Tropical Journal of Obstetrics & Gynaecology
ISSN-Print: 0189-5117
Online: 2543-148X
Official Publication of Society of Obstetrics & Gynaecology of Nigeria

■ Original Research Article

Histopathological Features of The Ovaries, Physical and Behavioural Patterns Following Administration of Cadmium in Wister Rats

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ABSTRACT

Background: Cadmium is an environmental pollutant, toxic to humans and animals. Cadmium toxicity affects tissues including reproductive system causing infertility. **Study Objective**: To determine the effect of cadmium on behavior and ovaries of Wister rats. **Materials And Methods:** Two groups - A and B, each of 5 Wister rats were studied at the Laboratory of Anatomy Department of Nnamdi Azikiwe University, Nnewi, Nigeria. Following acclimatization, rats in group A (control) were weighed and administered distilled water and laboratory chow while rats in group B were administered 50mg per kg of Cadmium Chloride. Both groups were fed daily and monitored weekly (for weight and behavior over seven weeks). Following final weighing, the rats were sacrificed, and the ovaries harvested and prepared for histology, carried out with photomicrography. Data analysis employed SPSS version 25, comprise of variables employed T dependent test with $p < 0.05$ considered significant. **Results:** Rats in group A showed significant weight gain, 144. 407±07g vs. 167.107 ±87g (P=0.009) between initial and final measurement. They also showed normal behavioral pattern, then rats in group B showed significant weight loss, 201.405 ± 48g vs. 163.801 ± 78g (P=0.04) and severely abnormal behavior. Ovarian sections in group A were normal while sections in group B showed degenerated follicles, absence of secondary ovarian follicles and presence of multiple cysts. **Conclusion:** Cadmium toxicity manifested as abnormal behavior and pathological ovarian changes in rats administered cadmium chloride. Cadmium toxicity is linked to human reproduction particularly polycystic ovary syndrome, and infertility. Further studies on effect of cadmium on steroidogenesis and counter effect of some antioxidants on cadmium toxicity are recommended.

Keywords - Histopathological features of the ovary; physical and behavioral patterns; Cadmium; Wister rats.

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INTRODUCTION

Industrialization and agriculture have to a large extent resulted in varying degrees of the pollution of the environment and reorganization of some toxic elements in the food chain[1] Exposure to toxic environmental and occupational pollutants has been identified to play significant role in the disruption of the reproductive system causing some public health issues[2]. Of notable concern is infertility, a condition that affects as much as 15-25% of couples globally.[2,3,4] Over the past 5 decades, there has been report of increasing trends of adverse effects on reproductive functions in particular; male reproductive organs related more to toxic environmental factors rather than genetic factors. It has been reported that the response of reproductive organs to toxic pollutants is different from that of other target organs which distinguishes it as a veritable benchmark for the measurement of the adverse effect of environmental pollutants on humans and animals[2].

Some widely studied environmental pollutants associated with diseases and disorders of the reproductive system are the heavy metals - notably cadmium, lead and mercury. In a recent review by Peter et al[2] experimental studies were carried out on a variety of animals- Rats, Mice, Birds and rabbits to demonstrate the effect of cadmium, lead and mercury on the structure and function of reproductive organs. Their findings, along with other reviews demonstrated the particularly sensitive nature of the gonads to the heavy metals (cadmium, lead and mercury) attributable to their distinct intense cellular activities involving the vital processes of sperm, follicles and oocytes production[2,5,6,7]. They also reported that specifically ovarian toxicity manifested as retarded follicular growth, occurrence of follicular atresia, Corpus luteal degeneration and cycle alteration[2].

Cadmium is a non-essential transitional metal that is toxic and constitutes a health risk to both humans and animals[8]. It is an environmental pollutant usually resulting from agricultural and industrial activities[8,9,10]. Cadmium usually occurs in the human and animal population through contaminated water and food such as meat and milk product, and inhalation of fumes from tobacco smoking[10,11,12]. In humans the absorption of cadmium from foods is about 3-5% and this is easily well reabsorbed by the kidneys with a biological half-life of a reasonably long period of approximately 10-30 years[13,14,15,16,17,18,19,]. Several studies had been carried out that have demonstrated the effect of cadmium on the structure of the ovaries in rats and rabbits. Parizek et al.[12] demonstrated massive hemorrhagic necrosis of the gonads of male rats following subcutaneous injection of cadmium salts together with an analogous destruction of the fetal surface of the placenta of pregnant rats but did not demonstrate any changes in the ovaries of experimental rats following the administration of similar or even larger doses of cadmium salts. Massanyi et al.[19,20] similarly carried out intra-peritoneal injection of varying doses of cadmium chloride on 32 experimental rats and observed a high concentration of cadmium in the rat ovaries 48 hours after administration. Similar observations were made in studies carried out on rabbits[22]. Findings in the ovaries included a relative reduction in volume of growing follicles with an increase in stroma. [23,24] Other findings include significant elevation in the number of atretic follicles and other ultra-structural alterations of granulosa, luteal, stroma and endothelial cells in the form of undulation of external nuclear membrane, together with the dilatation of perinuclear cistern and endoplasmic reticulum[25].

This study has been undertaken in female whisker rats to elicit the effect of cadmium chloride on the structure of the ovaries of the rats, and on their physical and behavioral patterns. Findings from this animal study can be extrapolated to humans. The implications of the findings to ovarian disease, and infertility in particular are discussed, and the literature reviewed.

RESULTS

Table 1 shows the distribution by physical features and behavioral pattern of the rats for the control (group A) and group B before and following the administration of cadmium chloride alone. The rats in the two groups exhibited healthy features and normal behavior during the period of acclimatization- healthy skin with normal color and smoothly laid hairs, pinkish eyes, increased physical size, active movement, normal breathing patterns and good appetite. Following the administration of the test substances, group A (control) remained normal showing no change in features and behavioral pattern, group B however manifested marked changes to severe degrees, which includes weight alterations, loss of skin hairs, changes in skin color, labored breathing, staggering gait, lethargy and loss of appetite manifesting as decreased food intake.

Table 1-The Distribution by physical changes and behavioral pattern of the rats, following the administration of test substances for the group A (control) and group B

Groups	Physical and behavioral pattern (following administration of test substance)	During Acclimatization (2wks)
Group A(control)	Normal	Nil
Group B(50mg/Kg of CDCL2)	Severe	Nil

Table 2 shows the distribution by the rats in the 2 groups for their initial mean body weight and final weight

following the administration of the test substances. Rats in group A (control group) showed a significant increase in the mean body weight from initial value of 144.407 to 167.107 following the administration of test substance-distilled water and laboratory chow, (P= 0.009). Rats in group b on the other hand showed a significant decrease in mean body weight from an initial value of 201.405± 48 to 163.801 ±78 following the administration of 50mg per kg of cadmium chloride (P= 0.046)

Table 2: Distribution by the rats in various groups for their initial mean body weight and final weight following the administration of the test substances.

Body weight (grams)	Mean	± SEM	P value	Test of Significance
Group A(control)	Initial-144.407 Final-167.107.	±07 ±87	0.009	Significant
Group B (50mg/kg of CdCl2)	Initial-201.405 Final-163.801	±48 ±78	0.046	Significant

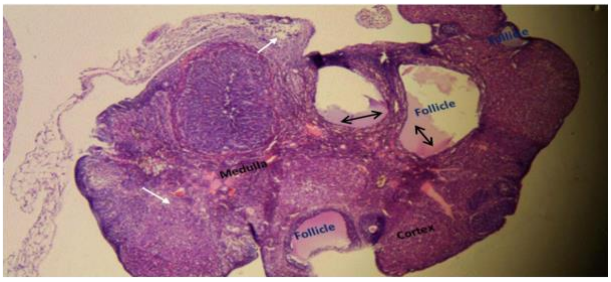
The distribution by the mean weight of the ovaries for the 2 groups as shown in table 3 indicates that the mean weight of the ovary in group B (rats administered with 50mg per kg of Cadmium Chloride) was relatively higher, 0.00170 compared to the mean weight of the ovaries of the rats in the control group, 0.00150. However, the difference was not statistically significant (p = 0.341).

Table 3: Distribution by the rats in the 2 groups for the mean weight of their ovaries

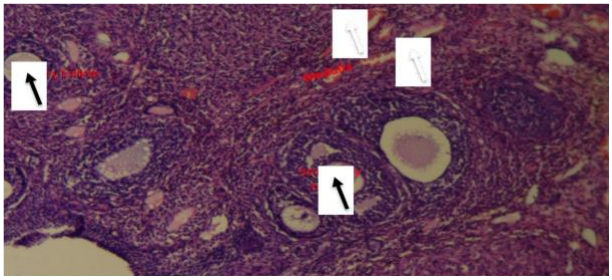
GROUPS	Mean Weight (grams)	± SEM	P-value
Group A (control)	0.00150	±0.002	0.341
Group B	0.00170	±0.002	

Figure 1 is a photo micrograph showing the histological pattern of the ovaries from rats in group A (control), section of the various tissues showed follicles at various stages of maturation, including primary and secondary follicles and appears normal with well intact ovarian blood vessels.

Figure 2 is a photomicrograph showing the histopathological changes in the ovaries of rats in group B- administered with 50mg per kg Cadmium Chloride. Sections show degenerated ovarian follicle, absence of secondary follicles together with the presence of multiple cysts.



Photomicrograph Section of Ovarian tissue for Group A (Control)


Photomicrograph section of ovarian tissues for Group B (administered 50mg per kg Cadmium Chloride)

DISCUSSION

Changes in physical and behavioral patterns demonstrated in this study following the administration of cadmium to the rats are a physical behavioral manifestation of cadmium toxicity observed in various studies. Mouloud et all[27] observed the depression-like, Anxiety-like, memory state and oxidative stress effect on Wister rats following the chronic administration of Cadmium. Severe alterations in physical and behavioral pattern observed in this study include weight alterations, loss of skin hairs, changes in skin color, labored breathing, staggering gait, Lethargy and loss of appetite manifesting as decreased food intake. Cadmium has been recognized to be one of the toxic element existing in the environment particularly in industrial areas through environmental pollution involving dispersion into the environment and contamination of the ground surrounding metal emitting industry[28] the low rate of excretion of cadmium from the body enables it to have a long biological half-life spanning through 10-30 years- a period long enough to cause toxic damage to the body.[29, 13]

Toxicity can occur from accumulation of cadmium over long period of time, at peripheral level in a variety of tissues including- liver, Kidneys ovaries and both Central and peripheral nervous systems[30]. The preliminary step in regulating the entry of cadmium into the CNS is through transportation into the blood brain barrier. The nasal mucosa through the olfactory pathway is also an alternative entry pathway of Cadmium into the Central Nervous System (CNS).[31] Heavy metals such as

Cadmium are known to act as catalysts to biochemical reaction; cofactors for many vital enzymes; second messengers signaling pathways and regulators of gene expression- all pathways recognized in the regulation of important physiological, pathological and behavioral functions. Chronic exposure to cadmium, therefore, affects several nervous system functions leading to symptoms such as headache, vertigo, pseudo-parkinsonism, unstable gait, peripheral neuropathy, loss of concentration and impaired learning ability[32,33,34]. The hippocampus of the brain has been observed to accumulate heavy metals such as cadmium leading to dysfunction manifesting as behavioral alteration which has been shown in animal studies such as ours[35,36,37].

Maoloud et al[27] observed that this derangement in behavior correlated with levels of oxidative stress markers in the hippocampus of their experimental rats and hypothesized that behavioral Neuro dysfunction occurring from administration of cadmium maybe linked with the elevation of the levels of oxidative stress markers in the hippocampus. Cadmium toxicity result from its generation of non-radical hydrogen peroxide which in turn generates free radicals through the Fenton reaction[38,39]. Cadmium indirectly induces oxidative stress through causing a decrease in intracellular levels of glutathione, combining with thiol groups of antioxidant enzymes e.g. catalase, glutathione peroxidase and superoxide dismutase ultimately preventing their normal function. Cadmium in addition obstructs complex 111 of the mitochondrial electron transport chain thereby enhancing the production of reactive oxygen species that will result in negative alteration of mitochondrial membrane and subsequently encouraging apoptosis[40]. Cadmium is capable of replacing Iron, Zinc, copper Magnesium and Calcium from a number of biomolecules and membrane proteins. This can alter the function of such biomolecules and cause an increase in the levels of such metals, this can, in some cases be related to production of oxidative stress through the Fenton reaction. Cadmium has a binding capacity that is 10 times more than that of Zinc and has even much greater difficulty to unbind[40].

The histological features of the ovary following the administration of cadmium in this study showed pathological changes that constitutes a clear departure from the normal pattern shown in the control and signifies cadmium toxicity. Sections of these ovaries showed degenerated ovarian follicle, absence of secondary ovarian follicle and presence of multiple cysts. Several studies carried out on experimental rats have demonstrated high concentration of cadmium in ovaries 48hours following intra-peritoneal administration of varying doses of cadmium chloride; and a relative reduction in volume of growing follicle, increase in stroma, significant increase in the number of atretic follicles, and ultra-structural alterations of other cells of the ovarian tissue- Granulosa, Luteal and endothelial cells[21 23,24]. Cadmium is a known endocrine disruptor associated with reproductive complication and in fact has been ranked as one of the top ten most toxic chemicals for human health[41] it has been reported that cadmium exposed workers can harbor blood levels of cadmium ranging between 2 and 50ug/L[42]. Environmental pollution with Cadmium is of major world health public concern because of its notoriety as an endocrine disrupting chemical (EDC) and reproductive toxicant[43].

In specific terms cadmium has been reported to cause dysfunction of the menstrual cycle; infertility and in recent times polycystic ovary syndrome and premature ovarian failure (POF)[44]. Polycystic Ovarian syndrome (PCOS) is a condition characterized by ovulatory dysfunction, excessive action of androgen hormones and development of multiple cysts in the ovary. The condition also features high levels of luteinizing hormones (LH) being stimulated by gonadotropin releasing hormones leading to high LH/FSH ratio[45]. PCOS and Primary Ovarian Failure (POF) occur in approximately 1 and 10% of women of reproductive age respectively[46] Recent studies have shown that exposure to cadmium has resulted to the development of PCOS and POF in mammalian models[47]. Data obtained from an even more recent study conducted on rats suggested that sub-acute cadmium exposure using doses found in workers occupationally exposed to cadmium disrupt the Human Pituitary Gonadotropin (HPG) axis function, leading to PCOS and POF features and other abnormalities in the female rats. The study further reported that in specific terms exposure to cadmium resulted in irregularity of the estrous cycle, abnormal hypothalamic gene expression, high levels of luteinizing hormone (LH), low levels of ant-Mullerian hormone (AMH) and abnormal development of the follicles. The study in addition observed a reduction in ovarian reserve and antral follicle number which suggests ovarian depletion. Cadmium exposure also caused a reduction in corpora luteal, thickness of the granulosa layer and an increase in cystic/atretic follicles. Other findings associated with cadmium exposure include inflammation of the reproductive tract (RT), Fibrosis and Oxidative stress[48].

CONCLUSION

This study has demonstrated the toxic effect of cadmium on the Nervous system and the ovaries of Wister rats evident from severe alterations in the physical features and behavioral patterns of the rats as well as pathological changes observed from photo micrographic studies of sections of the ovaries. Physical and behavioral manifestations observed in this rats following exposure to cadmium includes loss of body weight, alteration in skin color, loss of body hairs, staggering gait, labored

breathing, Lethargy and loss of appetite. Histopathologic manifestations of ovarian toxicity featured as degenerated ovarian follicles, absence of secondary follicles and presence of multiple cysts. The mechanism of action of cadmium in the causation of toxicity to tissues and in particular its role in increasing levels of oxidative stress markers has been discussed. The implication of ovarian toxicity, linked to human reproduction and in particular polycystic ovarian syndrome, primary ovarian failure and infertility has also been discussed extensively. Further studies in relation to the effect of cadmium on steroidogenesis and the possible effect of certain antioxidant substances such as curcuma longa in ameliorating the toxic effect of cadmium on reproductive function is recommended.

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