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Observed germ cell loss restoration with improved hematological indices by aqueous *Acalypha wilkesiana* leaf extract in cadmium-induced testicular toxicity on adult Wistar rats

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Abstract

Introduction: Cadmium (Cd) is an industrial pollutant of occupational and environmental concern while *Alcalypha wilkesinia* (AW) is a medicinal plant rich in natural antioxidant molecules.

Objective: To evaluate Anti-toxicity effect of aqueous extract of *Acalypha wilkesiana* leaf on cadmium.

Methods: 25 male wistar rats assigned into 5 groups (n=5) and treated as Control (Distilled water), Group-1 (2 mg/kg $CdCl_2$), Group-2 (2 mg/kg $CdCl_2$ + 400mg/kg AW), Group-3 (2 mg/kg $CdCl_2$+ 800mg/kg AW), Group-4 (2 mg/kg $CdCl_2$+ 1600mg/kg AW) for 14 days and sacrificed on the 15th day for heamatological parameters, biochemical assay and histological analysis

Results: Observed were significant decrease ($P < 0.05$) in mean abnormal sperm and increase in mean percentage motility in Control, Group -2 Group -3 and Group-4 when compared to Group-1 with same trend recorded for the mean testosterone and Hemoglobin (Hb) levels, Packed cell volume (PCV) ,Red blood cell (RBC) count and number of germ cell line. Distorted histoarchitecture of seminiferous tubules was seen in Group-1, 2, and 3, which were improved in Goup-4 compared to normal cells seen in control group. Also, the thickness of the germinal epithelium was significantly increased ($P < 0.05$) in Control and Group-4 compared to Group-1, 2, and 3.

Conclusion: Aqueous Acalypha wilkesiana improved biochemical parameters and testicular histoarchitecture in cadmium chloride exposed adult wistar rats.

Keywords: Germ cell; Pack Cell Volume; Cadmium; *Acalphya wilkesinia*; Wistar Rats

1. Introduction

Cadmium (Cd) is a toxic heavy metal with a biological half-life of 10–35 years in humans and animals (1). Generally, cadmium exposure occurs by ingesting contaminated water, inhaling contaminated air or by smoking. For example, it has been shown that tobacco smoking is a good source of Cd exposure (2). Cadmium (Cd) is also an industrial pollutant

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that accumulates in the body causing health hazards in organs such as brain and ovary of human and animals (3). Daily dietary intake of Cd varies with pollution (4). People working in mines and high-risk environments could wear mask to reduce inhalation of harmful substances (5). Cd enters the body through respiration and cause damage to the liver, kidneys (6), and testes. The testes have been shown to be very sensitive to cadmium toxicity (7). Also, exposure to small quantity of Cd could increase the mortality rate of prostate cancer and could be hazardous to female reproduction and embryonic development (8). In fact, it is known that Cd exposure at a minimal temperature is detrimental to the health, such as increasing the risk of conditions such as cardiovascular (9) and reproductive disease (10). Cd also causes damage to the testes leading to testicular dysfunction and the consequent impairment to the process of spermatogenesis occasioned with reduced testosterone level (11). While the kidney and liver are reportedly the main targets for Cd concentration, it was also shown to accumulates profoundly in the heart tissue (12). Accumulation of Cd in the heart is linked with high chance of heart failure, acute myocardial infarction and cardiovascular mortality (9). Furthermore, accumulation Cd has been shown to cause neurological defects in the brains (13), inducing the corpus luteum degeneration and deplete follicular growth (14). Marettova et al. also showed that exposure to CD induced nuclear fission leading to the formation of dense bodies and slight alteration in the size of endoplasmic reticulum in rats, mice and cock's sertoli cells (7).

Several studies have reported the diverse toxic effects of Cd on male reproductive organs, dysregulation of the hypothalamic-pituitary-testicular axis function, alterations in endocrine function and hormonal levels (15, 16). Exposure to Cd causes different types of cell death in germ cells. For example, it was shown that germ cell apoptosis was increased by downregulating sertoli cell autophagy after exposure to Cd (17). A study by Marcela et al. revealed that exposure to high dose of cadmium reduced male sexual behavior, sexual incentive motivation and reduced serum in adult male rats (18). Another study indicated the role of Cd in reducing mounting frequency in male rats (19). These studies all together showed the damaging role of cadmium on testicular function and consequently disrupting the process of spermatogenesis. Therefore, many recent studies now focus on exploring the possible protective effects of medicinal plants against testicular toxicity.

Different cultures have explored many herbal plants in folk medicine as potential remedies resulting in the discovery of varieties of pharmacological agents exerting their effects on renal tissues and hepatocytes via inhibition of free radicals (20). The different parts used includes fruits, stems, leaves, flowers, twigs, exudates and modified plant organs. While some of these raw plant materials are fetched in small quantities by the local communities and traded off, serving as raw materials for many herbal industries, others are collected in abundance to be processed industrially as pharmaceutical products (21).

*Alcalypha wilkesinia (*AW), which is a known medicinal plant commonly referred to as copper leaf or as fire dragon in the South Pacific Islands belongs to the genus Acalypha. AW leaves and are widely varied with cream, bronze, red and yellow coloration (22). This plant constitute the main source of natural antioxidant molecules and has been shown to prevent or control diseases related to their constituents antioxidant properties (23, 24). For example, in Southern Nigeria, the boiled leaves of AW is commonly used in traditional health care practices as therapeutic agent against gastrointestinal disorders, hypertension, skin infection and diabetes mellitus due to the anti-inflammatory and antioxidant properties of its bioactive constituents, such as tanins, steroids, terpenoids, phytates, alkaloids, flavonoids, cardiac glycoside and phenolic compounds (25, 26).

A study on Gas chromatography-mass spectrometry (GC-MS) analysis of the leaf extract of AW by Aknloye et al. (24) showed that in vitro antioxidant property of AW is characterized by the presence of 2-bromodecane, 2,6,10-trimethyldodecane, eicosane, nitric acid, hexadecane, methyltridecane, undecane, 2-bromonane, 6-methyl dodecane, and acetic acid. Therefore, this study was carried out to validate the therapeutic efficacy of the aqueous extract of AW on Cd-induced testicular dysfunction in adult male wistar rats.

2. Materials and methods

Twenty-five (25) healthy male adult wistar rats (5 weeks old) weighing 127±8g were used for this study. The rats were housed in the Experimental Animal unit of Anatomy Department, Olabisi Onabanjo University, Shagamu Ogun state, Nigeria. They were covered in cages that allows easy ventilation. During this period, the animals were acclimatized under standard condition of 12-hour light/12-hour darkness with a temperature range of 25±3^{0}C and mean relative humidity of 50±5%. The rats were administered feed and water during this period.

2.1. Plant materials

Fresh Acalypha wikesiana leaves were obtained from the horticulture garden of O.O.U campus, Shagamu, ogun state. Taxonomic identification and authentication of the plant was established in the Department of Pharmacology, Olabisi Onabanjo University, Shagamu, Ogun state, Nigeria. After plucking from the branches, the leaves were properly washed, dried under room temperature for 11 days, then grinded into fine powder with the aid of a mechanical blender and weighed.

2.2. Plant extraction

700 g of the dried, grounded plant materials was soaked in 5 liters of water in a plastic container with constant shaking from time to time for even distribution of solvent to sample. It was stirred and kept at the pharmacology laboratory overnight. The solution was filtered using a clean handkerchief, then poured into a rotatory evaporator which was used for the gentle removal of solvent from the solution by evaporation. The extract was then transferred into a pre-weighed

beaker and allowed to cool in a fume cupboard. Thereafter, the beaker containing the extract was weighed and the weight of the dried extract was derived:

$$\text{\% yield was calculated as: } \frac{\text{Weight of dried extract obtained}}{\text{Weight of plant material used}} \times 100\%$$

Subsequently, the extract was dissolved in Tween-80 and distilled water (Tween-80/water) to give a homogenous solution equivalent to the dose level of 100 mg/mL. The extract was reconstituted in distilled water to obtain various concentrations of the extract, given orally daily for fourteen days

2.3. Experimental design

The rats were randomly assigned into five groups: Control, Group-1, Group-2, Group-3 and Group-4 with 5 animals each. The animals in Control were fed with feed and water only for 14 days, animals in Group-1 were given 2mg/kg of cadmium chloride ($CdCl_2$), after 48 hours, they received another 2 mg/kg dose of $CdCl_2$, and then left untreated. Group-2, Group-3 and Group-4 animals were given 2 mg/kg dose of cadmium chloride, after 48 hours they received another 2 mg/kg dose of $CdCl_2$. In addition, after 24 hours, animals in Group-2, 3, and 4 were treated with 400mg/kg, 800mg/kg, 1600mg/kg of aqueous AW extract, respectively, for 14 days. 24 hours after the last treatment with AW, blood were collected from the eyes of all the animals for biochemical assay and full blood cell count after which the animals were sacrificed by cervical dislocation. The left testes were harvested through a long incision made in abdominal cavity, weighed and then immediately fixed into 10% formal saline solution for histological analysis.

2.4. Blood sample collection

24 hours after the last treatment with aqueous extract of AW leaf, blood samples were collected from the animal via inserting a capillary tube in the retro-orbital plexus. Blood sample was collected in a test tube, rinsed with heparin saline solution as anticoagulant, in the proportion of 50 µl for each 1.0 mL of blood and used for the complete blood count. Whole blood was used for analyses of the Packed cell volume (PCV), total Red Blood Cell (RBC), total White Blood Cells (WBC) or Leukocytes, Platelet (PLT), Neutrophil (NEU), Lymphocyte Percentage (LYM) and hemoglobin content (Hb). Using non-coagulant, the serum was separated by centrifugation at 3000 rpm for 20 minutes and kept at -200C until further biochemical analysis (Testosterone, FSH)

2.5. Tissue Sample collection

The rats were sacrificed via cervical dislocation. The left epididymides were removed to collect semen for sperm analysis (sperm motility and morphology). Also, the left testes were removed, weighed and fixed in Bouin's fluid for histomorphological evaluation.

2.6. Procedure of sperm analysis

The left epididymis of each rat was crushed in a container to get the sperm and poured in a container containing the mixture of sodium carbonate, formalin and water. This serve as diluting fluid and for preservation. The semen was put in the Neubauer Counting Chamber, cover slipped, and observed under microscope. The sperm motility and morphology were determined and expressed in percentage.

2.7. Hematological Parameters

2.7.1. Full Blood Count (FBC)

Blood sample from the animal groups were collected in EDTA bottle and mixed gently. For PCV, the plain capillary tube was used to collect the blood sample from the EDTA bottle with one end of the capillary sealed with the aid of burnsen burner. The sample was transferred to heamatocrit, and centrifuged for 5 minutes and the heamatocrit reader was used to read the level of the blood.

2.7.2. White Blood Cell (WBC) Total Count

20µl of blood was pipetted into 380µl of Tusk's fluid and mix gently until the counting chamber was loaded. Blood cells were then observed and counted under the light microscope (X40).

2.7.3. White Blood Cell Differential

A drop of blood was placed on a clean grease free slide, cover slipped and drag to make a thin film. The slides were allowed to air dried. After drying, Leishman stain was applied on the slide and left for 3 minutes, and then diluted with buffer H_2O and left for 8 minutes, then rinsed with buffer H_2O. The slides were allowed to air dried, then applied a drop of immersion OH on the slide and then observed under light microscope (X100)

2.8. Hormonal Assay

2.8.1. Determination of serum FSH and testosterone level

The serum was stored at -20 °C and assay was completed within three months. For determination of serum LH and testosterone by ELISA (Enzyme Linked Immunosorbant Assay), the kit was obtained from Fortrees Diagnostic Limited, United Kingdom and north Ireland and the procedure performed according to the methods of Saxema et al. (27)

2.9. Histomorphological evaluation

48 hours after fixation, testicular tissues were dehydrated in ascending grades of alcohol, then cleared in two changes of xylene and embedded with paraffin wax. Thereafter, Tissues were sectioned into 5µm via microtome. The tissue sections were deparaffinized, hydrated gradually and then stained with H & E. In each section, 50 round seminiferous tubule epithelia were observed by a pathologist who is blinded to the experiment with the aid of optical microscope (Olympus IX71) equipped with camera (Olympus E-3-). Furthermore, the number of spermatogonia, spermatocytes, spermatids, spermatozoa, sertoli, myoid and leydig cells were counted, and the thickness of the germinal layer and diameter of the seminiferous tubules were measured in all animal groups with the aid of ImageJ software (ImageJ 1.5.4). Results were expressed as mean ± standard deviation (SD) for each group.

2.10. Statistical analysis

Experimental data were analyzed by SPSS software (version 22.0 for windows; SPAA Inc. Chicago, IL, USA) and Microsoft Excel for windows (version 2016). Graphs were plotted with the aid of Graphpad Prism (5.0). All results were expressed as means ± SD. Statistical significant difference between groups were determined by one-way analysis of variance. Multiple comparisons were made using the Tukey post hoc test and the difference were considered significant at $P < 0.05$.

3. Result

3.1. Sperm motility and morphology

24 hours after oral treatment of animals with aqueous extract of AW, the epididymis of all the animals in all groups were observed for percentage of motile and abnormal sperms. The result showed that there was significant decrease ($P < 0.05$) in the mean percentage sperm motility of animals in Group-I, Group-2, and Group-3 when compared to that of Control. Also, the mean sperm motility in Group-2, Group-3 and Group-4 were significantly higher ($P < 0.05$) than mean sperm motility in Group-1 . 1However, there were no significant difference ($P > 0.05$) when the mean percentage sperm motility of animals in Control and Group 4 (Fig. 1A).

The mean percentage of abnormal sperm cells were significantly higher ($P < 0.05$) in animal Group-1, Group-2, Group-3 and Group-4 than the Control, whereas the mean abnormal sperm in Group-3 and Group-4 were significantly lower

(P < 0.05) than the mean observed in Group 1. There was no significant difference (P > 0.05) in the mean percentage of abnormal sperm cells observed in Group-1 and Group-2

3.2. Hormonal assay

The serum level of testosterone and FSH were determined in all animal groups as shown in Fig. 1C and 1D. Surprisingly, the result of our analysis showed no significant difference (> 0.05) in the mean FSH in all animal groups whereas the mean testosterone level of animals in Group 1, Group 2 and Group 3 significantly decreased (P < 0.05) when compared to the mean level of animals in Control group and Group-4. However, we observed no significant difference when the mean testosterone level of animals in Group-4 was compared to that of Control.

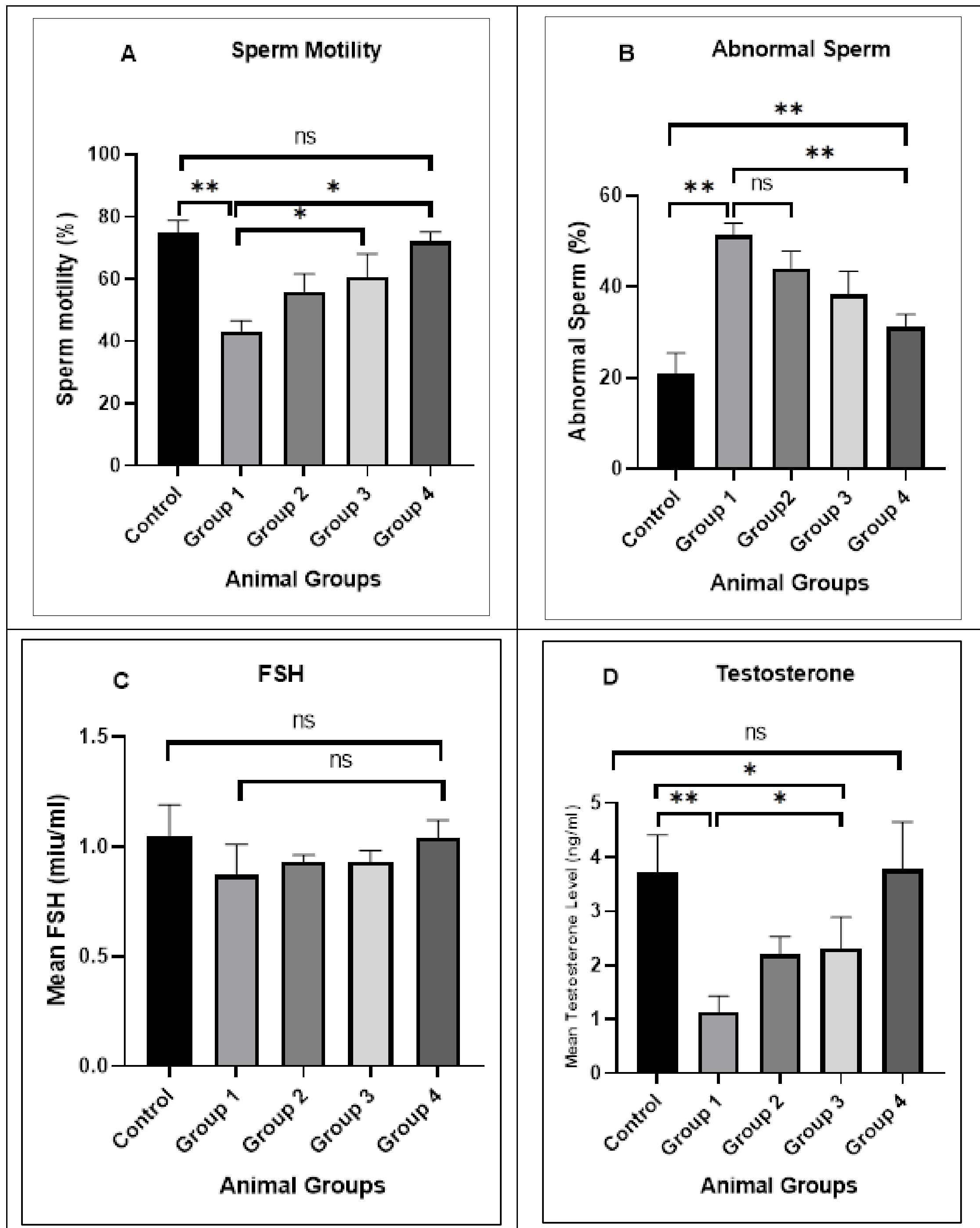

Figure1 A - Mean percentage sperm motility, **Figure1**B - Mean percentage of abnormal sperm, **Figure1** C - Mean follicle stimulating hormone (FSH) level (miu/ml) and **Figure1** D - Mean testosterone level (ng/ml) in all animal groups, assessed 24 hours after the last treatment with aqueous AW leaf extract

Control (feed and water only), Group-1 (2 mg/kg CdCl2/twice/orally only), Group-2(2 mg/kg CdCl2 twice + 400 mg/kg aqueous AW leaf extract/14 days/orally), Group-3 (2 mg/kg CdCl2/twice + 800 mg/kg aqueous AW leaf extract/14 days/orally), Group-4 (2 mg/kg CdCl2/twice + 1600 mg/kg aqueous AW leaf extract/14 days/orally), Data analyzed by One-way Anova followed by Post Hoc Tukey tests. ** = P < 0.001; * = P < 0.05; ns = P > 0.05

3.3. Hematological indices

The results of the hematological profile in all animal groups after treatment with aqueous AW leaf extract for14 days are presented in Table 1. The overall analysis for erythrocytes revealed significant decrease (P < 0.05) in the mean level of packed cell volume (PCV), red blood cell (RBC) and Hemoglobin (Hb) in animal Group 1, Group 2, and Group 3 when compared to the Control group. However, there was no significant difference in the mean level of these blood parameters between Control and Group 4. Similarly, the mean level of neutrophils was significantly reduced (P < 0.05) in Group 1, Group 2 and Group 3 when compared to Control whereas administration of 1600 mg/kg body weight of aqueous extract AW leaf extract restored neutrophil level in Group 4 animals with no significant difference (P > 0.05) when compared to the mean level of neutrophils in Control animals. Surprisingly, there is no significant difference in the mean level of platelets and lymphocytes in all the animal groups (Table 1).

Table 1 Mean values of hematological parameters (± SD)

Parameters	Control (Mean ± SD)	Group-1 (Mean ± SD)	Group-2 (Mean ± SD)	Group-3 (Mean ± SD)	Group-4 (Mean ± SD)
PCV (%)	63.00 ± 1.57	51.40 ±1.52**	53.00 ± 3.46**	56.00 ± 1.22**	61.40 ± 2.25#
RBC (x 10^6/mm^3)	10.60 ± 1.34	7.20 ± 0.45**	7.00 ± 1.00**	8.00 ±0.71#	10.20 ± 0.45#
WBC (x 10^3/mm^3)	15.04 ± 0.04	6.15 ± 0.13*	8.87 ± 1.47*	9.58 ± 1.79*	14.05 ± 0.08#
PLT (x 10^3)	209.60 ± 8.65	190.60 ± 9.04#	192.80 ± 12.21#	198.2 ± 15.47#	202.80 ± 12.83#
NEU (%)	69.4 ± 5.41	55.20 ±3.27**	53.20 ± 2.05**	57.20 ± 2.95**	70.6 ± 3.36#
LYM (%)	44.80 ± 3.96	41.20 ± 2.77#	39.80 ± 3.42#	39.40 ± 3.36#	44.60 ± 4.77#
Hb (g/dl)	19.63 ± 0.40	17.02 ± 0.41**	17.20 ± 0.70**	18.00 ± 0.39**	19.48 ± 0.73#

Control (feed and water only), Group-1 (2 mg/kg CdCl2/twice/orally only), Group-2(2 mg/kg CdCl2 twice + 400 mg/kg aqueous AW leaf extract/14 days/orally), Group-3 (2 mg/kg CdCl2/twice + 800 mg/kg aqueous AW leaf extract/14 days/orally), Group-4 (2 mg/kg CdCl2/twice + 1600 mg/kg aqueous AW leaf extract/14 days/orally), SD: standard deviation, PCV = packed cell volume, WBC = white blood cell, PLT = platelets, NEU = Neutrophil, LYM = Lymphocytes, Hb = Heamoglobin, * = P < 0.05 vs Control, ** = P < 0.001 vs Control, # = P > 0.05 vs Control

3.4. Histomophological assessment of the seminiferous tubules

H & E stained sections of seminiferous tubules of animals in all the groups were examined under light microscope. Histoarchitecture of seminiferous tubules of animals in Control revealed normal morphological appearance with substantial amount of germ cells (Fig. 2A). Spermatogonia, spermatocytes, spermatids, spermatozoa, sertoli, myoid and leydig cells were all observed in regular manner. Also, the seminiferous tubules of animals in Group 4 showed improved germ cells and normal morphological appearance. Likewise, the seminiferous tubules of animals in Group 3 showed improved cellularity when compared to the group $CdCl_2$ only group (Group-1) and that which received lower dose of the aqueous AW leaf extract (Group-2). The seminiferous tubules of animals in Group 1 and Group 2 showed abnormal histomorphology with obvious distorted germinal epithelium, very few germ cells, sertoli and leydig cells. The seminiferous tubules of animals in Group-3 also showed a lower degree of abnormal histoarchitecture but with more number of germ cells compared to Group-1 and Group-2.

The number of germ cells were counted in the seminiferous tubules of all animals in all groups and the results presented in (Table 2). The mean number of spermagonia, spermatocytes, spermatids, spermatozoa, sertoli, and leydig cells were significantly reduced (P < 0.05) in Group-1, Group-2 and Group-3 when compared to Control and Group-4. Although animals in Group-3 showed improved number of spermatogonia, sertoli, myoid and leydig cells. Surprisingly, there was no significant difference in the mean number of myoid cells in all animal groups. Furthermore, there were significant reduction (P < 0.05) in the thickness of the germinal layer of animals in Group-1, Group-2 and Group-3 compared to Control and Group-4. The mean diameter of seminiferous tubules was also significantly reduced (P < 0.05) in Group-1 and Group-3 when compared to Control and Group-4. Also, the mean weight of the testes of animals in Group-1 and Group-2 animals were significantly reduced (P < 0.05) when compared to the mean testes weight of animals in Control, Group-3 and Group-4.

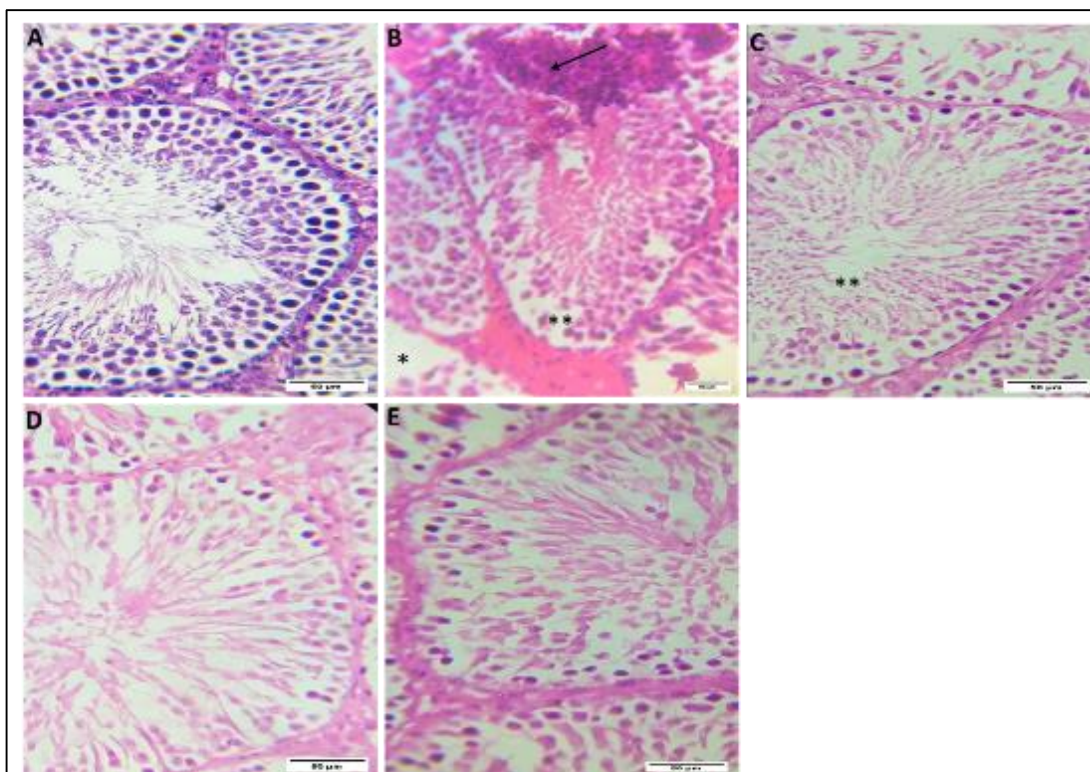

Figure 1 Light microscopy of H & E stained testicular tissue in experimental groups 24 hours after 14 days of treatment with aqueous AW leaf extract. A: Control - normal testicular histology, B: Group 1 – necrosis in epithelium (**), necrosis in interstitium (*), hemorrhage in epithelium (→), C: Group 2 – increased cellularity with organized and improved tubular structure, but with necrosis in epithlium and insteritum; Group 3: increased cellularity with organized and improved tubular structure, but with necrosis in epithelium and insteritum; Group 4: increased cellularity with organized and improved tubular structure

Table 2 Histomorphology of the Seminiferous tubules

parameters	Control (Mean ± SD)	Group-1 (Mean ± SD)	Group-2 (Mean ± SD	Group-3 (Mean ± SD)	Group-4 (Mean ± SD)
Testes weight	2.74 ± 0.21	1.66 ± 0.26*	1.84 ± 0.61*	2.54 ± 0.55#	2.86 ± 0.40#
Spermatogonia	31.20 ± 4.40	17.60 ± 7.16*	12.00 ± 4.80**	26.40 ± 9.94#	21.48 ± 9.22#
spermatocytes	61.80 ± 13.80	21.20 ± 7.60**	27.80 ± 12.80*	29.40 ± 8.80*	54.60 ± 10.50#
Spermatids	114.60 ± 11.10	40.20 ± 8.23**	62.4 ± 22.60*	65.20 ± 15.00*	110.80 ± 21.30#
Spermatozoa	115.40 ± 21.17	14.80 ± 3.80**	50.20 ± 8.40*	61.60 ± 8.30*	113.80 8.40#
Sertoli cell	13.20 ± 5.30	7.00 ± 1.22*	7.60 ± 1.34*	12.6 ± 12.9#	17.00 ± 3.39#
Myoid celll	13.00 ± 2.00	9.80 ± 2.20#	10.80 ± 3.00#	13.20 ± 5.10#	13.80 ± 1.90#
Leydig cell	14.40 2.90	6.20 2.20*	7.00 ± 1.73*	10.00 ± 1.58#	11.60 ± 4.40#
G.L.T (μm)	21.10 ± 1.95	12.60 ± 2.30**	13.60 ± 1.67**	15.15 ± 3.16*	19.40 ± 2.89#
S.T.D (μm)	372.80 ± 31.46	308.32 ± 34.45**	341.60 ± 4.39#	322.02 ± 9.85*	347.40 ± 5.50#

Control (feed and water only), Group-1 (2 mg/kg CdCl2/twice/orally only), Group-2(2 mg/kg CdCl2 twice + 400 mg/kg aqueous AW leaf extract/14 days/orally), Group-3 (2 mg/kg CdCl2/twice + 800 mg/kg aqueous AW leaf extract/14 days/orally), Group-4 (2 mg/kg CdCl2/twice + 1600 mg/kg aqueous AW leaf extract/14 days/orally), SD: standard deviation, PCV = packed cell volume, WBC = white blood cell, PLT = platelets, NEU = Neutrophil, LYM = Lymphocytes, Hb = Heamoglobin, * = P < 0.05 vs Control, ** = P < 0.001 vs Control, # = P > 0.05 vs Control

4. Discussion

This study showed the potential ameliorative role of aqueous extract of AW leaf against CdCl2-induced loss of germ cells and testicular dysfunction in adult male wistar rat model. Based on the result of this study, aqueous extract of AW increased testicular weight, restored germ cells, improved sperm motility and morphology, improved serum hormonal level of testosterone and FSH, improved hematological parameters and decreased the distortion of testicular histoarchitecture of the seminiferous tubules consequent upon oral CdCl2 administration.

In our study, CdCl2 exposure reduced testicular weight, induced apoptotic effect on germ cells and abnormal sperm morphology. Accordingly, the study of Behairy et al. (28) showed that cadmium had a profound negative effect on rats' testicular weights, semen quality. The histopathological findings in their study showed many multinucleated giant cells in the testes of animals exposed to cadmium. In another study, it was established that DNA replication in primary spermatocytes that failed to undergo meiotic division resulted to giant cell formation (29). Wang and colleagues also showed that Cd causes impairment in the physiological function of sperm-specific cation channel, a primary source of intracellular Calcium and potassium , both of which are important for regulating sperm physiology, consequently lowered sperm viability and motility (30). In line with the aforementioned studies, the result of our study showed that CdCl2 reduced the amount of normal germ cells and increased in the quantity of sperm cells with abnormal morphology, thereby reducing mean percentage sperm motility.

Previous studies on Phytochemical analysis of the aqueous leaf extract of revealed high presence of carbohydrates, tannins and Flavonoid, and moderate, presence of phlobatannins, Alkaloid, cardiac glycosides, Terpene and steroids which elicit anti-inflammatory reactions (31). In our study, administration of the aqueous leaf extract of AW showed some beneficial effects on testes by reversing the abnormal sperm morphology and low motility. Conversely, Olukunle et al. who showed that aqueous extract of AW leaf at doses of 400, 800 and 1600 mg/kg did not alter progressive sperm motility, percentage sperm live/dead ratio and sperm count (32). Moreover, some increased secondary morphological sperm abnormalities such as bent tail, headless tail, curved mid-piece and testicular lesions were observed in animals treated with 1600mg/kg dose. However, it should be noted that in the present study, animals were pre-induced with CdCl2 before treatment with aqueous AW leaf extract, creating a pathological condition which was lacking in the study of Olukunle et al. This probably suggest that aqueous AW leaf extract exert positive effect on tissues with known loss of function and which the same may be detrimental to healthy tissues, albeit in high dose. However, we suggest further studies to corroborate this.

Gonadotropins (FSH, LH) and testosterone are the main drivers of germ cell development (33). Previously, it has been reported that reduced sperm quality mediated by Cadmium may result from malfunction of H_2O_2 removal system leading to alteration in steroidogenesis function of leydig cells, membrane harm or macromolecular deterioration induced by ROS (34). For example, a study by Shupe and colleague showed that abnormal spermatogenesis is gonadotropins and testosterone dependent (35). For the first time, we have shown that aqueous extract of AW leaf restored serum FSH level in CdCl2-mediated loss of germ cells and disruption of testicular histoarchitecture in adult wistar rat, albeit with no significant alteration in testosterone level. This necessitate the need for further studies on the role of aqueous AW leaf extract on steroidogenesis function on CdCl2-induced loss of germ cells and relates testicular dysfunctions.

Assessment of hematological indices can be a good indication of the level of damaging effect of plant extracts such as AW on the blood components (36). It could also provide clues to blood relating functions of extracts of chemical compounds and plants (37). Hematological parameters provide information about the status of bone marrow activity and hemolysis. The different hematological parameters observed in this study are useful indices that could be utilized to assess the toxic potentials of plant extracts in living entities (38). In the present study, CdCl2 significantly reduced the mean blood levels of PCV, RBC, WBC, Neutrophils, and hemoglobin with insignificant reduction observed in the mean level of PLT and lymphocytes. Administration of aqueous leaf extract of AW was observed to reverse the negative effect of CdCl2 with the resultant significant increase in the levels of these blood parameters, albeit at an effective dose of 1600 mg/kg body weight with no significant alteration observed in the mean levels of platelets and lymphocytes. The result of our study is in line with the finding of Madziga et al, which showed that treatment of mice with AW leaf extract significantly increased the levels of mean Hb concentration, RBC, and PCV with no changes in platelets count (39). Apparently, unaltered level of platelet in our study signify that the oxygen carrying capacity of the blood was unaffected at the doses administered.

In the present study, the improvement in haematological indices likely indicate the potential curative role of the extract as a good blood enhancer in animals with reduced blood cell count resulting from adverse effect of $CdCl_2$. Previous studies have shown that anti-anaemic agents stimulate production of RBC and elevate the levels of Hb and PCV (40, 41).

This may be attributed to the presence of Carbohydrate owing to its immune role in eliciting immune reaction since and besides, it is known to play many roles in living things, such as storage and transportation of energy and structural components of cells (chitin in animals, cellulose in plants). Also, carbohydrates and its derivatives are essential in cascades of immune functions like blood clotting, fertilization, pathogenesis and development (42, 43). Furthermore, AW contains good amount of iron which could potentially contribute to the observed improvement in the haematoglogical parameters. The increased haematological profile in this study may also be attributed to the activities of the chemical constituents of the plant, saponins, as found in the extract, and are known to hydrolyze and produce sapogenins which may be steroid or triterpene.

5. Conclusion

The leaf of AW contains some important organic and chemical components which are pharmacologically active and may possess therapeutic values, which therefore could qqbe of immense medicinal value. The aqueous leaf extract increased testes morphology, germ cell count, sperm motility and reduced abnormal sperms and increased hematological parameters, thus it appears to have some beneficial effect on the testes, suggesting that it could be used as a therapeutic agent against Cadmium-induced loss of germ cells and consequently, infertility.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All animal experiments and protocols adhered to the guidelines and regulations set forth by the National Research Council in regards to laboratory animal care and utilization (2011) and Ethical approval gotten from the Department of Anatomy, Faculty of Basic Medical Sciences Olabisi Onabanjo University, Ago-Iwoye Ogun State.

References

[1] Zou H, Sun J, Wu B, Yuan Y, Gu J, Bian J, et al. Effects of Cadmium and/or Lead on Autophagy and Liver Injury in Rats. Biol Trace Elem Res. 2020, 198(1):206-15.

[2] Matt GE, Quintana PJE, Hoh E, Dodder NG, Mahabee-Gittens EM, Padilla S, et al. Tobacco smoke is a likely source of lead and cadmium in settled house dust. J Trace Elem Med Biol. 2021, 63:126656.

[3] Genchi G, Sinicropi MS, Lauria G, Carocci A, Catalano A. The Effects of Cadmium Toxicity. Int J Environ Res Public Health. 2020, 17(11).

[4] Mezynska M, Brzoska MM. Environmental exposure to cadmium-a risk for health of the general population in industrialized countries and preventive strategies. Environ Sci Pollut Res Int. 2018, 25(4):3211-32.

[5] Brdaric E, Sokovic Bajic S, Dokic J, Durdic S, Ruas-Madiedo P, Stevanovic M, et al. Protective Effect of an Exopolysaccharide Produced by Lactiplantibacillus plantarum BGAN8 Against Cadmium-Induced Toxicity in Caco-2 Cells. Front Microbiol. 2021, 12:759378.

[6] Gong ZG, Wang XY, Wang JH, Fan RF, Wang L. Trehalose prevents cadmium-induced hepatotoxicity by blocking Nrf2 pathway, restoring autophagy and inhibiting apoptosis. J Inorg Biochem. 2019, 192:62-71.

[7] Marettova E, Maretta M, Legath J. Toxic effects of cadmium on testis of birds and mammals: a review. Anim Reprod Sci. 2015, 155:1-10.

[8] Cheung MR, Kang J, Ouyang D, Yeung V. Association between urinary cadmium and all cause, all cancer and prostate cancer specific mortalities for men: An analysis of national health and nutrition examination survey (NHANES III) data. Asian Pac J Cancer Prev. 2014, 15:483-8.

[9] Lin HC, Hao WM, Chu PH. Cadmium and cardiovascular disease: An overview of pathophysiology, epidemiology, therapy, and predictive value. Rev Port Cardiol (Engl Ed). 2021, 40(8):611-7.

[10] Kumar S, Sharma A. Cadmium toxicity: effects on human reproduction and fertility. Rev Environ Health. 2019, 34(4):327-38.

[11] Benvenga S, Micali A, Pallio G, Vita R, Malta C, Puzzolo D, et al. Effects of Myo-inositol Alone and in Combination with Seleno-Lmethionine on Cadmium-Induced Testicular Damage in Mice. Curr Mol Pharmacol. 2019, 12(4):311-23.

[12] Ali S, Awan Z, Mumtaz S, Shakir HA, Ahmad F, Ulhaq M, et al. Cardiac toxicity of heavy metals (cadmium and mercury) and pharmacological intervention by vitamin C in rabbits. Environ Sci Pollut Res Int. 2020, 27(23):29266-79.

[13] Rinaldi M, Micali A, Marini H, Adamo EB, Puzzolo D, Pisani A, et al. Cadmium, Organ Toxicity and Therapeutic Approaches: A Review on Brain, Kidney and Testis Damage. Curr Med Chem. 2017, 24(35):3879-93.

[14] Massanyi P, Massanyi M, Madeddu R, Stawarz R, Lukac N. Effects of Cadmium, Lead, and Mercury on the Structure and Function of Reproductive Organs. Toxics. 2020, 8(4).

[15] Adamkovicova M, Toman R, Cabaj M, Massanyi P, Martiniakova M, Omelka R, et al. Effects of subchronic exposure to cadmium and diazinon on testis and epididymis in rats. ScientificWorldJournal. 2014, 2014:632581.

[16] Horri E, Esmaeilnejad Moghadam A, Talebpour Amiri F, Ebrahimzadeh MA. Protective effect of Feijoa sellowianan fruit on testicular toxicity-induced by cadmium chloride. Andrologia. 2021, 53(2):e13926.

[17] Zhou GX, Zhu HL, Shi XT, Nan Y, Liu WB, Dai LM, et al. Autophagy in Sertoli cell protects against environmental cadmium-induced germ cell apoptosis in mouse testes. Environ Pollut. 2021, 270:116241.

[18] Arteaga-Silva M, Limon-Morales O, Bonilla-Jaime H, Vigueras-Villasenor RM, Rojas-Castaneda J, Hernandez-Rodriguez J, et al. Effects of postnatal exposure to cadmium on male sexual incentive motivation and copulatory behavior: Estrogen and androgen receptors expression in adult brain rat. Reprod Toxicol. 2023, 120:108445.

[19] Ujah GA, Nna VU, Agah MI, Omue LO, Leku CB, Osim EE. Effect of quercetin on cadmium chloride-induced impairments in sexual behaviour and steroidogenesis in male Wistar rats. Andrologia. 2018, 50(2).

[20] Yousefi-Manesh H, Dehpour AR, Ansari-Nasab S, Hemmati S, Sadeghi MA, Shahraki RH, et al. Hepatoprotective Effects of Standardized Extracts from an Ancient Italian Apple Variety (Mela Rosa dei Monti Sibillini) against Carbon Tetrachloride (CCl(4))-Induced Hepatotoxicity in Rats. Molecules. 2020, 25(8).

[21] Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. J Ethnobiol Ethnomed. 2006, 2:14.

[22] H. Wu, H. Pang, Y. Chen, L. Huang, H. Liu, Y. Zheng, et al. Anti-inflammatory effect of a polyphenol-enriched fraction from Acalypha wilkesiana on lipopolysaccharide-stimulated RAW 264.7 macrophages and acetaminophen-induced liver injury in mice. Oxid Med Cell Longev. 2018, 2018.

[23] Ikewuchi JC, Uwakwe AA, Onyeike EN, Ikewuchi CC. Hepatoprotective effect of an aqueous extract of the leaves of Acalypha wilkesiana 'Godseffiana' Muell Arg (Euphorbiaceae) against carbon tetrachloride induced liver injury in rats. EXCLI J. 2011, 10:280-9.

[24] Akinloye DI, Osatuyi OA, Musibau OG, Yusuf AA, Adewuyi S. In vitro antioxidant activities, elemental analysis and some bioactive constituents of Acalypha wilkesiana Muell arg and Acalypha wilkesiana java white Leaf Extracts. J Chem Soc Nigeria. 2016, 41:150-7.

[25] Akinyemi O, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. BMC Complement Alternat Med. 2005, 5(1):1-7.

[26] Omage K, Azeke AM. Medicinal potential of Acalypha wilkesiana leaves. Adv Res. 2014, 2(11):655-65.

[27] Saxena BB, Demura H, Gandy M, Peterson RE. Determination of steroidal hormones by immunosorbant technique. J Clin Endocrinol Metab. 1968, 28:591-608.

[28] Behairy A, Hashem MMM, Abo-El-Sooud K, Soliman AM, Mouneir SM, El-Metwally AE, et al. Influence of titanium dioxide nanoparticles and/or cadmium chloride oral exposure on testicular morphology, oxidative stress, and apoptosis in rats: Ameliorative role of co-enzyme Q10. Heliyon. 2024, 10(1):e24049.

[29] Alkhedaide A, Alshehri ZS, Sabry A, Abdel-Ghaffar T, Soliman MM, Attia H. Protective effect of grape seed extract against cadmium-induced testicular dysfunction. Mol Med Rep. 2016, 13(4):3101-9.

[30] Wang HF, Chang M, Peng TT, Yang Y, Li N, Luo T, et al. Exposure to Cadmium Impairs Sperm Functions by Reducing CatSper in Mice. Cell Physiol Biochem. 2017, 42(1):44-54.

[31] HA M, S S, UK S. 2010. Phytochemical and elemental analysis of Acalypha wilkesiana leaf. Journal of American Science. 2010, 6(11):510-4.

[32] Olukunle JO, Jacobs EB, Adeleye OE, Oyewusi JA, Adenubi OT, Durotoye LA. Effects of aqueous leaf extract of Acalypha wilkesiana on semen morphology and characteristics in male Wistar albino rats. Nigerian Journal of Animal Production. 2019, 46(2):47-54.

[33] Shupe J, Cheng J, Puri P, Kostereva N, Walker WH. Regulation of Sertoli-germ cell adhesion and sperm release by FSH and nonclassical testosterone signaling. Mol Endocrinol. 2011, 25(2):238-52.

[34] M. El-Komy, H.O. Saad, El-sayed mohamed el-habibi. magda protective effect of date palm extracts on cadmium-induced infertility in male rats. The Egyptian Journal of Hospital Medicine. 2017, 69(4):2181-90.

[35] Shupe J, Cheng J, Puri P, Kostereva N, Walker WH. Regulation of Sertoli-germ cell adhesion and sperm release by FSH and nonclassical testosterone signaling. Mol Endocrinol. 2011, 25(2):238-52.

[36] Ashafa AOT, T. YM, S. GD, J. AA. Effects of aqueous leaf extract from the leaves of Chrysocoma ciliate L. on some biochemical parameters of Wistar rats. African Journal of Biotechnology. 2009, 8:1425-30.

[37] Yakubu MT, Akanji MA, Oladiji AT. Haematological evaluation in male albino rats following chronic administration of aqueous extract of Fadogia agrestis stem. Pharmacognosy Magazine. 2007, 3:34.

[38] Sunmonu TO, Oloyede OB. Performance and haematological indices in rats exposed to monocrotophos contamination. Human & Experimental Toxicology. 2010.

[39] Madziga HA, Chiroma M, Usman JG, Daniel N, Omeh IJ. Toxicity study of aqueous extract of Muell-Arg (Jacob's coat, Acalypha wilkesiana Copper leaf) leaves on some haematological parameters in mice. Advance Pharmaceutical Journal. 2020, 25(1):9-13.

[40] Jour AD, Vovor GM, Eklu-G A, Aklikokou K, Agbonon K, Abena A, et al. Effect of Tectona grandis on phenylhydrazine-induced anaemia in rats. Fitoterapia. 2008, 76:332.

[41] Brown BA. Haematology, Principles and Procedures. 2nd ed. Philadelphia, USA: Lea and Fibiger, 1976.

[42] Maton A, Jean CWM, Susan J, Maryanna QW, David L, Jill DW. Human Biology and Health. Englewood Cliffs, New Jersey, USA: Practice Hall, 1993.

[43] Patil UK, Muskan K. Essential Biotechnology 2009. Available from: books.google.com.ng books.