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Original Research

Cadmium-induced testicular toxicity, oxidative stress and histopathology in Wistar rats: Sustained effects of polyphenol-rich extract of Vernonia amygdalina (del.) leaf

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ABSTRACT

Background: Cadmium (Cd) is a toxic heavy metal of both environmental and occupational concerns. The health impact of ethno-botanical approaches in attempts to ameliorate its deleterious effects in biological systems should be an area of scientific interest since established therapies are often burdened with undesirable side effects. **Aim:** To determine the effects of polyphenol-rich extract of the leaf of *Vernonia amygdalina* (PEVA) on Cd-induced testicular toxicity, oxidative stress, and histopathology in Wistar rats. **Materials and Methods:** A total of twenty five (25) male Wistar rats were divided into five groups as follows: Group 1 (Control) received distilled water (0.2 ml/100 g i.p.) for 5 consecutive days and thereafter left untreated for 28 days. Group 2 received Cd alone at 5 mg/kg (i.p.) for 5 consecutive days. Group 3 was pre-treated with Cd as Group 2 and thereafter left untreated for a period of 28 days, whereas Groups 4 and 5 were pre-treated with Cd as Group 2 and thereafter received PEVA (orally) at two dose levels (200 and 400 mg/kg, respectively) for 28 days. **Results:** Cd administration induced reproductive toxicity as evidenced by lowered level of follicle stimulating hormone, luteinizing hormone, and testosterone ($P < 0.05$); perturbation of sperm characterization ($P < 0.05$); deleterious disruptions of the antioxidant system as evidenced by lowered levels of reduced glutathione and superoxide dismutase as well as elevation in thiobarbituric acid reactive substances level ($P < 0.05$); decrease in relative testicular weight ($P < 0.05$); and severe disseminated necrosis of the seminiferous tubules with terminally undifferentiated/necrotic cells as revealed by the histopathological examination. These conditions were sustained following administration of the two dose levels of PEVA. **Conclusion:** PEVA administration is not a suitable therapeutic choice for fertility enhancement in male Wistar rat model of Cd-induced decline in reproductive function.

KEY WORDS: Cadmium, polyphenol-rich extract, rats, testicular toxicity, *Vernonia amygdalina*

INTRODUCTION

The incidence of chemically-induced infertility appears to be on the increase worldwide [1]. Therefore, the toxic effects of environmental toxins and drugs on the human reproductive system have become a major health concern [2,3]. Cadmium (Cd), a toxic heavy metal of both occupational and environmental concern, has found its relevance in several industrial processes such as in electroplating and manufacturing of paint pigments, batteries, plastic, and fertilizers. However, in regions of inadequate exposure control, it readily bioaccumulates in biological systems where it induces deleterious health effects [4]. When released into the environment following occupational activities, it is readily absorbed from the soil by the root of plants; making food consumption a major source of its exposure [5,6]. It is readily absorbed by the body via

oral route (by means of Cd-contaminated water) and inhalation (particular in cigarette smoke) [7]. Unlike most heavy metals, once absorbed by the body Cd exposure can induce deleterious effects at relatively lower doses [8-10]. Its toxic effects are expressed in the testes even before pathological changes occur in other organs [11], although its main repository organ is the kidney [6,12,13].

Cd is reputed to exert its toxic effects by inducing reactive oxygen species (ROS) generation through oxidative damage [14]. These ROS, mainly O_2^+, H_2O_2 and OH^+ [15], initiate reactions with cellular biomolecules, and consequently, results in lipid peroxidation, altered the antioxidant system, membrane protein damage, DNA damage, and apoptosis [14,16]. It was, therefore, hypothesized that a potent antioxidant could retard or inhibit the basic mechanism of Cd-induced deleterious alterations (generation of ROS) and possibly ameliorate its toxic effects in biological systems. This hypothesis was tested using dietary polyphenols which are relatively cheaper, readily available, and considered to have considerable lesser side effects [17] as opposed to already established models (such as the use of dimercaptosuccinic acid) which are often more expensive, not readily available, and burdened with undesirable side effects [18].

The function of dietary polyphenols as defense against ROS is attributed to the fact that they are potent antioxidants [19]. Rich sources of polyphenols include vegetables, fruits, and whole grains [20]. *Vernonia amygdalina* leaf contains abundant polyphenols which most study considers the basis for its vast medicinal properties such as immune system strengthening [21], antidiabetic, and anti-inflammatory effects [22]. Stimulating additional research, these studies have pioneered a focus on the health effects of polyphenol-rich foods, supplementation/combination of several types of polyphenols as well as specific phenolic compounds [23]. Nevertheless, there is dearth of literature on the effects of dietary polyphenols on the reproductive function of male subjects that are exposed to Cd toxicity. This study, therefore, seeks to bridge this gap in knowledge using rat model.

MATERIALS AND METHODS

Plant Material and Chemicals

Fresh leaves of *V. amygdalina* were harvested from a private garden in Ile-Ife, Osun State Nigeria and certified by a Taxonomist in the Department of Botany, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria.

Assay kits for hormone analyses were purchased from Monobind Inc., Lake Forest CA 92630, USA (Accu-Bind ELISA Microwells). Cadmium sulfate salt was purchased from Guangzhou Fischer Chemical Co., Ltd, Guangdong, China.

Preparation of Polyphenols-rich Extract

The adopted procedure for obtaining polyphenol-rich extract of the leaves of *V. amygdalina* (PEVA) was as described in our previous study [4]; where the total phenol content of the extract was determined to be 681.70 ± 47.36 (mg of gallic acid equivalent/gram of the extract), and the total flavonoids content was determined to be 23.70 ± 1.78 (mg of quercetin equivalent/gram of the extract).

Stock Solutions of the Extract and Cd Salt

The choice of therapeutic doses of PEVA for this study (200 and 400 mg/kg) was guided by the predetermined oral LD_{50} of PEVA [4]; this was determined to be ≥4242.64 mg/kg in adult Wistar rats. Therefore, dose levels ≤10% of the oral LD_{50} 200 and 400 mg/kg were adopted for this study. The stock solution was prepared such that every 100 g rat received 0.2 ml of the extract orally. This is as described below:

Stock solutions of 200 and 400 mg of PEVA were prepared by dissolving 2 and 4 g of the extract in 20 mL of distilled water, respectively. Samples were stored in a deep freezer after use, and fresh samples were prepared every 48 h throughout the study period.

Stock solution of Cd salt was prepared by dissolving 50 mg of the salt in 20 mL of distilled water and was administered at 0.2 mL/100 g via intraperitoneal route for 5 consecutive days. Therefore, each rat received 5 mg/kg/day of Cd solution.

Experimental Design and Animal Management

All experimental protocols were in strict compliance with the guidelines for animal research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (National Academy of Sciences and National Institutes of Health Publications, 2011) [24] and approved by local Institutional Research Committee.

A total of 25 male Wistar rats, weighing 150-170 g, were used for this study. They were purchased from the Animal Holdings of the College of Health Sciences, OAU, Ile-Ife, Osun State, Nigeria, where the study was carried out. They were housed in plastic cages under natural light/dark cycle and allowed to have access to standard laboratory rat chow (Caps Feed PLC Osogbo, Nigeria) and water *ad-libitum*. The rats were divided into 5 groups of 5 rats each as follows: Group 1 (Control) received intraperitoneal administration of distilled water at 0.2 mL/100 g for 5 consecutive days after which they were left for 28 days before sacrifice. Group 2 (Cd) received intraperitoneal administration of Cd at 5 mg/kg/day for 5 consecutive days after which they were sacrificed. Group 3 (Cd + Recovery) was pre-treated with Cd as Group 2 and thereafter left untreated for 28 days. The PEVA-treated Groups 4 and 5 were each pre-treated with Cd as Group 2 and thereafter received oral two dose levels of the extract at 200 and 400 mg/kg, respectively, for 28 days before they were sacrificed.

Blood samples were collected by cardiac puncture into separate EDTA bottles after the rats were euthanized. These were centrifuged at 4000 rpm for 15 min at −4°C using cold

centrifuge (Centurion Scientific, Model 8881). The plasma was collected into separate plain bottles for the assessment of the reproductive hormones using enzyme-linked immuno sorbent assay (ELISA) technique. The left caudal epididymis of each rat was carefully excised for the collection of sperm that was used for sperm characterization. The left testis of each rat was kept in a cooler for homogenate preparation while the right testis was fixed in Bouin's fluid for histopathological examination using hematoxylin and eosin (H and E) staining technique. The experimental design and dose regimen is summarized in Table 1.

Body Weight and Testicular Weight Measurements

The body weight of the rats was determined weekly using a digital weighing balance (Hanson, China) while at the point of sacrifice; the testicular weight was determined using a sensitive weighing balance (Camry, China). The relative testicular weight (RTW) and percentage weight change (PWC) were calculated using the formulae below;

$$\text{Relative testicular weight } (\%) = \frac{\text{Left testis} + \text{Right testis}}{\text{Body weight at the point of sacrifice}} \times 100\%$$

$$\text{Percentage weight change } (\%) = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100\%$$

Sperm Characterization

Sperm fluid from the caudal epididymis was released onto a microscope slide, and epididymal sperm counts were made using hemocytometer and expressed as million/mL of suspension. The epididymal sperm motility was assessed by calculating motile spermatozoa per unit area and expressed as motility in percentage. By the method of Bloom and Fawcett [25], sperm viability was determined by preparing uniform smear spermatozoa on the slides using Eosin-Nigrosin stain.

Assay of Reproductive Hormones

The plasma levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were determined using standard laboratory protocols that were provided by the appropriate (aforementioned) kits. The adopted technique was ELISA technique.

Table 1: Experimental design and dose regimen

Groups	5 days	28 days
Control	DW	*
Cd	Cd*	-
Cd+Recovery	Cd	RP*
Cd+200 mg/kg PEVA	Cd	200 mg/kg PEVA*
Cd+400 mg/kg PEVA	Cd	400 mg/kg PEVA*

DW: Distilled water (0.2 mL/100 g via intraperitoneal route), Cd: Cadmium (5 mg/kg via intraperitoneal route), RP: Recovery period, PEVA: Polyphenol-rich extract of leaf of *Vernonia amygdalina* (oral administration), *Point that rats were sacrificed

Assessment of Oxidative Stress Indicators

The left testis of each rat was carefully excised and weighed. Using an electric homogenizer (SI601001), the testicular tissue was homogenized with 10 mL of sucrose solution (0.25 M). Thereafter, 10% homogenate in phosphate buffer (100 Mm) was prepared at pH of 7.4. The homogenate was centrifuged at 3000 rpm for 20 min, and the supernatant was collected for the assessment of the following indicators of oxidative stress;

The reduced glutathione (GSH) level was estimated by the method of Beutler *et al* [26]. The activity of superoxide dismutase (SOD) was determined by the method of McCord and Fridovich [27], whereas thiobarbituric acid reactive substances (TBARS) were determined as described by Ohkawa *et al* [28].

Histopathological Examination

Sample of the testicular tissue was dehydrated in graded alcohol and embedded in paraffin wax. Sections >4 μm thick were stained with H and E and thereafter viewed under a Leica DM750 Camera Microscope. Photomicrographs were taken at magnifications of ×40, ×100, and ×400.

Statistical Analysis

Results were expressed as mean ± standard error of mean and subjected to one-way analysis of variance. The data were thereafter subjected to Tukey's *post-hoc* test, and values at $P < 0.05$ were considered statistically significant. Graph pad Prism 5.03 (GraphPad Software Inc., CA, USA) statistical package was used for the statistical analysis.

RESULTS

Plasma Levels of FSH, LH, and Testosterone

The plasma level of FSH was significantly higher in Group 2 (0.49 ± 0.02) and lower in Group 3 (0.16 ± 0.01) when both were compared with Group 1 (0.27 ± 0.02) ($F = 131.9$; $P < 0.0001$). The PEVA-treated Groups 4 and 5 recorded no significant difference in plasma FSH level (0.18 ± 0.02 and 0.15 ± 0.01, respectively) when compared with Group 3 (0.16 ± 0.01) ($F = 1.566$; $P = 0.249$). Plasma level of LH was significantly lower in Groups 4 and 5 (0.18 ± 0.02 and 0.15 ± 0.01, respectively) when compared with Group 1 (0.27 ± 0.02) ($F = 29.95$; $P < 0.0001$). This is depicted in Figure 1.

There was a significantly higher level of plasma LH in Group 2 (0.38 ± 0.01) and a significantly lower level in Group 3 (0.20 ± 0.01) when both were compared with Group 1 (0.29 ± 0.01) ($F = 160.3$; $P < 0.0001$). The PEVA-treated Groups 4 and 5 recorded no difference in plasma LH levels (0.23 ± 0.01 and 0.21 ± 0.004, respectively) when

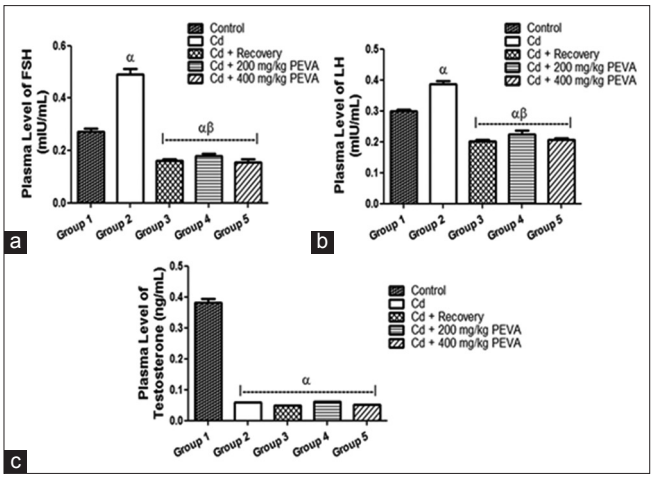


Figure 1: (a-c) Effects of polyphenol-rich extract of leaf of *Vernonia amygdalina* on the plasma level of follicle stimulating hormone, luteinizing hormone, and testosterone of rats exposed to cadmium toxicity. Each bar represents mean±standard error of mean (n = 5); α: Significantly different from Group 1 ($P < 0.05$); β: Significantly different from Group 2 ($P < 0.05$)

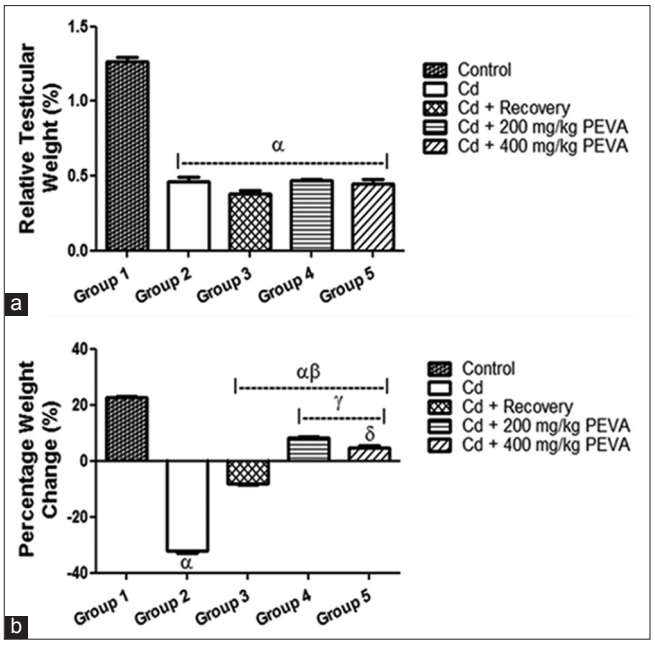


Figure 2: (a and b) Effects of polyphenol-rich extract of leaf of *Vernonia amygdalina* on the relative testicular weight and percentage weight change of rats exposed to cadmium toxicity. Each bar represents mean ± standard error of mean (n = 5); α: Significantly different from Group 1 ($P < 0.05$); β: Significantly different from Group 2 ($P < 0.05$); γ: Significantly different from Group 3 ($P < 0.05$); δ: Significantly different from Group 4 ($P < 0.05$)

compared with Group 3 (0.20 ± 0.01) ($F = 2.608$; $P = 0.115$). Plasma level of LH was significantly lower in Groups 4 and 5 (0.23 ± 0.01 and 0.21 ± 0.004, respectively) when compared with Group 1 (0.29 ± 0.01) ($F = 37.40$; $P < 0.0001$). This is depicted in Figure 1.

Plasma testosterone level was reduced significantly in Group 2 (0.06 ± 0.001) when compared with Group 1 (0.38 ± 0.01) ($P < 0.05$). This reduced level was found to be significantly sustained in Groups 3, 4, and 5 (0.05 ± 0.001; 0.06 ± 0.001; and 0.05 ± 0.001, respectively) when compared with Group 1 (0.38 ± 0.01) (F = 561.1; $P < 0.0001$). This is depicted in Figure 1.

RTW (%) and PWC (%)

Following exposure to Cd toxicity, Group 2 recorded a significant decrease in RTW (0.47 ± 0.02) when compared with Group 1 (1.26 ± 0.03) ($P < 0.0001$). This reduced level was significantly sustained in both Group 3 and the PEVA-treated Groups 4 and 5 (0.38 ± 0.02; 0.47 ± 0.01; and 0.45 ± 0.04, respectively) when compared with Group 1 (1.26 ± 0.03) ($F = 250.5$; $P < 0.0001$). This is depicted in Figure 2.

Groups 2 and 3 (−32.15 ± 0.72 and −8.32 ± 0.24, respectively) never recovered from the significant decrease that was recorded in the PWC when compared with Group 1 (22.50 ± 0.70) ($F = 2098$; $P < 0.0001$). Although the PEVA-treated Groups 4 and 5 recovered from the Cd-induced significant decrease in PWC (8.10 ± 0.62 and 4.70 ± 0.90, respectively), this was found to be significantly lower than Group 1 (22.50 ± 0.70) ($F = 158.5$; $P < 0.0001$). This is depicted in Figure 2.

Sperm Counts (million/mL), Motility (%), and Viability (%)

As summarized in Table 2, exposure to Cd toxicity was accompanied by a significant lowering ($P < 0.05$) of the aforementioned sperm characterization. This observed decrease was sustained in Group 3 and the PEVA-treated Groups 4 and 5 ($P < 0.05$).

Testicular Levels of GSH, SOD, and TBARS

The indicators of oxidative stress (GSH, SOD, and TBARS) in the testicular tissue were found to be significantly lowered following exposure to Cd toxicity in Groups 2 and 3 ($P < 0.05$). The PEVA-treated Groups 4 and 5 never recovered from the deleterious effects of Cd on the aforementioned indicators of oxidative stress in the testicular tissue as summarized in Table 3.

Histopathological Examination

Plate [1] shows evidence of apparently intact seminiferous tubules (ST) as well as active cell division and maturation of the germ cells as evidenced in abundance of terminally differentiated cells/spermatozoa (yellow arrow). Plates [2-5] show feature of severe disseminated necrosis of the ST (black arrows) as well as terminally undifferentiated, and necrotic cells, which are evidence of inactive/inhibition of cell division and maturation of germ cells (red arrow). Worthy of note is the fact that plate [3] reveals evidence of lump formation within the testicular interstitium (green arrow). There is no apparent evidence of tissue healing/restoration in the experimental groups [2-5] when compared with that of the control [1]. This is depicted in Figure 3.

Table 2: Effects of PEVA on sperm counts (million/mL), motility (%), and viability (%) of rats exposed to Cd toxicity

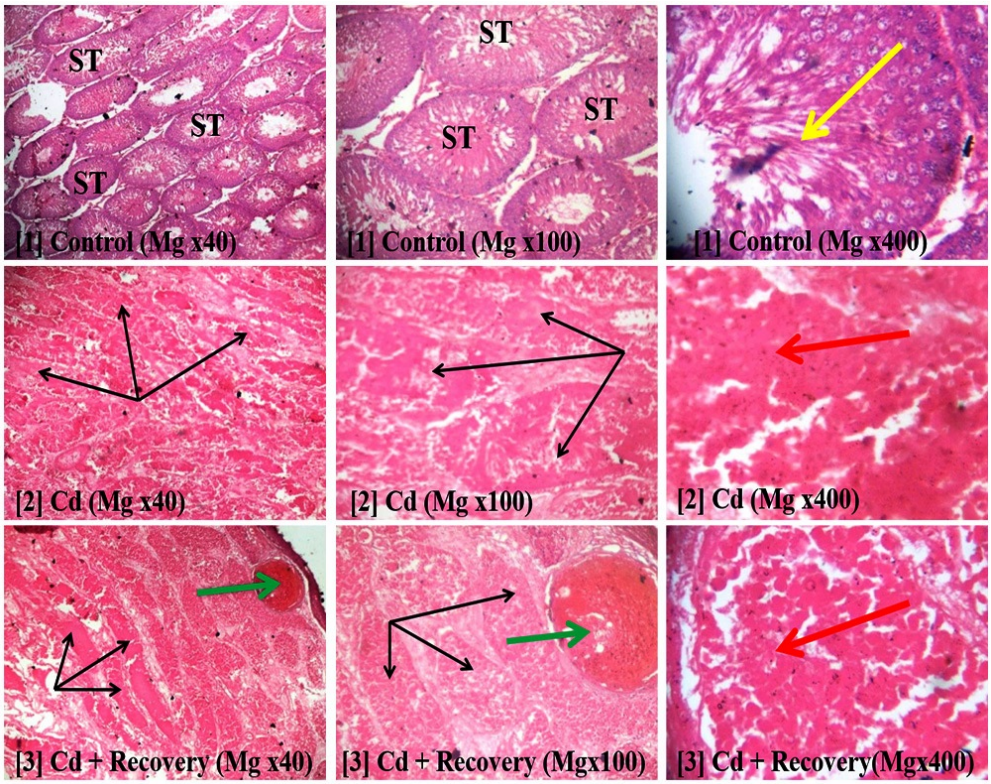
Sperm characterization	Groups (n=5)				
	Control	Cd	Cd+Recovery	Cd+200 mg/kg PEVA	Cd+400 mg/kg PEVA
Sperm counts (million/mL)	74.20±2.35	14.60±1.54$^{\alpha}$	16.40±1.86$^{\alpha}$	19.60±0.75$^{\alpha}$	18.80±1.20$^{\alpha}$
Sperm motility (%)	72.00±1.67	6.40±1.29$^{\alpha}$	8.60±0.81$^{\alpha}$	10.80±0.37$^{\alpha}$	9.80±0.86$^{\alpha}$
Sperm viability (%)	79.60±0.51	8.40±0.51$^{\alpha}$	9.20±0.58$^{\alpha}$	10.20±0.37$^{\alpha}$	10.00±0.45$^{\alpha}$

$^{\alpha}$Significantly different from Group 1 (P<0.05); n: Number of rats in each group. Cd: Cadmium, PEVA: Polyphenol-rich extract of leaf of *Vernonia amygdalina*

Table 3: Effects of PEVA on testicular levels of GSH, SOD and TBARS of rats exposed to Cd toxicity

Oxidative stress indicators	Groups (n=5)				
	Control	Cd	Cd+Recovery	Cd+200 mg/kg PEVA	Cd+400 mg/kg PEVA
GSH (μg/mg protein)	2.84±0.06	0.99±0.05$^{\alpha}$	0.88±0.06$^{\alpha}$	1.02±0.03$^{\alpha}$	0.95±0.02$^{\alpha}$
SOD (U/mL)	3.00±0.08	0.97±0.06$^{\alpha}$	0.86±0.02$^{\alpha}$	1.00±0.07$^{\alpha}$	0.91±0.06$^{\alpha}$
TBARS (nmol/mg protein)	0.10±0.009	0.35±0.01$^{\alpha}$	0.32±0.01$^{\alpha}$	0.31±0.02$^{\alpha}$	0.34±0.01$^{\alpha}$

$^{\alpha}$Significantly different from Group 1 (P<0.05); n: Number of rats in each group, Cd: Cadmium, PEVA: Polyphenol-rich extract of leaf of *Vernonia amygdalina*, GSH: Reduced glutathione, SOD: Super oxide dismutase, TBARS: Thiobarbituric acid reactive substances



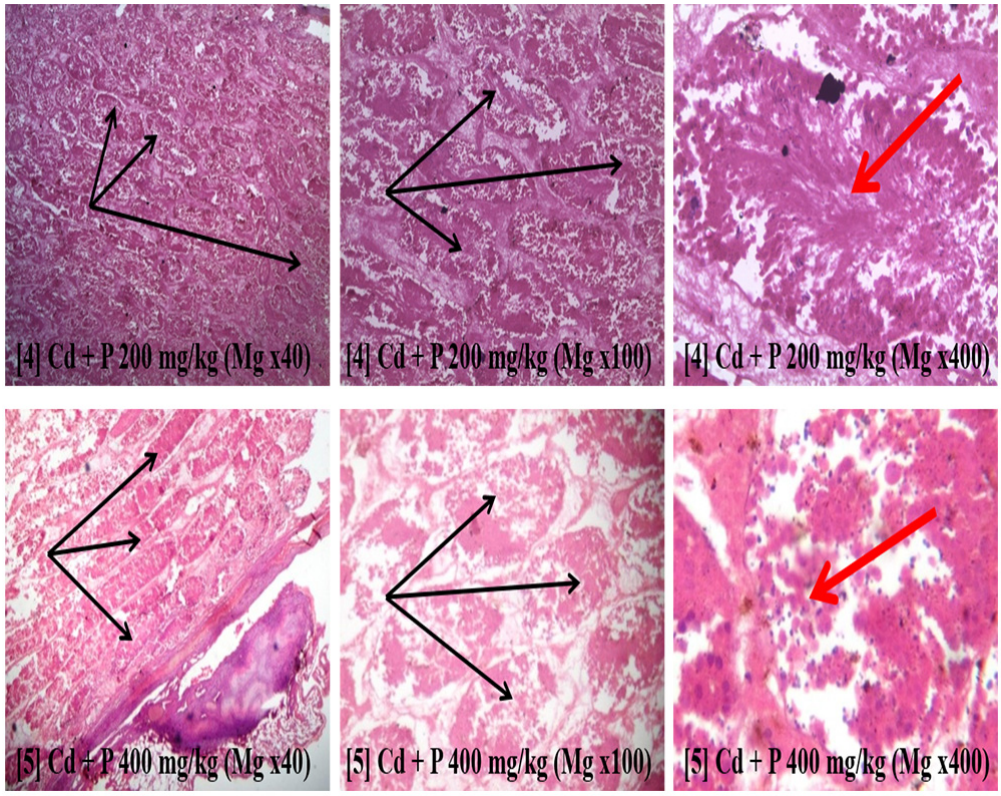


Figure 3: Photomicrographs showing the effects of PEVA on the testicular tissue of Wistar Rats exposed to cadmium toxicity. Mg: Magnification; PEVA: Polyphenol-rich extract of leaf of *Vernonia amygdalina*

DISCUSSION

The study investigated the effects of oral administration of polyphenol-rich extract of the leaf of *V. amygdalina* (PEVA) on Cd-induced reproductive dysfunction in male Wistar rats. This is apparently the first report of its kind. Overall, the findings of this study showed that PEVA administration sustained testicular toxicity, oxidative stress, and histopathology in the Wistar rat model of Cd-induced reproductive toxicity.

Basically, the biological activity of polyphenols is anchored on their remarkable antioxidant potential. There seem to be two generally accepted mechanisms of polyphenol antioxidant action; the first is the free radical-scavenging mechanism, whereby the polyphenols interferes to break any existing free radical chain reaction. The second being suppression of free radical formation by chelating metal ions that are involved in free radical production or regulation of enzyme activity.

However, another possible antioxidant pathway for these compounds (polyphenols) has been proposed to be a possible interaction with other physiologic antioxidants [29,30].

A biological connection exists between the reproductive hormones (follicle stimulating hormone, LH, and testosterone) such that any significant deleterious disturbance in one factor will initiate a possible deleterious alteration(s) in the other factors [31,32]. Follicle stimulating hormone (FSH), secreted by the anterior pituitary, is responsible for the initiation of spermatogenesis which is maintained by testosterone production by the Leydig cells in the testis. However, LH, also from the anterior pituitary, is responsible for the initiation of testosterone secretion by the Leydig cells [31,32]. The initialelevation of plasma FSH level, as recorded in this study, can be attributed to the sustained feedback loop to the anterior pituitary from the testis that was initiated by the decreased plasma testosterone level, in an attempt to restore normal plasma testosterone level. This was also accompanied by an initial increase in plasma LH level during the period of exposure to Cd toxicity. The representative photomicrograph showed evidence of severe disseminated necrosis of the ST, which is the sperm-producing tubes in the testes that are bordered by Leydig cells which secrete testosterone. This explains the decrease that was observed in the testosterone level (consequent availability of necrotic Leydig cells in the ST, which cannot respond to available plasma FSH and LH). The recovery period was associated with a sustained decrease in the plasma testosterone level and a later decrease in both FSH and LH levels. A possible explanation for this observation is subsequent deleterious effects of Cd toxicity on the anterior pituitary. The effects of Cd toxicity on the pituitary have been well demonstrated by Lafuente *et al* [33] and Farombi *et al* [34]. This result is consistent with the report of Prozialeck *et al* [11], who stated that the toxic effects of Cd are expressed in the testes even before pathological changes manifest in other organs. This study demonstrated that reproductive hormone aberrations associated with Cd-induced reproductive toxicity are irreversible in untreated rat model. There was, however, a sustained decrease in these reproductive hormones in the PEVA-treated groups

after the study period; evidence showing the unsuitability of PEVA as an amelioration therapy for subjects exposed to Cd-induced reproductive toxicity. This consequence may be a result of reduced efficacy resulting from inadequate interaction(s) with the available but reduced physiologic antioxidants and or an increased testicular threshold for the activation and expression of polyphenol antioxidant activity due to a relatively higher affinity of Cd for testicular biomolecular to bring about generation of ROS. This is subject to further verification.

Spermatozoa production in the epithelium of ST and androgen biosynthesis by the Leydig cells are two synchronized but independent functions in a biological system that controls overall testicular function [35]. Highly correlated with fertility, sperm counts provide information on the cumulative result of all stages in sperm production. This makes it one of the most reliable sensitive tests for spermatogenesis [36,37]. The study demonstrated that exposure to Cd toxicity was associated with reduced sperm counts which were sustained even after a recovery period in the untreated rats. As depicted by the representative photomicrographs, severe disseminated necrosis of the ST was accompanied by evidence of inactive/inhibition of cell division and maturation of germ cells. Pyruvate, a compound containing pyruvic acid (an intermediate compound formed during the metabolism of carbohydrates and proteins), is known to be the preferred substrate that is essential for the activity and survival of sperm cells [38,39]. Cd-toxicity could have been associated with highly decreased pyruvate production to bring about reduced sperm motility and viability. Sperm motility and viability are integral part of some reproductive toxicity guidelines and are considered as crucial in sperm characterization [40,41]. The treatment with two dose levels of PEVA was associated with a sustained decrease in the aforementioned sperm characterization. It is, therefore, hypothesized that severe disseminated necrosis of the ST accompanied by evidence of inactive/inhibition of cell division and maturation of germ cells may be associated with decreased pyruvate production with a remarkable potential to mask the expression of testicular antioxidant activity of apparently potent antioxidants (such as PEVA).

Exposure to Cd toxicity was associated with over 2-fold decrease in the RTW of the rats and was further decreased in the recovery group by over 3-fold; a feature that was sustained in the PEVA-treated groups. The mass of differentiated spermatogenic cells in the ST is the principal determinant of testis weight [42]. Furthermore, the availability of testosterone in the circulation is required for the growth, development, and maintenance of male reproductive organs [43]. Hence, the observed decrease in RTW can be attributed to the severe disseminated necrosis of the ST (with inhibition of spermatogenic cell differentiation) associated with a sustained decrease in plasma levels of testosterone. Although from a renal study, PEVA has been reported to show evidence of ameliorative potential on Cd-induced nephrotoxicity [44], the fact that testicular necrosis (associated with reduced RTW) was sustained following the two dose levels of PEVA treatment in rats model of Cd-induced testicular toxicity is worthy of further investigation.

The rats were observed to undergo a state of depression and isolation during the period of Cd administration. Irregularities in feeding pattern were also observed as feeds and water were left (almost) untouched in the feeding trough and drinkers, respectively, during this period. The Cd-induced decrease in PWC of the rats may be attributed to its neurotoxic effect. This may have suppressed the feeding center in the lateral hypothalamus (Katherine *et al*, 2008) with a consequent inhibition of appetite. Cd has been reported to cause deleterious alteration in the feeding pattern of Wistar rats [4]. Since weight gain or loss is determined by a balance between dietary intake and energy expenditure [45], the decrease in PWC as observed in this study may be attributed to the deleterious alteration in feeding pattern that was Cd-induced. However, the study recorded an increase in PWC in the recovery group when compared with the toxic group, corresponding to the gradual restoration of the feeding pattern that was observed; an observation that was improved upon treatment with PEVA. Thus, this study demonstrated that PEVA-administration is associated with improved appetite in rat model of Cd-induced deleterious alteration in feeding pattern. This is consistent with our previous finding [4].

Superoxide dismutase (SOD), a free radical scavenger and a chain reactor terminator, is known to eliminate superoxide by converting it to hydrogen peroxide [46] which is effectively removed by GSH [47,48]. GSH is considered to probably be the most important antioxidant that is present in the body cells since it effectively removes hydrogen peroxide and serves as a cofactor for glutathione transferase, which helps to remove certain reactive molecules, drugs, and chemicals from cells [47,48]. Oxidative stress results in toxicity when the rate of ROS production exceeds the cell capacity for their removal. The consequence is lipid peroxidation which is an autocatalytic process that results in cell death. TBARS is one of the end products of lipid peroxidation [49]. The decreased level of GSH in the testis, as recorded in this study, can be attributed to its excessive use by the testicular tissue to scavenge the free radicals that were generated following exposure to Cd toxicity and or reduced GSH production by the tissue; a consequence evidently enhanced by the increased use of SOD in the oxidative process which resulted in its reduced testicular level. On the other hand, the increased TBARS activity shows evidence of enhanced lipid peroxidation [48] leading to tissue damage and failure of the antioxidant defense system to prevent ROS production. This result is in contrast to the report of Imafidon *et al* [44] on the antioxidant effects of PEVA in rats with Cd-induced nephropathy.

To reduce the risk of sustained/irreversible infertility, as recorded in this study, it is recommended that Cd-exposed subjects resort to prompt and efficacious treatment/management therapy. Evidently, PEVA treatment is an unsuitable therapeutic choice of amelioration for subjects with Cd-induced testicular toxicity. Based on the fact that its antioxidant potential(s) was not masked in kidney tissue with the same condition of toxicity [44], PEVA therapy can be an option for subjects of Cd toxicity that are indifferent about fertility outcome(s) post treatment.

CONCLUSION

Exposure to Cd toxicity is associated with attenuation of reproductive function which is irreversible in untreated rat model. Furthermore, treatment with PEVA sustained testicular toxicity, oxidative stress, and histopathology in rat model of Cd-induced toxicity. This can be attributed to a high testicular threshold for the activation and expression of "polyphenol antioxidant activity," enhanced by high activity of lipid peroxidation which possibly resulted from a relatively higher affinity of Cd to testicular biomolecules. Therefore, PEVA administration is not a suitable therapeutic choice for fertility enhancement in male subjects with Cd-induced decline in reproductive function.

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