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HISTOMORPHOLOGICAL AND BIOCHEMICAL ASSESSMENT OF TESTICULAR PARAMETERS FOLLOWING ORAL ADMINISTRATION OF CADMIUM NITRATE TO ADULT MALE WISTAR RATS

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ABSTRACT

Background: Available evidence shows that cadmium nitrate (CdN) is locally used in Africa as a supplement in the preparation of herbal mixture. This study focused on histomorphological and biochemical assessment of testicular parameters following oral administration of CdN in adult male wistar rats. **Materials and Methods:** Twenty (n=20) adult male wistar rats were randomly divided into four groups (A-D) of five (n=5) rats each. Group A served as control was given 10 ml/kg/day of distilled water. Group B, C, and D [cadmium nitrate (CdN) group] were given 100 mg/kg/bw of CdN, 200 mg/kg/bw of CdN and 300 mg/kg/bw of CdN respectively. Administration was done by gastric gavage once a day, for fifty-six consecutive days. Testicular weight and semen analysis revealing the sperm count, sperm motility, and sperm morphology were assessed. Malondialdehyde (MDA),Superoxide dismutase(SOD) GSH(Glutathtone reductase) were also examined. The Testis of each rat were harvested and fixed in Bouin's fluid for histological analysis. **Results:** Histological result revealed depletions in spermatogenic cells in germinal epithelium. The body weights of the experimental group significantly increased throughout the period of the experiment when compared with the control. Testicular weights and sperm quality of the experimental group were significantly decreased in a dose dependent manner. There is significant increase in the activity levels of MDA, SOD, and GSH in the experimental groups when compared with the Control. **Conclusion:** This study showed that CdN induced alteration in testicular weight, semen parameters and oxidative stress parameters in the testis of adult male wistar rat.

KEYWORDS: Cadmium Nitrate; testes; spermatogenic; cells; sperm.

INTRODUCTION

Research has revealed that excess cadmium exposure produces adverse health effects on human beings.[1] At significant high exposure, virtually all chemicals have adverse health effects. For certain elements such as copper and zinc, which are essential to human life, a deficiency, as well as an excess can cause adverse health effects.[2] Cadmium (Cd) is a heavy metal and a major environmental toxicant.[3] The general population is exposed to Cd via contaminants found in drinking water and Blanco food, whereas occupational exposition to Cd usually takes place during mining and manufacturing of batteries and pigments that contain Cd.[4] Industrial activities, such as smelting and refining of metals, and municipal waste incineration also release Cd into the atmosphere.[5] Tobacco smoke is another important source of Cd exposure.[6] Cd toxicity is associated with severe damage in various organs, particularly the testes, in both humans and animals.[7] Rodent testes are especially sensitive to the toxic effects of Cd exposure.[8] Cd impairs reproductive capacity by causing severe testicular degeneration, seminiferous tubule damage and necrosis in rats.[9]

Cadmium exposure has been reported to be a risk factor for infertility.[10] Studies have shown that exposure to cadmium causes lipid peroxidation, which is associated with cadmium toxicity in testes.[11] Its effects on the testis appear to be manifested mainly in the sertoli cells, which present more morphological changes under scanning electron microscopy and also causes derangement in spermatogenesis and spermiogenesis.[12] At the Cellular level, Cadmium induces oxidative stress in many organisms.[13][14], which might result in

physiological damage to different organs among which are the Brain, Kidneys, Liver, Lungs, Pancreas, Testes, Placenta, and Bone.[15][16][17]

Cadmium nitrate (CdN) occurs as a colorless solid that is very soluble in dilute acids and soluble in ethanol, acetone, water, diethyl ether, and ethyl acetate.[18] Cadmium nitrate is available in technical and reagent grades with a purity of 99% or higher.[19] It has been reported by[20] that Cadmium Nitrate caused inflammations, distortions, congestions, and degenerative changes in myocardium tissue and significantly increased the heart weight following the oral consumption of Cadmium Nitrate in wistar rats. CdN also caused distortions of the liver cytoarchitecture, decreased in body weights of experimental rats, significant decreased in mean liver weight, the serum levels of AST and ALT were significantly increased.[21]

The present study therefore focused on investigating the histomorphological and biochemical assessment of testicular parameters in oral administration of cadmium nitrate in adult male wistar rats.

MATERIALS AND METHODS

Cadmium Nitrate

Procedure

The cadmium Nitrate used in this research was purchase from a pharmaceutical store along Cathedral Bus Stop, Akure , Ondo State, Nigeria on 1st October, 2016.It was taken to the Department of Chemistry, Federal University Of Technology, Akure, Ondo State, Nigeria for authentication. It was thereafter taken to the Laboratory of Department of Anatomy, School of Health and Health Technology, Federal University of Technology for use.

Animals and Diet

Twenty (n=20) adult male wistar rats were obtained from a breeding stock maintained in the animal house of Department of Human Anatomy, School of Health and Health Technology, Federal University Of Technology , Akure, Ondo State, Nigeria. The animals had free access to rat chow and tap water and they were randomly divided into four (4) groups (A-D) of five (n=5) rats each in a separate room at a constant temperature (22.0± 1.0ºc) under a 12h light/dark cycle. Rats in group A which served as control was given 10ml/kg/day of distilled water while groups B, C, and D (Cadmium Nitrate group) were given 100mg/kg bw cadmium nitrate, 200mg/kgb.w of cadmium nitrate and 300mg/kg/bw of cadmium nitrate respectively. Administration was done orally through an orogastric cannula inserted into the esophagus of each rat for 56days consecutive days. The average daily oral intake of cadmium by non-smokers living in unpolluted areas was estimated to 10–25 µg[22] while in Laboratory animal oral LD_{50} values for mice and rats range from 60 to over 5000 mg/kg of body weight.[23] All experimental investigations were done in compliance with humane animal as stated in the "Guide to the care and use of Laboratory Animals Resources". National Research Council, DHHS, Pub.No NIH 86-23[24] and in accordance with the guideline and approval of Nigeria Medical Ethical Association for Accreditation of Laboratory Animal Care.

Animal Sacrificed and Sample Extraction

Twelve (12) hours after the administration of the last dose of cadmium nitrate, the rats were weighed and sacrificed by cervical dislocation according to the ethical humane animal euthanasia. The abdominal cavity of each rat was opened up through a midline thoracic incision to expose the testis. The testis was excised and weighed with an electronic analytical and precision balance. The testis of each animal was fixed in Bouins fluid for histological examination.

Histological Procedures and Analysis

This was done as described by.[25] The organs were cut in slab of about 0.5 cm thick transversely and fixed in Bouin's fluid for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into moisten paraffin wax for 20 minutes each in an oven at 57°C. Serial sections were cut at 5 microns. Slides were prepared from these tissues. The slides were dewaxed and passed through absolute alcohol (2 changes); 70% alcohol and then to water for 5 minutes. The slides were then stained with hematoxylin. Photomicrographs were taken with a JVC colour video digital camera (JVC, China) mounted on an Olympus light microscope [Olympus UK Ltd, Essex,UK] to demonstrate the testicular damage.

Determination of sperm characteristics

The caudal epididymis was minced in pre-warmed normal saline (37°C). 1 drop of sperm suspension was placed on a glass slide to analyze 200 motile sperm in 4 different fields. The motility of the epididymal sperm was evaluated microscopically within 2 - 4 minute of their isolation from the epididymis, and data was expressed as percentage motility.[26]

Epididymis sperm was obtained by mincing the epididymis in normal saline, and filtering through a nylon mesh (80-µm pore size). The sperm were counted using a hemocytometer. The number of sperm in 5 squares (4 corners and the center) in the center grid of both sides were counted and averaged following the method of[27] Sperm morphology was studied using 2 drops of Walls and Ewas stain, air-dried, and examined under the microscope. The normal sperm cells were counted and the percentage calculated.

Determination of testicular malondiadehyde (MDA) and testicular antioxidant enzymes

This was done as described by.[28] The lipid perioxidation products were estimated by measuring TBARS and were

determined by modifying the method of.[29] Antioxidants - reduced glutathione (GSH) and superoxide dismutase (SOD) were estimated by employing modified methods of[30] and[31] respectively.

Statistical Analysis

Data is expressed as mean ± S.D. Statistical analyses were performed with SPSS software. $P < 0.05$ was considered statistically significant. Overall Differences between groups were determined by one-way ANOVA.

RESULTS

Table.1. Shows Mean Body Weight and Standard Deviation of the experimental and control rats following 56days oral Administration of Cadmium Nitrate

GROUPS	DAY 7 (MEAN ± SD)	DAY 14 (MEAN ± SD)	DAY 21 (MEAN ± SD)	DAY 28 (MEAN ± SD)	DAY 35 (MEAN ± SD)	DAY 42 (MEAN ± SD)	DAY 49 (MEAN ± SD)	DAY 56 (MEAN ± SD)
A(CONTROL)	118±8.19	182±9.11	202±8.94	206±0.84	213±1.58	214±0.84	221±1.67	227±0.84
B(150 mg/kg CdN)	160±6.60	150±7.3*	156±3.57	157±1.14*	163±2.24*	168±0.89*	175±1.67**	176±1.58**
C(225 mg/kg CdN)	138±5.39	162±3.34	178±5.90*	172±1.58	173±1.30**	180±1.14*	179±1.14	181±2.07*
D(300 mg/kg CdN)	174±6.70	136±4.32	146±5.21*	149±1.14*	154±1.22	163±084*	167±1.92	173±1.67*

*Value are expressed as n=5 Mean±SEM *p<0.05, p<0.001*

Body Weight

The difference in body of animals subjected to different treatments were shows in Table 1. During the course of present investigations. It was observed that Weights of the experimental group B, C, D were significantly increased throughout the period of the experiment and significantly lower than the control.

Table 2: Testes weights and Epididymal Sperm Count, Motility, Viability and Morphology in the experimental groups on the left and right sides following 56days oral Administration of Cadmium Nitrate.

Sperm Parameters /Groups	Testis Location	Testis Weight (mg)	Sperm Count x 10^{12}	Sperm Viability (%)	Abnormal Morphology (%)	Sperm Motility (%)
A(control)	Left	1.62±0.01	85.24±0.01	92.22±0.22	10.46±0.05	70.49±0.04
	Right	1.62±0.01	85.32±0.02	92.34±0.15	10.43±0.01	70.51±0.04
B (150 mg/kg CdN)	Left	1.60±0.02**	80.30±0.16*	85.32±0.18*	13.33±0.02*	65.26±0.05**
	Right	1.61±0.01**	80.26±0.15*	85.30±0.14*	13.28±0.10*	65.22±0.01*
C (225 mg/kg CdN)	Left	1.57±0.02*	75.24±0.11*	75.36±0.11**	15.52±0.01**	50.43±0.12**
	Right	1.57±0.01*	75.16±0.11*	75.32±0.15*	15.48±0.05**	50.46±0.03*
D (300 mg/kg CdN)	Left	1.46±0.04**	70.28±0.13*	70.42±0.08**	16.18±0.06**	42.33±0.10**
	Right	1.46±0.03**	70.28±0.13*	70.42±0.08**	16.18±0.06*	42.24±0.14**

*Data represented as MEAN±SD, n=5, *significant at p<0.05, ** significant at p<0.001*

Testis Weight

Weights of testis experimental group B, C, D were significantly decreased in dose dependant order (Table 2).

Sperm parameters

Testes Mean Epididymal sperm count, motility, viability and morphology. Obtained in cadmium Nitrate -treated rats (Groups B, C and D) were significantly decreased in dose dependant manner.Percentages of abnormal sperm cells (sperm morphology) in CdN-treated rats (Groups B, C and D) were significantly different from control rats (Table 2)

TABLE 3.MDA and testicular antioxidant enzymes analysis following 56days oral Administration of Cadmium Nitrate.

GROUP	MDA(nmol/ml)	SOD(min/mg/protein)	GSH(µmol/ml)
A(CONTROL)	10.64±0.29	41.33±0.80	0.166±0.004
B (150 mg/kg CdN)	18.54±0.49*	58.90±0.93*	0.145±0.004*
C (225 mg/kg CdN)	24.48±0.67*	62.46±0.45*	0.175±0.001*
D (300 mg/kg CdN)	27.34±0.52*	64.39±0.61*	0.195±0.001*

*Data represented as MEAN±SD, n=5, *significant at p<0.05*

MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutathtone reductase

MDA Levels and Antioxidant Levels (CAT, SOD, GSH)

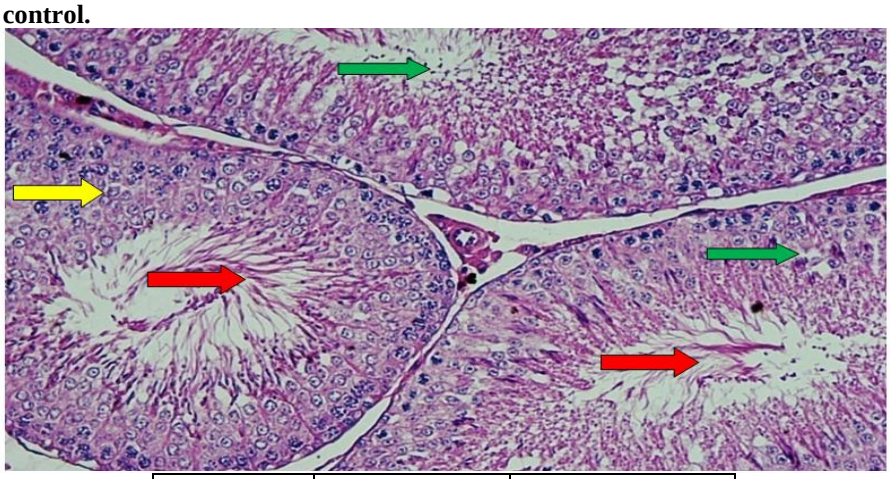
At the end of 56days oral Administration of Cadmium Nitrate there was significant increase in MDA level of group B, C, D when compared to group A. SOD level in group B, C, D significantly increased compared to group A. GSH level of group B significantly decreased compared to group A however GSH level of group C and D significantly increased compared to group A.

Testicular histology

Cross section of the testis of animals in groupA after 56days of administration showed, normal germinal epithelium, lumen fill with sperm cells and interstitial cell of Laydig in the interstitium. However, animals in groupB, C, D, showed a marked depletion of spermatogenic cells.(Fig.2,3,4)

PHOTOMICROGRAPY DEMOSTRATIONS

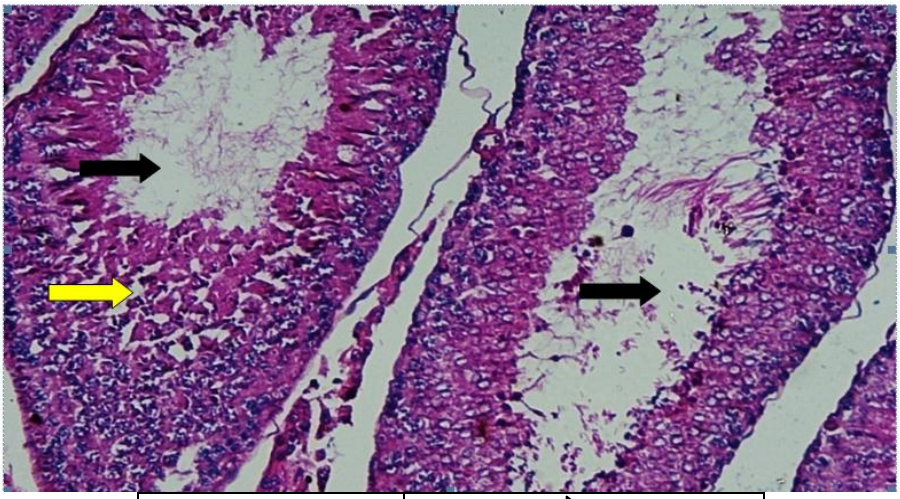
GROUP A: (CONTROL) Photomicrographs of the Testis of experimental animal administered 10ml/kg/day of distilled water as control.



Lumen	**Germinal Epithelium**	**Interstitial Cells of Ladyig**

Fig.1. Histological demonstration of the photomicrograph of longitudinal section of the Testis at light microscope level using H & E staining techniques [X400] after 56days of administration showing, normal germinal epithelium (yellow arrow), lumen (Red arrow), fill with sperm cells and interstitial cell of Laydig (Green arrow) in the interstitium.

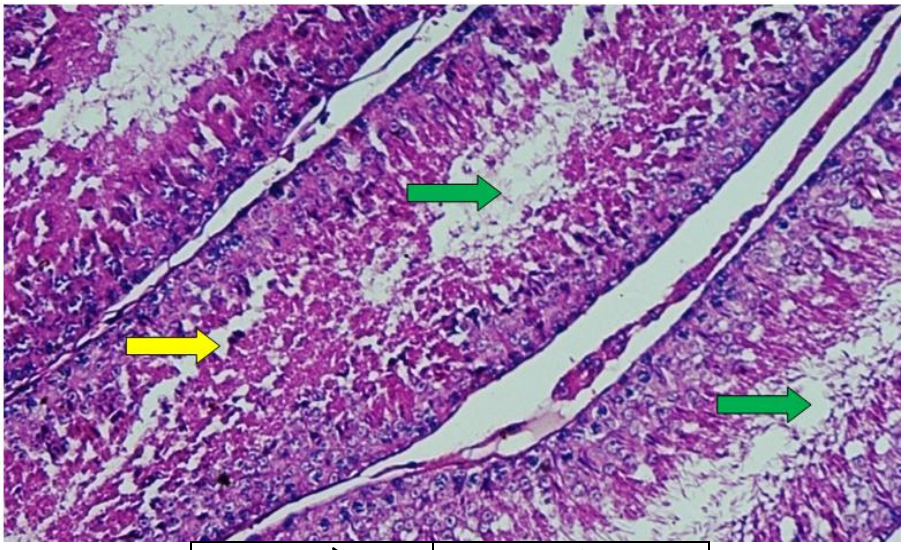
GROUP B: Photomicrographs of the Testis of experimental animal administered 100mg/kg/bw cadmium nitrate for Fifty-six days.



Lumen	Germinal Epithelium

Fig.2. Histological demonstration of the photomicrograph of longitudinal section of the Testis at light microscope level using H & E staining techniques [X400] after 56days of administration showing, depleted spermatogenic cells in germinal epithelium (yellow arrow), lumen (Red arrow), fill with scanty sperm cells.

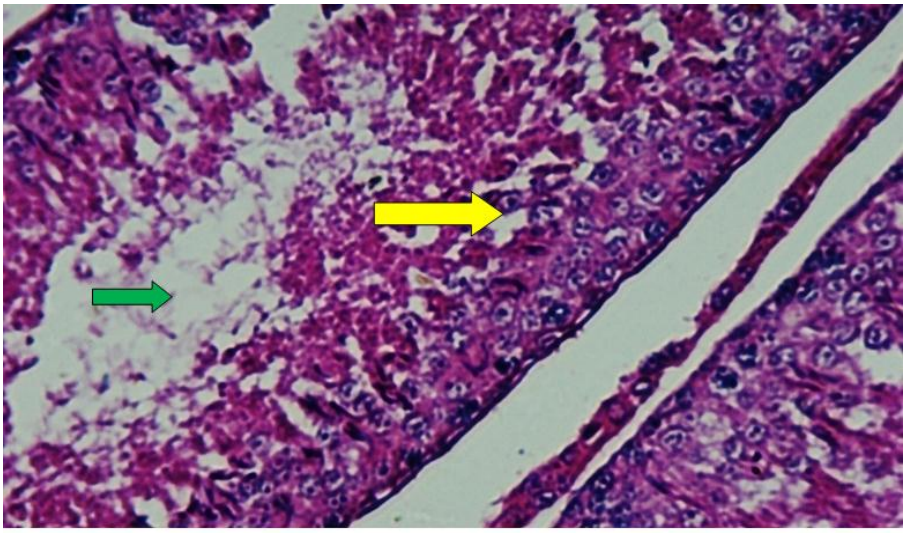
GROUP C: Photomicrographs of the Testis of experimental animal administered 200mg/kg/bw cadmium nitrate for fifty-six day.



Lumen	**Germinal Epithelium**

Fig.3. Histological demonstration of the photomicrograph of longitudinal section of the Testis at light microscope level using H & E staining techniques [X400] showing, depleted spermatogenic cells in germinal epithelium (Yellow arrow), Lumen with no sperm cells (Green arrow).

GROUP D: Photomicrographs of the Testis of experimental animal administered 300mg/kg/bw cadmium nitrate for fifty-six days.



Lumen	**Germinal Epithelium**

Fig.4. Histological demonstration of the photomicrograph of longitudinal section of the Testis at light microscope level using H & E staining techniques [X400] showing, depleted spermatogenic cells in germinal epithelium (Yellow arrow), Lumen with no sperm cells (Green arrow).

DISCUSSION

Cadmium (Cd), a heavy metal, is toxic to both humans and animals.[32] Cadmium in its elemental form occurs naturally in the earth's crust and it is unusual to find it in its pure form.[33] It is commonly found in combination with other element such as oxygen (cadmium oxide), sulfur (cadmium sulfate), chloride (cadmium chloride), Nitrate (cadmium nitrate) and carbon (cadmium carbonate).[34] Testicular function is examined in part by analysis of spermatic parameters including sperm count, motility, viability and morphology.[35][36] Measurements of these indices in the spermatozoa give an indication of the quality and functionality of the sperm. As normal sperm motility and count are vital for male fecundity[35],

the results of this study revealed that Cadmium nitrate administration significantly decreased sperm count, sperm motility, and sperm morphology of the rats. The changes observed in the above is in accordance with the previous reports, which demonstrated that cadmium impairs testicular function.[37][38] The significant reduction in sperm count, motility and morphology observed in this study following oral CdN administration may be associated to impairment of spermatogenesis consequent to reduced secretion of testosterone (from testes) caused by administration of CdN[39] also reductions in the number and motility of sperms by cadmium Nitrate in this study indicates that the metal could alter normal testicular function. In addition, the percentages of viable sperms (i.e., spermatozoa with intact cell membrane) and morphologically abnormal sperms are critical indicators of testicular function.[40] Oral consumption of Cadmium nitrate also significantly reduced sperm count and sperm motility by subjecting the spermatozoa to increased oxidative stress-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFAs)[41][42] and their cytoplasm contains lowconcentrations of scavenging enzymes[43][44] Increased formation of ROS has been correlated with a reduction of sperm motility.[45][46] The link between ROS and reduced motility may be dueto a cascade of events that result in a rapid loss of intracellular ATP leading to axonemal damage and sperm immobilization.[47] Organ weights are indices often employed in toxicological evaluations.[48] Reductions in testis weights in cadmium nitrate treated rats in this study demonstrate cadmium nitrate toxicity. This observation also suggests that cadmium nitrate may cause atrophy of the testis and ultimately affect their physiological functions.

Previous Studies revealed exposure to cadmium causes lipid peroxidation, which leads to oxidative stress.[49] SOD is an enzymatic antioxidant that converts superoxide radicals into hydrogen peroxide and oxygen, which is further converted into water. This study shows that oral administration of Cadmium Nitrate significant increased the activities of SOD and GSH. MDA level also significantly increased, this is in accordance has reported by[10] that $CdSO_4$ administration significantly increased testicular MDA concentration.

In this study oral consumption caused depletions of germinal epithelium in the somniferous tubule,degenerated germ cells and decreased the number of Leydig cells. Fordecades, it has been made clear that testosterone which is producedby the interstitial cells of Leydig is a necessary prerequisite for themaintenance of established spermatogenesis[28][50] The reduced cellularity of the interstitium in testis of animals that were treated with cadmium nitrate would consequently lead to a decrease in testosteroneresulting in the poor spermatogenesis observed. This study therefore showed that Cadmium Nitrate induced alteration in testicular weight, sperm count, sperm motility, sperm morphology, testis histo-morphology and MDA, SOD, GSH level.

CONCLUSION

This study therefore showed that Cadmium Nitrate induced alteration in testicular weight, sperm count, sperm motility, sperm morphology, testis morphology and oxidative stress parameters in the testis of adult male wistar rat.

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