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Effect of *Vigna angularis* Seed Extract on Cadmium-Induced Toxicity in Rat Testes

M. A. Adaikpoh*, N. J. Orhue and L. C. Odinko

Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.
*Author for correspondence (e-mail: martina.adaikpoh@uniben.edu)

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ABSTRACT: Cadmium, an established environmental toxin is also known to be toxic to the testes. This study investigated the effect of *V. angularis* on cadmium-induced testicular toxicity in rat. The animals were administered with cadmium chloride (4 mg/kg bwt.) in normal saline, subcutaneously while administration of aqueous extract of *V. angularis* which commenced 24 hours post-cadmium administration was done daily by gavage. Cadmium caused a significant ($p< 0.05$) reduction in Superoxide Dismutase (SOD) activity and a significant ($p< 0.05$) increase in catalase activity and malondialdehyde levels in rat testes relative to the control. The administration of *V. angularis* (300 mg/kg bwt.) resulted in a significant ($p < 0.05$) increase in SOD activity compared to the cadmium-only treatment group. Cadmium also significantly ($P< 0.05$) increased testes and prostate cholesterol levels, serum testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels relative to the control. However, treatment with *V. angularis* at 300 and 450 mg/kg bwt reduced serum LH and FSH levels when compared with the cadmium-only group. All the *V. angularis* treated groups showed significant ($P< 0.05$) decrease in prostate cholesterol concentration and increase in testes cholesterol levels relative to the cadmium-only treated group. Sperm count was also significantly ($p< 0.05$) reduced in all the cadmium-treated groups relative to the control. These results show that the aqueous extract of *V. angularis* was unable to effectively remove the toxic effect of cadmium on the testes of rats within the 12 day treatment period used in this study.

Keywords: Cadmium, *V. angularis,* antioxidant, hormones, sperm count

Introduction

It has been suggested that at least half of the cases of human male infertility of unknown etiology may be attributable to various environmental and occupational exposure to toxic metals (Gagnon, 1998). In cadmium-exposed mammals, many target organs are affected including the testes, brain, liver and kidneys (Liu *et al.,* 1996; Oteiza *et al.,* 1999; Shaikh & Tang, 1999). One of the proposed mechanisms by which cadmium causes testicular toxicity is induction of reactive oxygen species (Ikediobi *et al.,* 2004), which reacts with cellular biomolecules causing damage to the cell including lipid peroxidation, membrane protein and DNA damage, consequently inducing cell death (Stohls *et al.*, 2001). Other proposed mechanisms of cadmium toxicity in the testes include circulatory failure due to vascular damage and decreased utilization of Zn by spermatogenic cells (Lee and Dixon, 1973), alteration in the hypothalamic-

pituitary-testicular axis function (Lafuente *et al.,* 2001) and increase in the total cholesterol levels in the testes of rats (Adaikpoh and Obi, 2009).

Currently, due to the toxic side effects of chelating agents used in the management of cadmium toxicity (Jones *et al.,* 1988), numerous research have focused on the use of nutrients and plant phytochemicals in the treatment of cadmium toxicity.

Vigna angularis is an important food staple that is well endowed with proteins, calories, fibre, vitamins and minerals. It is rich in polyphenols especially, pro-anthocyanidins (Sato *et al.,* 2005) catechins and glucosides (Kojima *et al.,* 2006), suggesting a health promoting effect. Studies have shown that it has antioxidant properties (Wu *et al.,* 2015), serum cholesterol lowering effect (Matsumoto and Ono, 2002) and stimulates melanogenesis in cultured mouse B16 melanoma cells (Itoh and Furuichi, 2005). However, its effect on testicular damage induced by cadmium has not yet been investigated. Therefore, the aim of this study was to investigate the effect of aqueous extract of *Vigna angularis* seed on cadmium- induced testicular toxicity in rat.

Materials and Methods

Thirty-five adult male albino rats (Wistar strain; weighing 140 – 200g) bred in the animal house unit of the Department of Biochemistry, University of Benin, Benin City Nigeria were used for the study. The rats were allowed one-week acclimatization period. Thereafter, they were randomly divided into five groups of 7 rats each. They received water and chow *ad libitum* throughout the period of the experiment. The rats were housed in cages with wire mesh floor. The rats in group 1 (Control) were cadmium and extract free. Rats in group 2 received only cadmium (Cd). Those in group 3 received cadmium plus 300 mg/kg bwt. *V. angularis* aqueous extract (Cd+VA300) while those in groups 4 (Cd+VA_450) and 5 (Cd+VA_600) received cadmium plus 450 and cadmium plus 600 mg/kg bwt. *V. angularis* aqueous extract respectively. Aqueous extract of *V. angularis* was administered twice daily (morning and evening) for 12 days by gavage but the cadmium in form of $CdCl_2$ dissolved in normal saline was administered (just once) to the rats at a dose of 4 mg kg^{-1} bwt, 24 hours before the commencement of administration of the extract. These treatments were carried out in accordance with the principles of laboratory animal care (NIH publication no. 85-93, revised 1985). At the end of the study period, the animals were sacrificed under chloroform anaesthesia. Fresh blood was collected from the aorta into plain tubes for serum preparation. The testes, epididymis, and prostate were excised for analysis.

Preparation of *V. angularis* Seed Extract

The *V. angularis* aqueous extract used in this study was obtained by boiling *V. angularis* bean seeds in water for 2 hours 30 minutes. The extract obtained from boiling was allowed to cool and the concentration of the extract determined. Graded doses were then administered to the rats.

Preparation of tissue homogenate

Weighed portions of the tissues were homogenized in 5ml of ice cold normal saline. The homogenate was centrifuged at 3000 rpm for 15 minutes. The clear supernatant obtained was used for biochemical analysis.

Biochemical Assay

Superoxide dismutase (SOD) activity in the testis was estimated by the method of Misra and Fridovich (1972) and computed as described by Baum and Scandalios (1981). In this procedure, one unit represents the amount of the enzyme required for 50% inhibition of the conversion of epinephrine to adrenochrome during one minute. Catalase activity was determined by the method of Cohen *et al.* (1970). Each catalase (CAT) unit specifies the relative logarimatic disappearance of hydrogen peroxide per minute and is expressed as K min^{-1}. Malondialdehyde was determined according to the method of Buege and Aust

(1978) while total cholesterol concentration in the testis and prostate were estimated as described by Richmond, (1973) using Randox kit (Randox Laboratories LTD, UK). Testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels in rat serum were determined by Enzyme Immunoassay method (ELISA).

Sperm Analysis

Freshly excised epididymis was punctured in 20 different locations and the content flushed out with a gentle stream of phosphate buffered normal saline. The suspension was diluted 20 times with buffered saline and with the aid of an improved Neubauer Counting Chamber, the total sperm in 5 squares of $1mm^2$ each was determined and multiplied by 10^6 to express the number of spermatozoa / ml.

Histological Examination

A portion of the testis and epididymis, fixed in Bouin's solution, were prepared for histological studies using Haematoxylin and Eosin stain (H&E) **(**Bancroft & Gamble, 2008). Slides were studied under light microscopy (Glaurt and Lewis, 1998).

Statistical Analysis

Values were expressed as mean ± SD (SPSS version 16 was used for statistical analysis). Significant differences between the means were analyzed using one-way ANOVA and the level of significance was fixed at $P < 0.05$.

Results

Fig 1 showed that treatment with cadmium significantly ($P < 0.05$) decreased SOD activity in rat testes. However, treatment with 350 mg/kg bwt. of *V. angularis* significantly ($P < 0.05$) increased SOD activity compared to cadmium-only treated group; it restored it to levels not significantly different from the control group. Treatment with only cadmium also caused a significant increase in catalase activity and malondialdehyde levels (Fig 2) compared to control. Administration of 350 mg/kg bwt. of *V. angularis* decreased catalase activity to levels not significantly different from control and did not cause a decrease in malondialdehyde levels in rat testis. Treatment with 450 mg/kg bwt. of the extract caused a significant ($P < 0.05$) increase in both catalase activity and malondialdehyde levels in the rat tests.

Remarkable changes in the rat serum hormonal levels were observed upon treatment with cadmium. Treatment with only cadmium caused a significant ($P < 0.05$) increase in testosterone, LH and FSH levels compared to that of the control. *V. angularis* treatment at the doses of 300 and 450 mg/kg bwt. was able to reverse this effect. However 600 mg/kg bwt. of *V. angularis* showed a significant ($P < 0.05$) decrease in testosterone levels and a significant increase in LH and FSH levels compared to both the group treated with only cadmium and the control.

As shown in Fig 4, The cholesterol levels in the testis of the cadmium–exposed rats was elevated relative to the control. This effect on the testis was not ameliorated by the *V. angularis* treatment. Interestingly, the opposite effect was observed in the prostate, where the cholesterol level was significantly elevated relative to the control. In this tissue, the *V. angularis* exract effectively reduced prostate cholesterol.

Sperm count was significantly ($P < 0.05$) decreased in the cadmium-only treated rats compared to control. Treatment with *V. angularis* at the doses studied, though increased sperm count, the increase was not significant when compared with the cadmium only group.

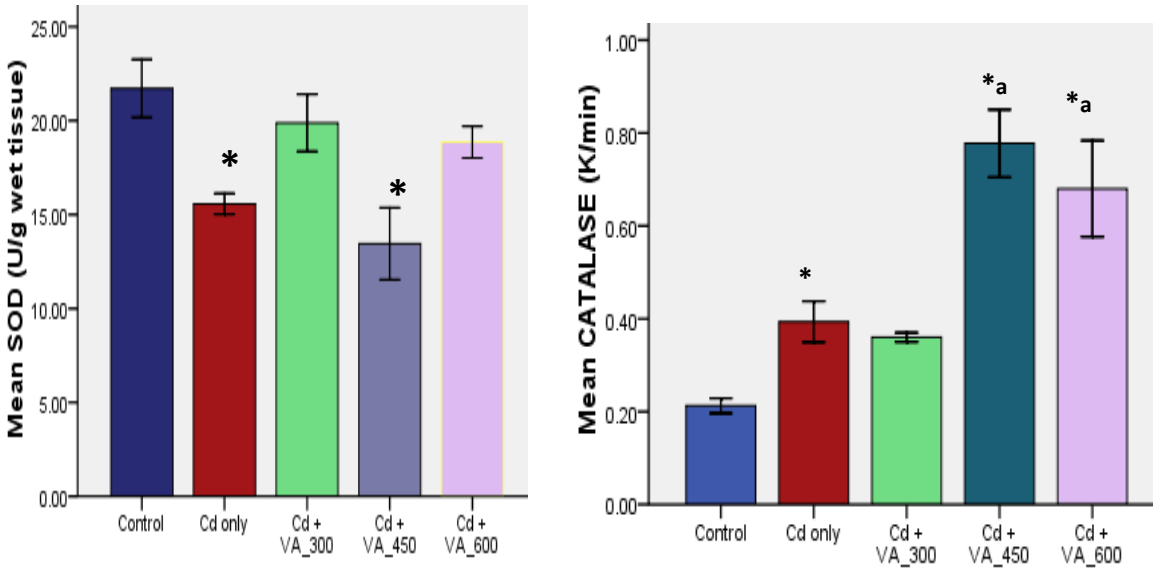

Fig 1: Effects of Cadmium and Cadmium plus *V.angularis* Treatments on SOD and Catalase Activity in Rat Testis

* Significantly ($P < 0.05$) different from control

[a] Significantly ($P < 0.05$) different from only cadmium treated group

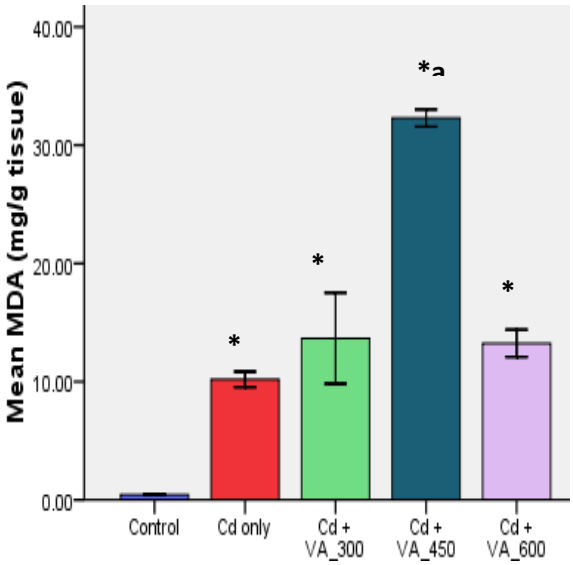

Fig 2: Effects of Cadmium and Cadmium plus *V. angularis* Treatments on Malondialdehyde and Testosterone Levels in Rat Testis

* Significantly ($P < 0.05$) different from control

[a] Significantly ($P < 0.05$) different from only cadmium treated group

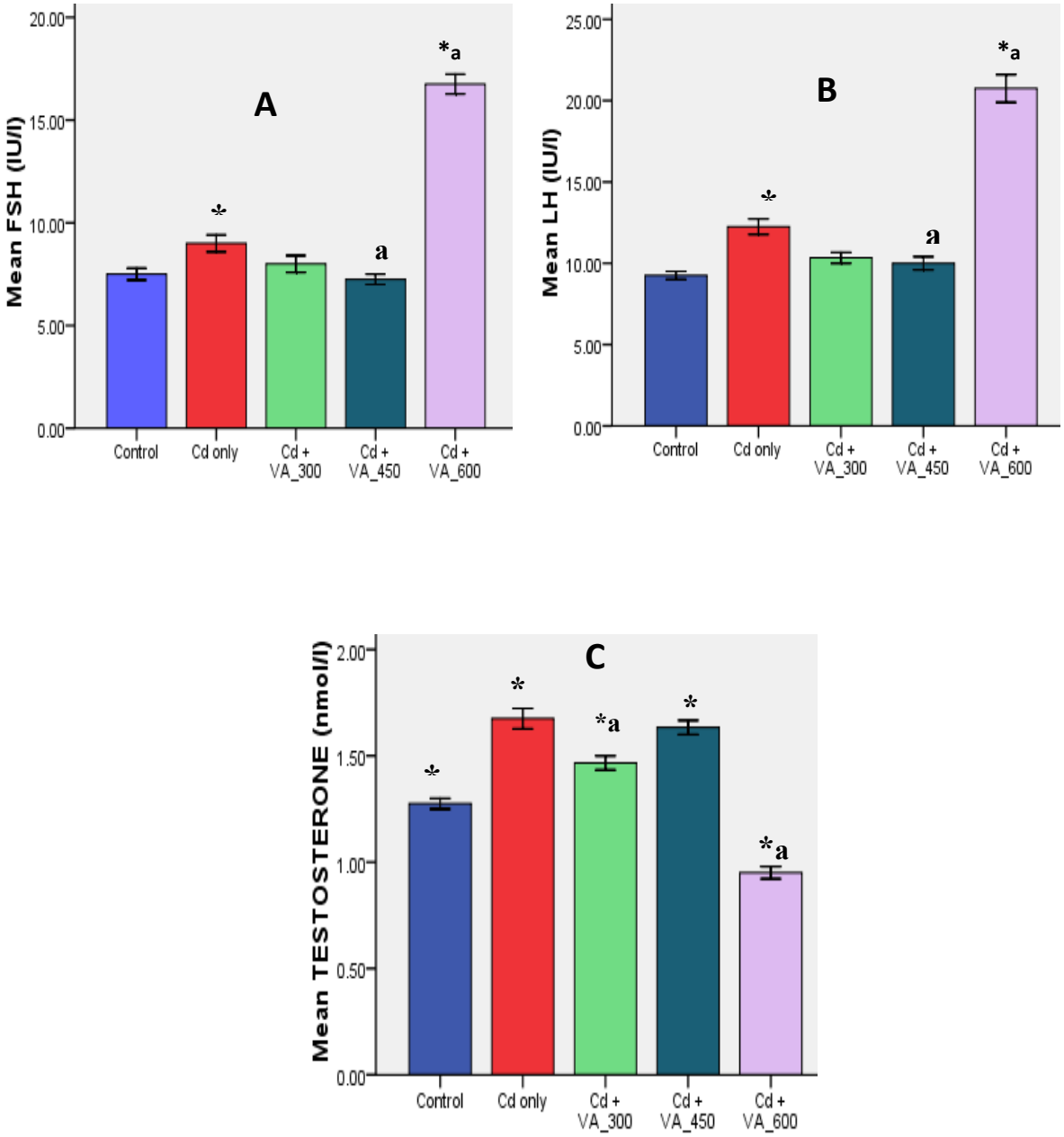

Fig 3: Effects of Cadmium and Cadmium plus *V. angularis* Treatments on Rat Serum FSH (A) LH (B) and Testosterone (C) levels.
* Significantly ($P < 0.05$) different from control
[a] Significantly ($P < 0.05$) different from only cadmium treated group

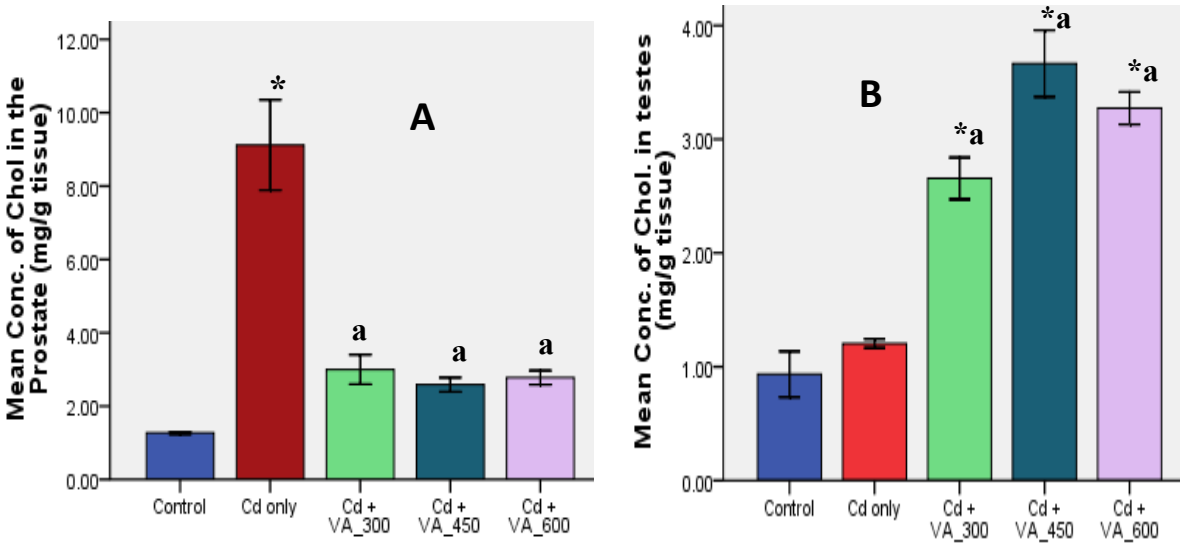

Fig 4: Effects of Cadmium and Cadmium plus *V. angularis* Treatments on cholesterol Levels in rat prostate (A) and testes (B)
* Significantly ($P < 0.05$) different from control
[a] Significantly ($P < 0.05$) different from only cadmium treated group

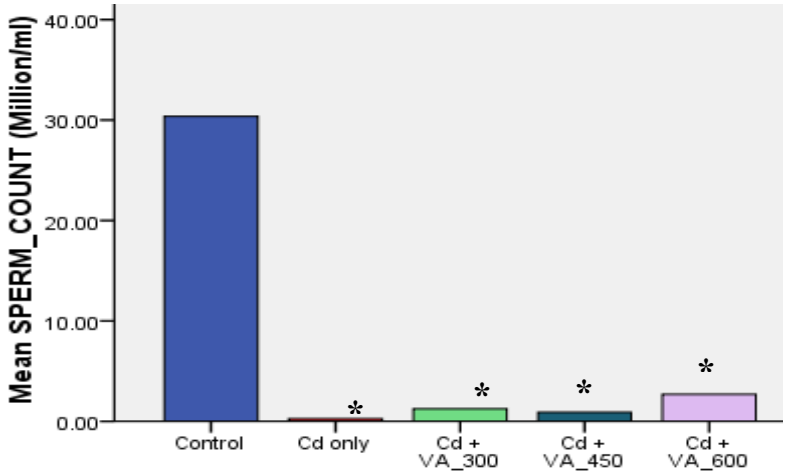

Fig 5: Effects of Cadmium and Cadmium plus *V. angularis* Treatments on Sperm Count
* Significantly ($P < 0.05$) different from control
[a] Significantly ($P < 0.05$) different from only cadmium treated group

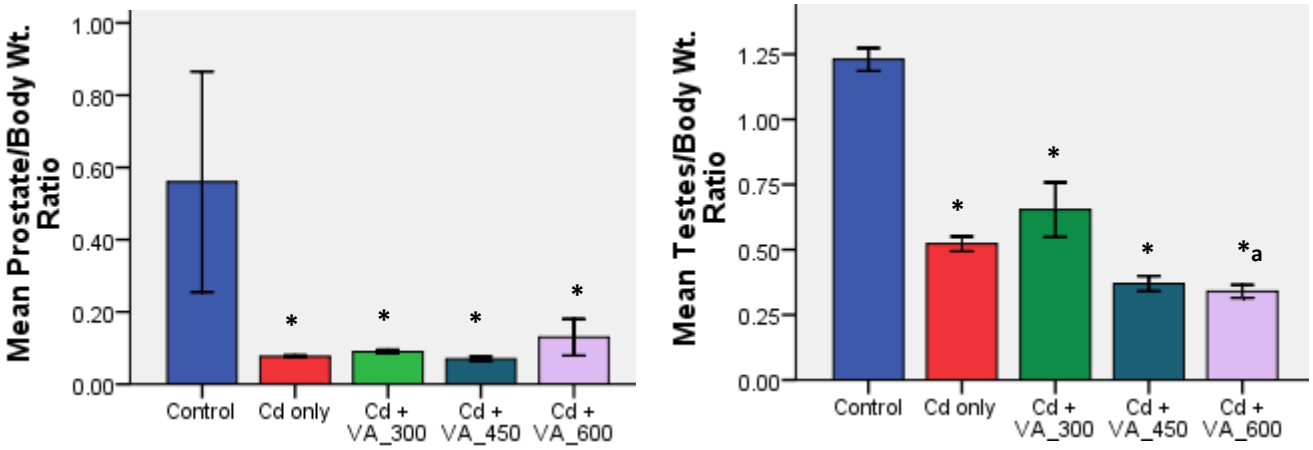

Fig 6: Effects of Cadmium and Cadmium plus *V. angularis* Treatments on Testis/Body wt. ratio and Prostate/Body wt. ratio
* Significantly ($P < 0.05$) different from control
[a] Significantly ($P < 0.05$) different from only cadmium treated group

Results of the Effects of Cadmium and Cadmium plus Aqueous Extract of *Vigna angularis* Treatment on the Histology of Rat Testes and Epididymis

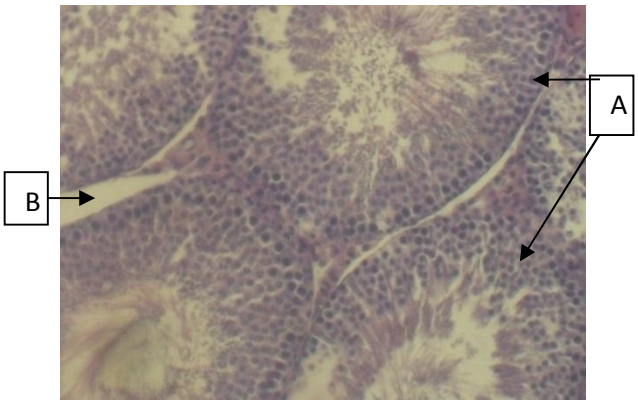

Plate 1 (Control): Rat testis composed of A, seminiferous tubules and B, interstitial space (H&E x 100)

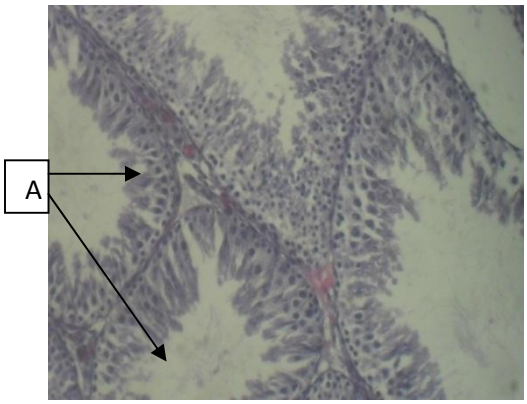

Plate 2: Rat testis given only Cadmium showing A, patchy spermatogenic arrest (H&E x 100)

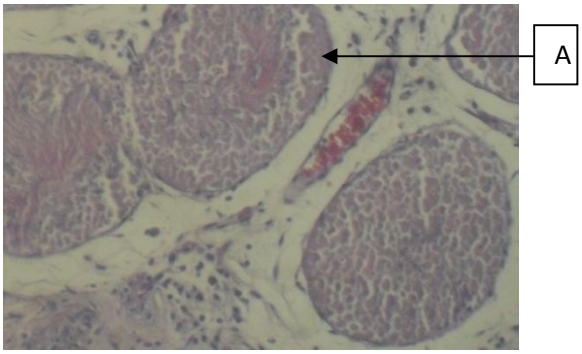

Plate 3: Rat testis given Cadmium plus 300mg/kg aqueous *V. angularis* extract showing A, moderate testicular degeneration (H&E x 100)

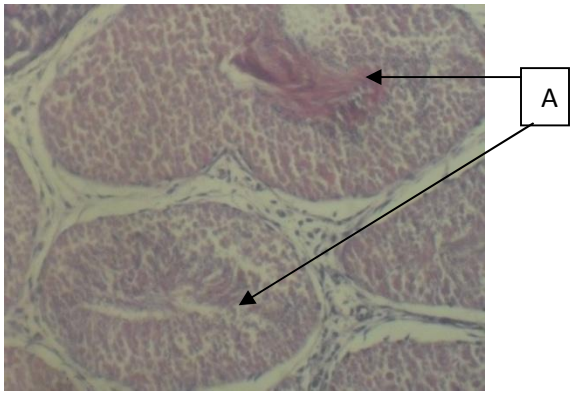

Plate 4: Rat testis given Cadmium plus 450mg/kg aqueous *V. angularis* extract showing A, many tubules with normal sequential maturation (H&E x 100)

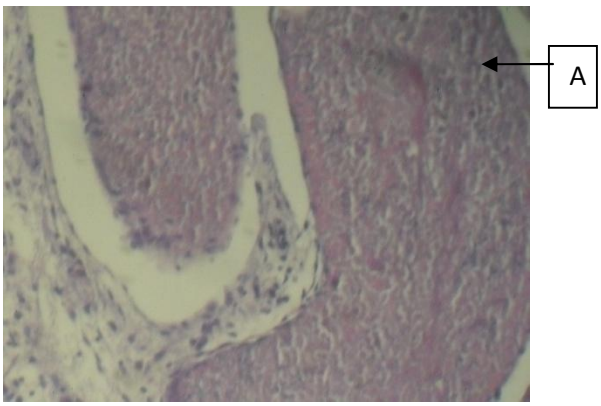

Plate 5: Rat testis given Cadmium plus 600mg/kg aqueous *V. angularis* extract showing A, moderate tubular degeneration (H&E x 100)

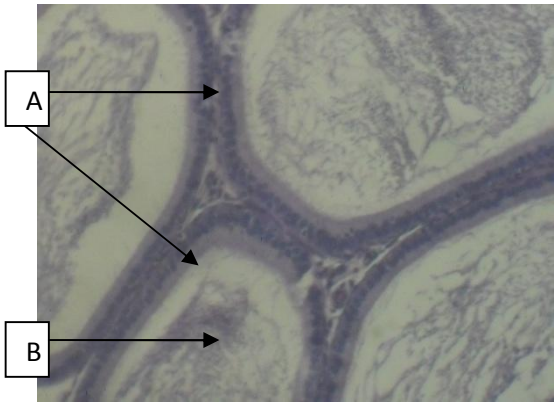

Plate 6 (Control): Rat epididymis composed of A, tubules packed with mature spermatozoa and B, interstitial space (H&E x 100)

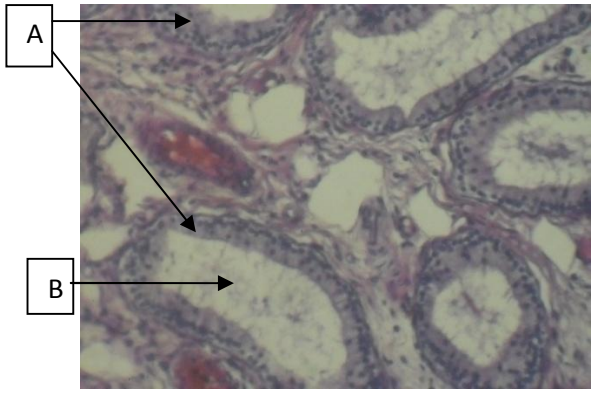

Plate 7: Rat epididymis given only Cadmium showing A, tubules with B, empty lumen (H&E X 100)

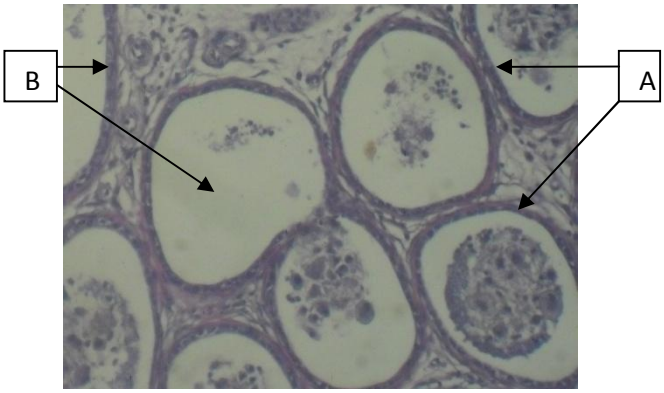

Plate 8: Rat epididymis given Cadmium plus 300mg/kg aqueous *V. angularis* extract showing A, many tubules with B, depleted luminal spermatozoa (H&E x 100)

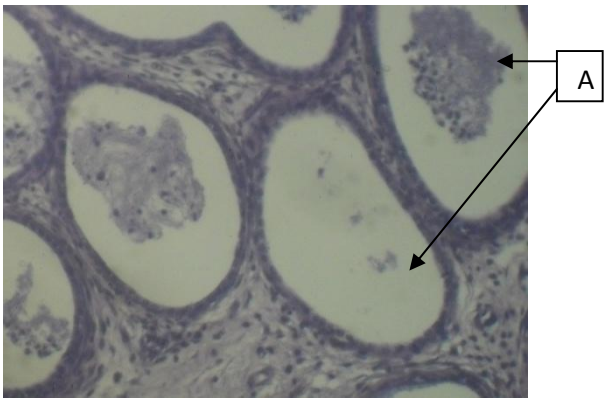

Plate 9: Rat epididymis given Cadmium plus 450mg/kg aqueous *V. angularis* extract showing A, some tubules with depleted luminal spermatozoa (H&E x 100)

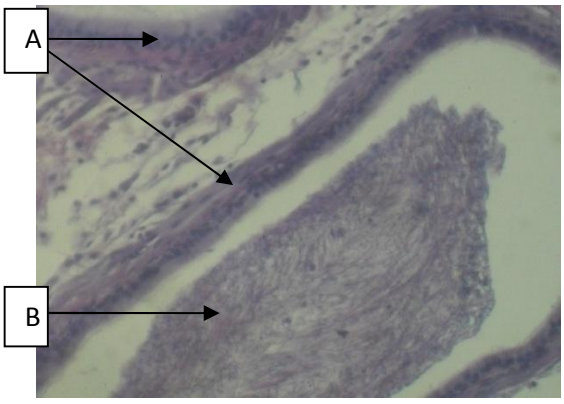

Plate 10: Rat epididymis given Cadmium plus 600mg/kg aqueous *V. angularis* extract showing A, tubules packed fairly full with B spermatozoa (H&E x 100)

Discussion

The protective effect of aqueous extract of *Vigna angularis* on cadmium-induced testicular changes in rat was investigated in this study. The gravimetric evaluation of the testes and prostate show significant reduction in relative organ weight. Previous studies (Blaco *et al.*, 2007) of acute exposure to the toxicant, has revealed a dose-dependent reduction in testes weight. The reports attributed these effects to the necrotic and degenerative changes occasioned by cadmium.

It is no longer news that cadmium increases reactive oxygen species (ROS) in the testes. In order to address this increase, cells have developed a sophisticated array of enzymatic and non-enzymatic antioxidant systems. The antioxidant enzyme system include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPx) and glutathione-s-transferase (GST). The biochemistry of these antioxidant enzymes involve the rapid conversion of the superoxide anion to hydrogen peroxide in the presence of SOD. The H_2O_2 formed in this way can easily form the destructive hydroxyl radical which is a powerful oxidizing agent. In order to prevent oxidative damage to DNA, proteins and unsaturated lipids in cells, the H_2O_2 is readily eliminated by either catalase or glutathione peeoxidase. In the testes, the predominating enzyme for the elimination of H_2O_2 is catalase (Peltola *et al.*, 1992).

The results of the present study showed that cadmium has an inhibitory effect on SOD activity in rat testes compared with the control. This effect is in line with our earlier reports (Adaikpoh *et al.,* (2007) and the reports of others (Ige *et al.,* 2012; Dzobo and Naik, 2013). In eukaryotes, two forms of the enzyme exist; Cu/Zn SOD and Mn SOD. The Cu/Zn SOD is found in virtually all eukaryotic cells. One of the proposed mechanisms of cadmium toxicity is that the metal competes with/and displaces zinc from enzymes, thereby inhibiting the activity of these enzymes (McMurray and Tainer, 2003; Casalino et al, 2002). This may explain the observed reduction in SOD activity, since SOD contains zinc in its active site. Similarly, cadmium in the testes causes a reduction in the uptake of zinc by spermatogenic cells (Lee and Dixon, 1973 and Ekhoye *et al.,* 2013). Zinc has been implicated in testicular development, sperm maturation and testosterone synthesis (Ebisch *et al*,. 2007) Administration of aqueous extract of *Vigna angularis* at the doses of 300 and 600mg/Kg body wt. effectively lifted the inhibition on the testes by cadmium. The elevated catalase activity observed in all the cadmium-exposed rats in this study, is probably an adaptation by the testes to mop-up the ROS generated by the exposure to the toxicant, especially in the presence of reduced SOD activity.

The fact that cadmium increases lipid peroxidation, as indicated by the elevated malondialdehyde levels in this study, is in consonance with earlier reports (Adaikpoh *et al,* 2007; Adaikpoh and Obi, 2009). However, treatment with graded doses of aqueous extract of *V. angularis* could not effectively prevent lipid peroxidation in the testes. Excessive production of free radicals has been implicated in infertility in man. Oxidative stress in the testes is capable of impairing the ability to produce viable spermatozoa (Aitken and Roman, 2008). It is therefore conceivable that this might be responsible for the low sperm count observed in all the cadmium exposed rats in this study. The low sperm count observed in this study agrees with the reports of Ekhoye *et al.,* (2013 and Doaa *et al.,* (2014) and is supported by the results of the histopathological study of the epididymis and testes reported in the present study. The histological study showed tubules with grossly depleted spermatozoa in the epididymis as well as degeneration of tubules and patchy spermatogenic arrest in the testes of all the cadmium exposed groups, except the group that was given the highest dose of the extract. This result of the histological evaluation of the testes is in line with the reports of Ekhoye *et al.,* (2013), which showed a dose dependent cadmium-induced severity in testicular morphological derangement.

There was a significant increase in testosterone, LH and FSH levels in the cadmium-only treated animals, relative to the control. This is in agreement with the reports of Oluyemi *et al.,* (2006). Testosterone is produced by the interstitial leydig cells of the testes and by the adrenal cortex. Since cadmium damages the testes, it is expected to impair the production of testosterone by the leydig cells and therefore, the serum testosterone increase may be from the adrenal gland which is the second site of testosterone production besides testes in males. It is also possible that the damage to the testes, caused by cadmium inhibits testicular uptake of LH and FSH hormones released by the anterior pituitary, leading to

an increase in serum LH and FSH levels in the group treated with only cadmium. Treatment with 300 and 450 mg/kg bwt of *V. angularis* aqueous extract was able to reverse these hormonal changes caused by cadmium.

Administration of cadmium to rats also resulted in elevated levels of cholesterol in the prostate and the testes which were successfully reduced in the prostate but not in the testes. This is in agreement with the results of Adaikpoh and Obi, (2009) who suggested that the increase in cholesterol might be an adaptation to resist oxidative stress induced by cadmium.

In this study, the results of the biochemical and histological analysis of the epididymis, the testes and its accessory organ, the prostate have shown that the treatment of cadmium-exposed rats with aqueous extract of *V. angularis* for 12 days could not effectively ameliorate the toxic effects of cadmium. However, it is not known if the treatment with the extract for a longer period will be more effective. This requires further investigation.

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