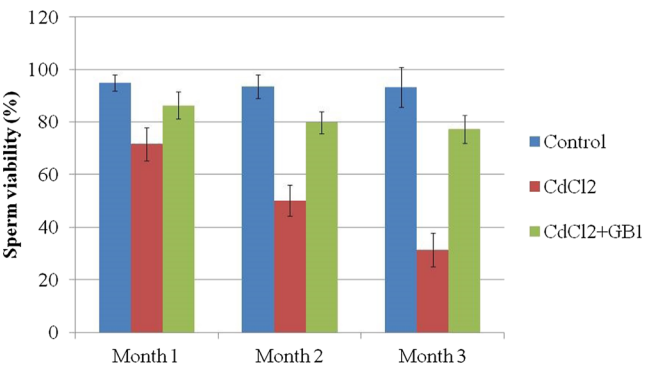

Fig. 8 Effect of GB1 on percentage sperm viability in $CdCl_2$-induced toxicity in Wister rats

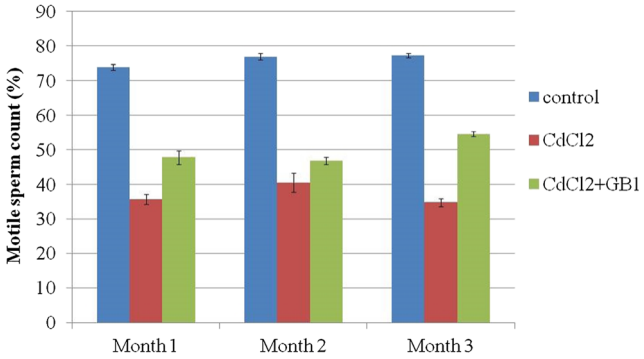

Fig. 9 Effect of GB1 on percentage sperm motility in Wister rats with $CdCl_2$-induced toxicity

spermatogenic cells (Fig. 15d). Within the epithelium were numerous spermatogonia, primary spermatocytes, round spermatids and elongated spermatids indicating unimpeded spermatogenesis.

Discussion

The results showed that exposure to $CdCl_2$ at 2.5 mg/kg b.w. for 90 days negatively affected the mean body and testicular weights, while addition of 2 mg/kg b.w. of GB1 to the treatment protocol offered protection against cadmium-induced weight losses. We observed that the daily food intake did not significantly differ across the groups (data not shown), suggesting that the reduced weights observed in the cadmium-treated group is unconnected to food intake. This observation implied that the $CdCl_2$ may have caused the decreased weight loss seen in the cadmium-treated group. This inference is supported by studies on lambs, calves and pigs fed cadmium with similar results (Doyle et al. 1974; Lymberopoulos et al. 2003). The decrease in testicular weight was probably due to degeneration of testicular tissues. The mechanism was believed to be either through the interference of cadmium in electron transport and energy metabolism or by alternative homeostatic and endocrine processes

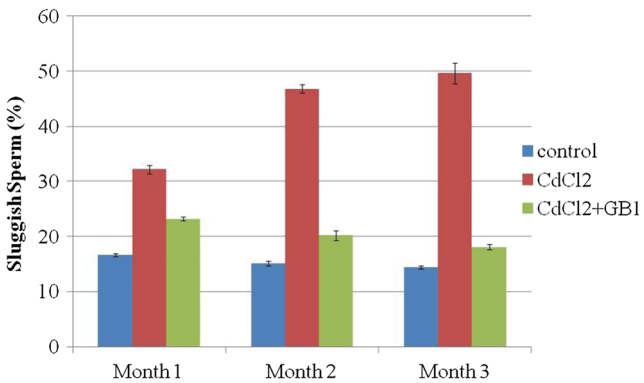

Fig. 10 Effect of GB1 on percentage of sluggish sperm in Wister rats with $CdCl_2$-induced toxicity

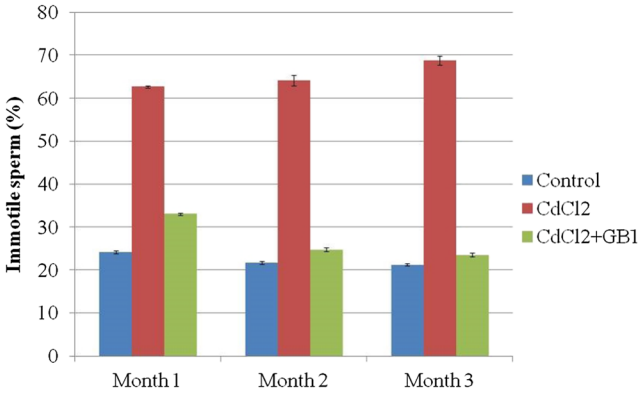

Fig. 11 Effect of GB1 on percentage of immotile sperm in Wister rats with $CdCl_2$-induced toxicity

(Lymberopoulos et al. 2003). However, the mean body and testicular weights of the $CdCl_2$ + GB1–treated rats were not affected by the cadmium even at the third month of administration. This probably indicates a protective mechanism of the GB1 on the cells of the testes from the actions of the $CdCl_2$.

The result of this study indicated that there was significant decrease in the levels of testosterone, gonadotrophin releasing hormone, follicle stimulating hormone and luteinising hormone in the $CdCl_2$-only-treated rats when compared to the $CdCl_2$ + GB1–treated and control rats. This indicates that cadmium probably alters the hypothalamic-pituitary-gonadal activities by disrupting the release of gonadotrophin releasing hormone from the hypothalamus. This action on the GnRH will also alter the secretion of the FSH and LH from the pituitary which then affects the function of Leydig cells in steroidogenesis of testosterone. Reports from other researchers agreed with this observation that cadmium is an endocrine disruptor (Thompson and Bannigan 2008; Waye and Trudeau 2011) with direct and indirect effects. It has a direct effect since cadmium replaces Ca^{++} and Zn^{++} by mimicking their physiological processes in the cells (Valko et al. 2005). It has an indirect effect since cadmium affects the hypothalamus and pituitary negatively thus affecting reproductive function through suppressed release of FSH and LH and inhibition of

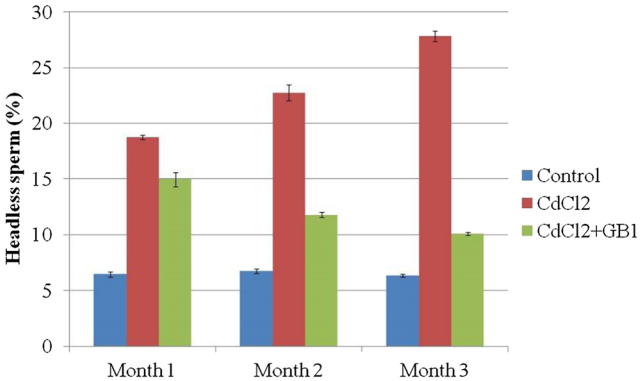

Fig. 12 Effect of GB1 on percentage headless sperm in Wister rats with $CdCl_2$-induced toxicity

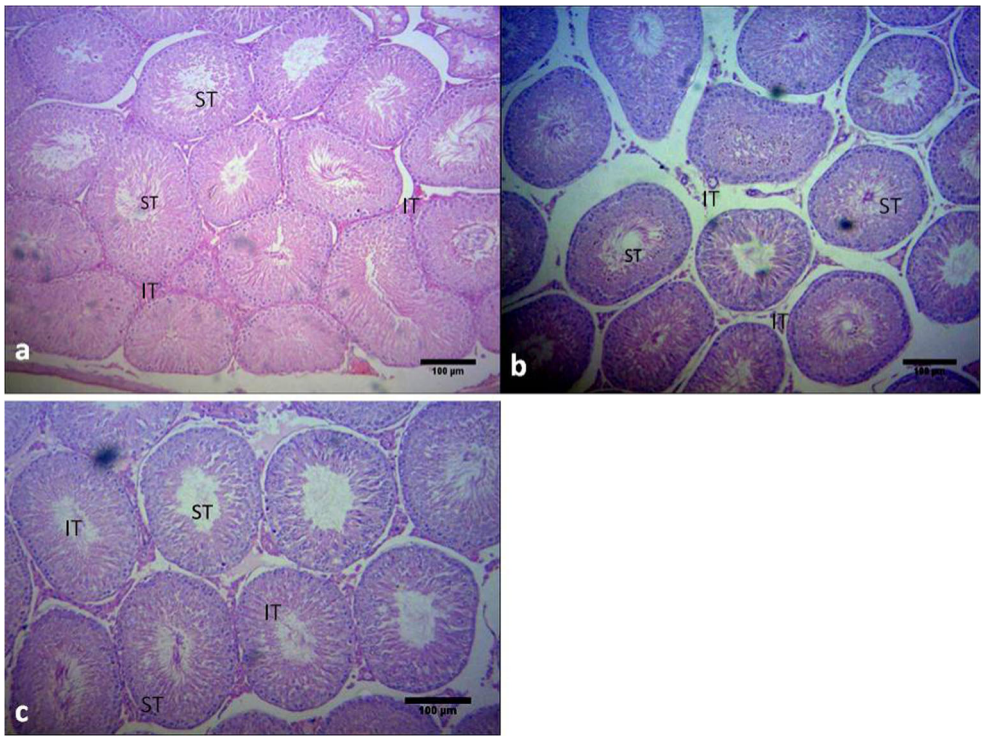

Fig. 13 Photomicrograph of the testes of control (**a**), $CdCl_2$-only-treated rats (**b**) and $CdCl_2$ + GB1–treated rats (**c**) at the end of month 1 showing seminiferous tubules (ST) and the interstitial connective tissues (IT). Note the levels of disorganisation of the interstitial connective tissues in the three groups

androgen production in the Leydig cells (Hoyer 2005; Takiguchi and Yoshihara 2006; Benoff et al. 2008).

Numerous studies showed that cadmium interferes with the reproductive hormones LH, FSH and testosterone (Lafuente et al. 2003; Hachfi and Sakly 2010; Gollenberg et al. 2010; Gallagher et al. 2010; Jackson et al. 2011). The increased level of these hormones in $CdCl_2$ + GB1–treated rats suggests that the extract prevented the interference of cadmium with reproductive hormones. The extract may have achieved this by exhibiting antioxidant and protective mechanism on the

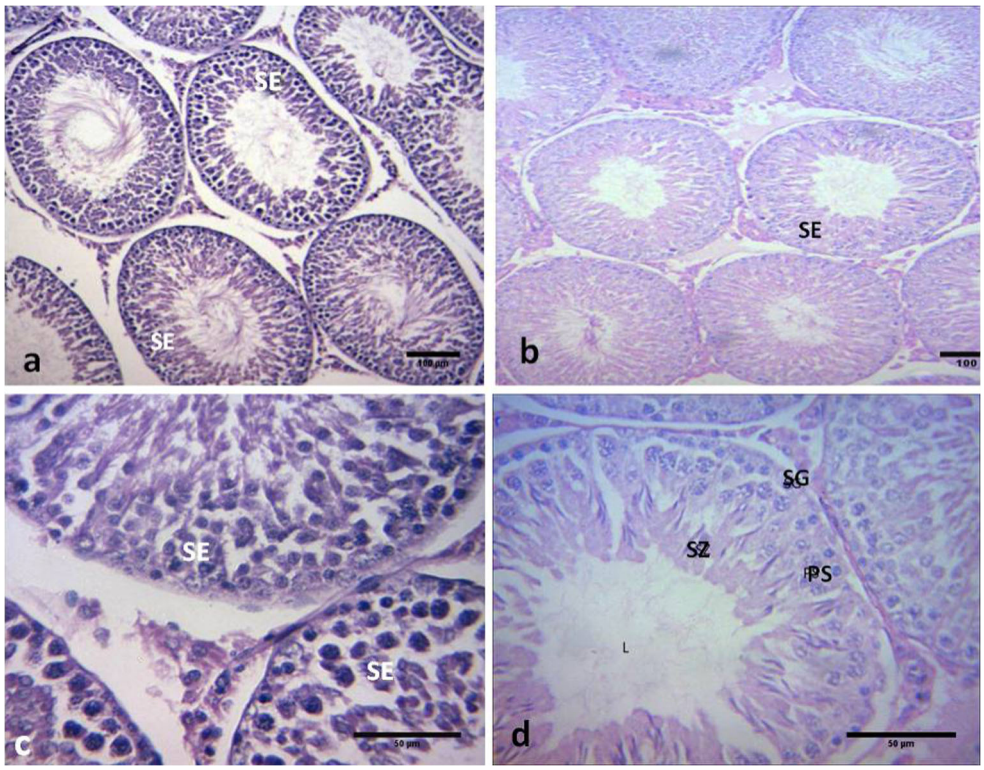

Fig. 14 The seminiferous tubules of $CdCl_2$-only-treated rats (**a**) and $CdCl_2$ + GB1–treated rats (**b**) at the end of month 2. Note the severely compromised and eroded seminiferous epithelium (SE) of $CdCl_2$-only-treated rats (**c**) with disorganised spermatogenic cells when compared with $CdCl_2$ + GB1–treated rats (**d**) showing well-organised spermatogonia (SG), primary spermatocytes (PS) and matured spermatozoa (SZ). H&E

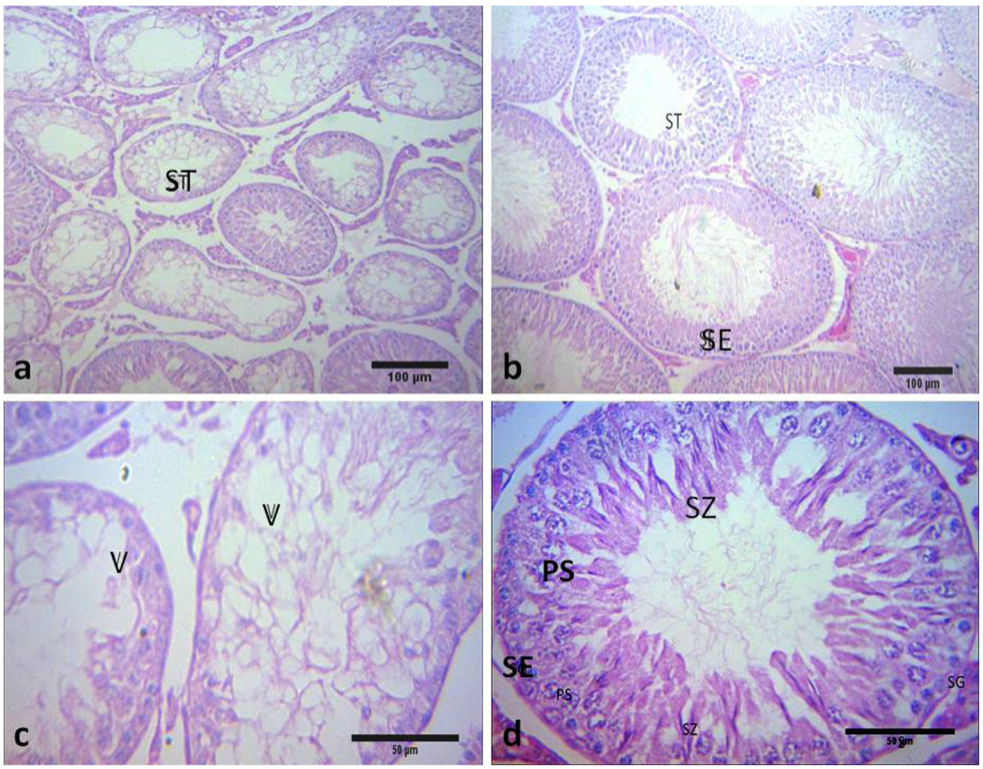

Fig. 15 The necrotised seminiferous tubules of $CdCl_2$-only-treated rats (**a**) and normal tubules of $CdCl_2$ + GB1–treated rats (**b**) at the end of month 3. Note the necrotised seminiferous tubules of the $CdCl_2$-only-treated rats (**c**) are lined by degenerated seminiferous epithelium containing few spermatogenic cells and numerous vacuoles (V) while tubules of the $CdCl_2$ + GB1–treated rats showed well-organised seminiferous epithelium (**d**) in which are numerous spermatogonia (SG), primary spermatocytes (PS) and elongated spermatids (SZ). H&E

organs against the known oxidative and disruptive activities of cadmium on these reproductive organs. Even though there are no existing study on the effects of GB1 on the hypothalamus-pituitary-testicular activities, the increased level of GnRH, testosterone, FSH and LH (though lower than the control rats) probably indicates an ameliorative action of the GB1 against a known endocrine disrupter.

Garcinia kola extracts had been reported to be potent antioxidants capable of increasing testosterone production (Guyton and Hall 1998; Ganong 2003; Akpantah et al. 2003). GB1 is known to significantly prevent drug and chemical-induced organ toxicity and oxidative damage in experimental models (Farombi et al. 2012; Adedara et al. 2013; Adaramoye and Arisekola 2013; Olayinka and Ore. 2014). The damaging and inhibitory effects of cadmium on the hormone-producing tissues may have been prevented and ameliorated by GB1 as this extract had been reported to show stimulatory effect on liver cell regeneration and subsequent stimulatory effect on the protein synthetic apparatus by increasing the rate of protein synthesis (Madubunyi 2012).

Results of this study showed a significant reduction in sperm count, sperm motility, sperm viability and number of morphologically normal spermatozoa in Wistar rats exposed to cadmium chloride when compared to control and $CdCl_2$ + GB1–treated groups. This result indicates that administration of cadmium resulted in degeneration of the seminiferous epithelium and spermatogenic cells within the epithelium. These results are in line with earlier studies which showed a degenerative reaction of testicular tissues to cadmium, thereby contributing to male infertility by reducing sperm quality (Roychoudhury et al. 2010; Mendiola et al. 2011). All testicular germ cell populations can be affected by cadmium as numerous studies had shown decreases in the number of spermatogonia and spermatocytes, aberrant morphology in all developing stages, release of immature cells into the lumen (Aoyagi et al. 2002; Zhou et al. 2004; Marettová et al. 2010) and failure in spermiation (Hew et al. 1993b). Furthermore, cadmium studies showed presence of elongated and round spermatids, as well as spermatocytes in the tubular lumen in >98% of tubules (Mruk and Cheng 2011).

$CdCl_2$ + GB1–treated rats in this study showed a significant increase in the sperm quality when compared to the $CdCl_2$-only-treated rats. Numerous studies on the effect of the crude extracts of *Garcinia kola* on the testes had shown increased sperm volume with highly enhanced sperm concentration, motility, sperm count and libido (Adesanya et al. 2007; Sewani-Rusike et al. 2016; Mesembe et al. 2013; Ebenebe et al. 2017; Aprioku 2018). Thus, the increased sperm quality in the $CdCl_2$ + GB1–treated rats when compared to $CdCl_2$-only-treated rats may be attributed to the antioxidant activities of GB1 extract which is capable of stimulating and increasing the process of spermatogenesis (Oluyemi et al. 2007). Apart from stimulating metabolism in the testicular cells and scavenging on free radicals (Iwu et al. 1990), GB1 may also be acting by stabilizing the membranes of the

spermatozoa and membranes of the Leydig cells which are involved in spermatogenesis.

The low live/dead ratio observed in the cadmium-only-treated rats is indicative of the genotoxicity of cadmium to spermatozoa. This indicates that cadmium probably had negative effects on the various processes involved in spermatogenesis leading to production of more dead spermatozoa when compared to the $CdCl_2$ + GB1–treated and control rats. This is in line with various reports that elucidated the mechanism of cadmium toxicity. The toxic effects of cadmium include alterations in permeability of plasma membranes, damage to nuclear and mitochondrial membranes, increase in chromatin condensation with incorporation of cadmium into the chromatin, ladder-like splitting of DNA and decrease in the total DNA content respectively (Fasanya-odewumi et al. 1998; Habbeebu et al. 1998; Klimova and Misurova 2004; Thompson and Bannigan 2008). These toxic effects of cadmium directly lead to disturbances in the mitotic and meiotic processes of spermatogenesis and ultimately numerous defective or dead spermatozoa. However, the GB1 addition probably stabilised the plasma membranes thereby reducing the known alterations associated with cadmium toxicity. This will ultimately lead to fewer disturbances to the mitotic and meiotic processes of spermatogenesis and ultimately more live and active spermatozoa.

The histopathological study revealed the effect of cadmium toxicity on the testes. When exposed to 2.5 mg/kg over time, there was gradual deterioration of the testicular tissue. Thus, exposing the rats to 2.5 mg/kg of $CdCl_2$ for 90 days showed a gradual destruction of the testicular tissues. Initially, the testicular tissue damage was minimal at the end of the first month but increased with the duration of exposure to cadmium. The effects observed included destruction of interstitial tissue which ultimately affected the Leydig cell population and consequent reduction in testosterone production. Also observed was the destruction of the seminiferous epithelium leading to the disorganisation and degeneration of the spermatogenic cells, degeneration of germinal cells and progressive sloughing of germ cells from the basement membrane, dysfunction of the organ and vacuolation of seminiferous epithelium. These results were similar to studies by other researchers on the effects of cadmium exposure on the testes (Xu et al. 1996; Yan et al. 1997; Zhou et al. 1999; Li et al. 2000; Waalkes 2000; Toman et al. 2002; Goyer et al. 2004; Marettová et al. 2010, 2013). These observations had led to studies inferring a negative association between cadmium concentration and sperm concentration, sperm motility and percentage abnormal spermatozoa and consequently infertility (Akinloye et al. 2006; Pant et al. 2015). Cadmium affects the testes by disrupting spermatogenesis via a mechanism that involved the induction of lipid peroxidation, depletion of ROS scavengers and disruption of testicular antioxidant enzyme activity (Koizumi and Li 1992; Santos et al. 2004; Linares et al. 2006; Marchlewicz et al. 2007). The cellular damage may be due to an improper balance between ROS generation and scavenging activities (Pajavic and Saicic 2008).

The $CdCl_2$ + GB1–treated rats when compared to $CdCl_2$-only-treated rats had unaffected testicular tissues. There were minimal signs of deterioration of the testicular tissues and destruction of interstitial tissue and of the seminiferous epithelium. The seminiferous tubules showed near normal seminiferous epithelium with numerous spermatogenic cells in an organised manner without obvious signs of degeneration and vacuolation of seminiferous epithelium. The result can only be attributed to the antioxidant and protective effect of the GB1 extract that was administered to the rats. The GB1 extract was found to show a high scavenging and protective effect (Adaramoye et al. 2005). Since cellular damage to the spermatogenic cells from cadmium toxicity is probably due to an improper balance between ROS generation and scavenging activities (Pajavic and Saicic 2008), inclusion of GB1 led to increased antioxidant properties and proper balancing between scavenging activities and ROS generation.

Conclusion

Cadmium-intoxicated rats showed a significant decrease in their relative body and testicular weights, reproductive hormones, sperm quality and testicular disorganisation, disruption of the seminiferous epithelium and spermatogenic cells. However, the addition of Garcinia hydroxybiflavanonol (GBI) probably prevented decrease in the relative body and testicular weights, reproductive hormonal levels and sperm quality, thus leading to stabilization of the seminiferous epithelium and spermatogenic cells towards normalcy. This can lead to the inference that GB1 is an antioxidant that protects the testes from the oxidative stress caused by cadmium toxicity. Overall, our data demonstrated that GB1 of *Garcinia kola* acts as a potent antioxidant against oxidative activities of cadmium. However, the results from the control group encourage advocacy for policies that will discourage or reduce environmental contamination with cadmium.

Authors' contribution All authors contributed to the study conception and design. Material preparation and data collection were performed by Clifford N. Abiaezute, Kenneth O. Anya, Edwin A. Uwagie-Ero. Analysis was by Chinaka O. Nwaehujor. The first draft of the manuscript was written by Clifford N. Abiaezute and Kenneth O Anya and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

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

Informed consent Not applicable.

Ethical approval None. Experimental procedures were in accordance with the guidelines for animal care as contained in the animal ethics handbook of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria.

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