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Evaluation of cadmium toxicity and its association with iron on the gonads of female rats

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ABSTRACT: Cadmium has been identified as one of the endocrine-disrupting chemicals. Several studies on heavy metals focus on individual metals neglecting the fact that they occur in association with other metals in the environment, a situation that can affect the toxic capacity of each metal. The current study was therefore designed to examine the possible influence of iron (Fe) on cadmium (Cd) toxicity in the gonad of female rats. Twenty adult female albino rats used in this study were divided into four groups. The groups were designated as group A-control (rats administered Cd-free water), group B rats were exposed to Cd-tainted water, group C rats were exposed to Fe-tainted water and group D rats were exposed to combined Cd+Fe tainted water. The treatments were done daily for four weeks. At the end of 4 weeks of exposure, there was significant increase in ovary CAT activity of rats exposed to Cd+Fe when compared to the control. The malondialdehyde (MDA) level in ovary of rats exposed to Fe only was significantly increased ($p \leq 0.05$) relative to the control. Similarly, there was a significant increase in serum cholesterol level of rats exposed to Cd+Fe simultaneously when compared to the Cd only and Fe only treated groups. The levels of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were significantly increased ($p \leq 0.05$) in sera of rats exposed to Cd only, Fe only and Cd+Fe when compared to the control. The levels of estradiol were significantly decreased in all the treated groups relative to control. The level of testosterone was significantly increased ($p \leq 0.05$) in Cd+Fe group relative to the control. Histological study revealed atretic and disintegrating follicles in ovary of rats exposed to Cd only and combined Cd+Fe. The results from this study suggest that cadmium only as well as combined cadmium and iron are responsible for the biochemical changes induced in the ovary. The presence of Cd caused oxidative stress in the ovary and an imbalance in serum levels of the reproductive hormones analyzed. Since Cd only was able to cause the changes observed and similar changes was also observed in presence of iron, it can therefore be suggested that Cd is responsible for the changes since iron was unable to ameliorate its effect. Findings from histological examination of the ovaries that there was profound disintegration with follicular damage appear to corroborate the biochemical observation made in this study. In conclusion, it appears that cadmium in the presence of iron can still exhibit its gonadal toxicity without antagonism or synergism with iron as shown in the rats exposed via water.

Keywords: Cadmium, iron, water, gonads, hormones.

Introduction

Cadmium is released into the environment as a result of human activities and it is routinely found as a contaminant in tissues collected from the human population throughout the world (Michael *et al*., 2002). It is a toxicant that has a long biological half-life (15–20 years) and accumulates over time

within the blood, kidneys, liver, and reproductive organs (Michael and Jorge, 2004). It caused a poisoning in various tissues of humans and animals. Acute administration of Cd often induces lethal toxicity in mice and rats (Jung, 2001). Cd, like other endocrine disruptors, is able to modify the basal activity of the ovaries and even the testes (Anunciación *et al.,* 2001). The gonad is considered the main target for environmental toxins and rodent ovaries or testes are especially sensitive to the toxic effects of Cd exposure. Cd impairs reproductive capacity by causing severe testicular degeneration, seminiferous tubule damage and necrosis in rats (Anunciación *et al.,* 2001; Burukoğlu and Bayçu, 2008).

Heavy metals do not occur alone but exist along with other pollutants in water, land and even in air. Any organism exposed to it will suffer the combine toxic effect of these pollutants (Ogunbiyi *et al.,* 2019). Cadmium (Cd) present in the air, drinking water and food has the potential to affect the health of people, mainly those who live in highly industrialized regions (Lara *et al*., 2004). Cd affects placental function (Lara *et al*., 2004) and even gonadal function. Cd itself do not occur in isolation but also influenced by the presence of other heavy metals such as iron, lead, zinc, copper, manganese, chromium and nickel (Okocha and Adedeji, 2011).

However, since heavy metals do not occur alone, it is therefore better to look at their combine toxic effect rather than their single effect. The purpose of the present study was therefore aim to evaluate the effect of cadmium (Cd) and iron (Fe) toxicity singly and combined on the reproductive organs (gonads) of female rats, as a model for human representative. The rats were exposed to Cd (0.229 mg/L) and Fe (1.900 mg/L) tainted water through drinking water for 4 weeks. The study investigates the gonadal effects of water– mediated exposure of rats to cadmium and iron.

Materials and Methods

Materials

Wistar rats

Twenty adult female albino rats was obtained from Animal Science department, University of Ibadan, Oyo State. The animals were acclimatized for a period of one week before the commencement of the treatments.

Chemicals/Reagents

All chemicals used for this study were of analytical grade. Cadmium chloride hemidihydrate ($CdCl_2.2.5H_2O$) was the product of Kermel, Germany. Iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$) was the product of JHD, China. Total cholesterol assay kit and ELISA kits were products of Monobind Inc, USA.

Methods

Preparation of stock cadmium/iron solution and cadmium/iron tainted water

Stock solution of cadmium/iron and cadmium/iron tainted water were prepared as described by Ogunbiyi *et al.,* (2019)

Treatment of Rats

The rats were divided into 4 experimental groups of 4 rats each preceded by one-week acclimatization period. The groups were designated as group A-control (rats exposed to exogenous Cd-free water), group B (rats exposed to Cd-tainted water), group C (rats exposed to Fe-tainted water) and group D (rats exposed to combined Cd and Fe- tainted water). The animals were kept under standard laboratory conditions in a well-ventilated wooden cages and fed with standard rat pellet diet (Bendel Feed Flour Mill, Ewu, Edo State) and drinking water *ad libitum* prior to and during the period of the study. Each rat receives the equivalent of 0.043 ml/g body weight which is administered daily by gavage orally. The exposure period lasted for 4 weeks.

All experiments were carried out in accordance with National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH publication no. 85-93, revised 1985). All rats were weighed at the end of four weeks of exposure and anaesthetized in chloroform saturated chamber. While under anaesthesia, the blood were collected by heart puncture and transferred to plain sample bottles. Subsequently, the ovary was weighed and homogenized on a pre-cooled mortal and pestle.

Preparation of Blood Serum and Tissue Homogenates

The clotted blood samples collected were centrifuged at 3,000 rpm for 15 minutes and the supernatant obtained were stored at -20°C overnight for biochemical assays. Ovary tissues was chopped into very small pieces and homogenized in ice-cold physiological saline (0.9 %) to obtain 10% homogenates (1: 9 w/v). The resulting homogenate were centrifuged at 5,000 rpm for 15 minutes and the supernatants obtained stored at -20°C overnight for biochemical assays.

Biochemical Assays

Determination of Serum/Ovary Cholesterol

Serum/ovary cholesterol was estimated based on the method described by Allain *et al.,* and Roeschlaw *et al.,* (1974).

Determination of Malondialdehyde level and Antioxidant Enzyme Activities

Malondialdehyde (MDA) level was estimated based on the amount of thiobarbituric acid reactive substances (TBARS) that will be produced as an indicator of lipid peroxidation as described by Guttridge and Wilkings (1982). Values of TBARS are quantitated using a molar extinction coefficient of 1.56×10^5 M/cm and expressed in terms of Malondialdehyde (MDA) units per gram tissue. Superoxide dismutase (SOD) activity was assayed by the method of Misra and Fridovich (1972) and the activity computed and expressed as described by Baum and Scandalios (1981) in which one unit represents the amount of the enzyme required for 50% inhibition of epinephrine during 1 min. Catalase (CAT) activity was assayed by using the method of Cohen *et al.,* (1970). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2), after the addition of the material containing the enzyme.

Determination of Reproductive Hormone Status

The levels of the reproductive hormones assayed in the sera samples were estimated based on the standard principle of enzyme- linked immunosorbent assay (ELISA) of Braunstein *et al.*, (1976), Winter and Faiman (1973), Klopper and Fuchs (1977) and Tietz (1995) as described in ELISA kit leaflets for luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol and testosterone respectively.

Histological Examination

Histological study on ovaries obtained from experimental rats was carried out following the method described by Kiernan (2008). The ovaries were removed from the animals and immersed in Bouin's fixative for 12–24 h. Tissues were dehydrated, embedded in paraffin, sectioned (5 µm), and stained with haematoxylin and eosin and analyzed under Omax research microscope.

Statistical Analysis

The experimental were expressed as mean ± standard error of the means (SEM) and were subjected to one-way analysis of variance (ANOVA). Significant levels were set at $p \leq 0.05$ using Tukey's multiple range comparison tests obtained from Graphpad Prism 6.0.

Results

Cholesterol Levels

The cholesterol level in serum and ovary of rats exposed to Cd and Fe via water are presented in Table 1.

Table 1: Cholesterol Level in Serum and Ovary of Rats after 4 weeks Exposure

Group Designation	Cholesterol Level		
	Treatments	Serum (mmol/L)	Ovary (mmol/L of tissue homogenate
Group A	–Cd –Fe	2.53 ± 0.28	2.41 ± 0.35
Group B	+Cd –Fe	2.54 ± 0.22	1.90 ± 0.27
Group C	–Cd +Fe	3.43 ± 0.55	2.13 ± 0.25
Group D	+Cd +Fe	1.35 ± 0.20c	2.40 ± 0.05

Mean ± SEM (n=5). ([a])Values with superscript 'c' within a column are statistically significantly different ($p \leq 0.05$) relative to the values of group C.

There was a significant decrease ($p \leq 0.05$) in serum cholesterol level of rats exposed to the combined cadmium and iron when compared to rats exposed to iron only.

MDA Levels, SOD and CAT Activities

The MDA levels, as well as SOD and CAT activities of the serum and ovary of rats at the end of 4 weeks exposure to Cd and Fe via water were presented in Table 2 and Table 3.

Table 2: Effects of Cd and Fe on MDA Levels and Antioxidant Enzyme Activities in Serum of Rat after 4 weeks Exposure

Group Designation	Group A*	Group B	Group C	Group D
MDA (mol/dL serum x 10^{-5})	24.26 ± 10.30	20.30 ± 3.55	14.08 ± 1.32	19.18 ± 4.51
Superoxide Dismutase (Units/dL serum x10^{-3})	50.08 ± 19.06	32.36 ± 4.36	26.36 ± 1.89	38.14 ± 7.81
Calatase (mol/dL serum)	9.84 ± 5.24	13.22 ± 6.31	6.57 ± 3.93	15.72 ± 6.55

*The groups are as described in Table 1.

Mean ± SEM (n=5). Values within a row are not statistically significantly different from each other ($p > 0.05$).

Exposure of rats to cadmium and iron had no effect on the serum MDA levels, SOD and CAT activities of rats when compared to control.

Table 3: Effects of Cd and Fe on MDA Levels & Antioxidant Enzyme Activities in Ovary of Rat after 4 weeks Exposure

Group Designation	Group A*	Group B	Group C	Group D
MDA (mol/mg tissue)	26.86 ± 4.57	26.70 ± 6.00	66.07 ± 16.85[a]	36.95 ± 6.39
Superoxide Dismutase (units/mg tissue)	22.68 ± 8.32	34.60 ± 2.22	50.88 ± 5.69	47.56 ± 8.22
Calatase (mol/mg tissue)	1.40 ± 0.35	7.02 ± 1.05[a]	13.23 ± 4.66[a]	12.05 ± 7.14[a]

*The groups are as described in Table 1.

Mean ± SEM (n=4). ([a])Values with superscript 'a' within a row are statistically significantly different ($p \leq 0.05$) relative to the value of Group A.

There was significant increase ($p \leq 0.05$) in MDA levels of ovary of rats exposed to iron-tainted water when compared to the control but was not statistically different from those exposed to cadmium- and cadmium-iron tainted water. Exposure of rats to both metals caused a significant increase in CAT activities of ovary of rats exposed to cadmium-, iron- and cadmium-iron via water when compared to the control but not statistically different from cadmium- and iron-groups (see Table 3).

Reproductive Hormone Status

Effects of Cd and Fe on Serum Reproductive Hormone Status of Rats

The level of reproductive hormone in serum of rats after 4 weeks exposure via water are presented in Figure 1.0, 2.0, 3.0 and 4.0 respectively.

Exposure of rats to both Cd and Fe increases the LH level and FSH level. When compared to control, there were significant increase ($p \leq 0.05$) in both LH and FSH levels of rats exposed to cadmium- and cadmium-iron tainted water whereas a slight significant increase was observed in rats exposed to iron.

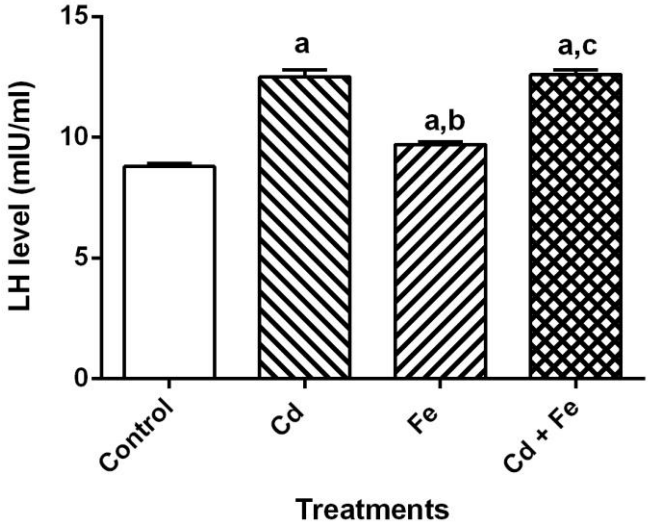

Figure 1: LH Level in Serum of Rat

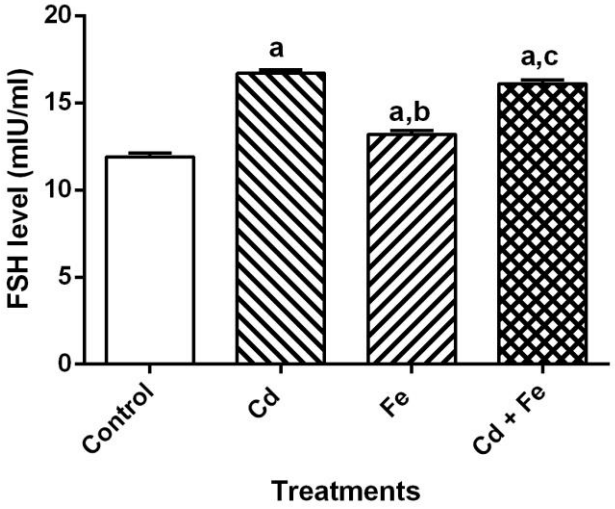

Figure 2: FSH Level in Serum of Rat

Mean ± SEM (n=3). ([a])Values with superscripts 'a, b and c' are significantly different ($p \leq 0.05$) relative to the values of control, cadmium only (Cd) and iron only (Fe).

Estradiol levels significantly decrease in all the treated groups (see Fig. 3.0). There was significant decrease ($p \leq 0.05$) in serum estradiol level in the rats exposed to the metals when compared to control. However, when exposed singly and combined to the metals, the testosterone level was not significantly different when compared to control.

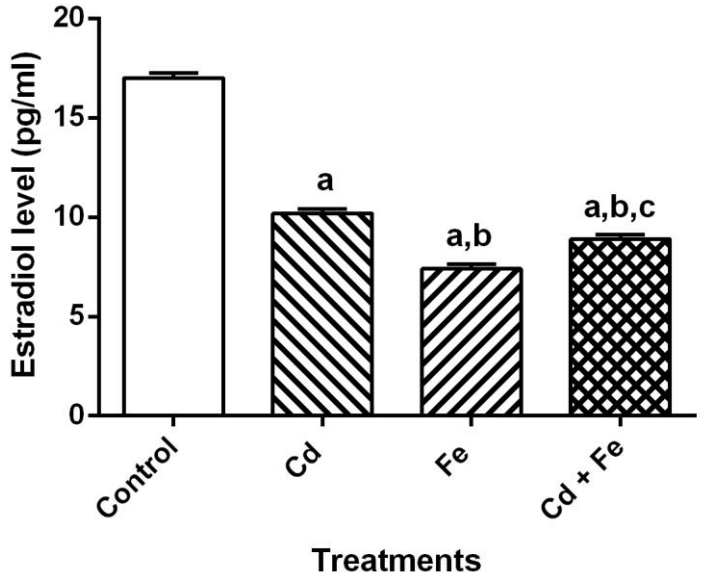

Figure 3: Estradiol Level in Serum of Rat

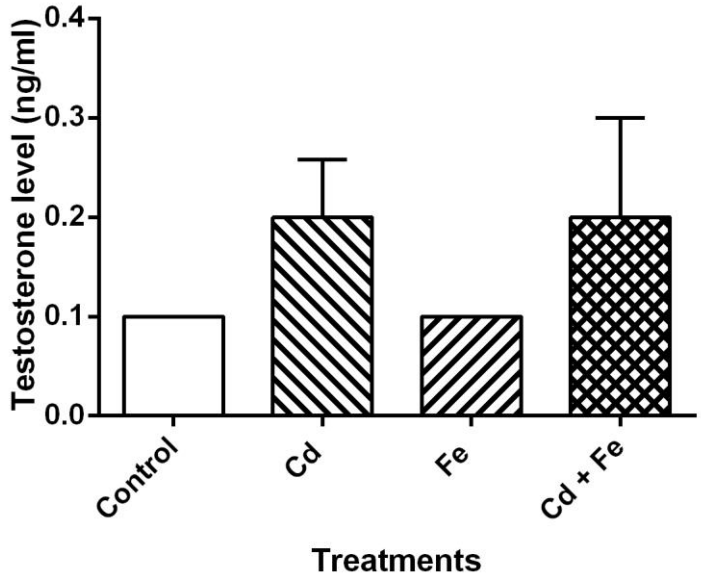

Figure 4: Testosterone Level in Serum of Rat
Mean ± SEM (n=3). ([a])Values with superscripts 'a, b and c' are significantly different ($p \leq 0.05$) relative to the values of control, cadmium only (Cd) and iron only (Fe).

Histology of Ovary of Exposed Rats

Ovary of Exogenous Cd and Fe-free Rat

Examination of the photomicrograph (Plate 1) of ovary obtained from rat that were not exposed to Cd and Fe revealed the presence of follicles at different stages of development.

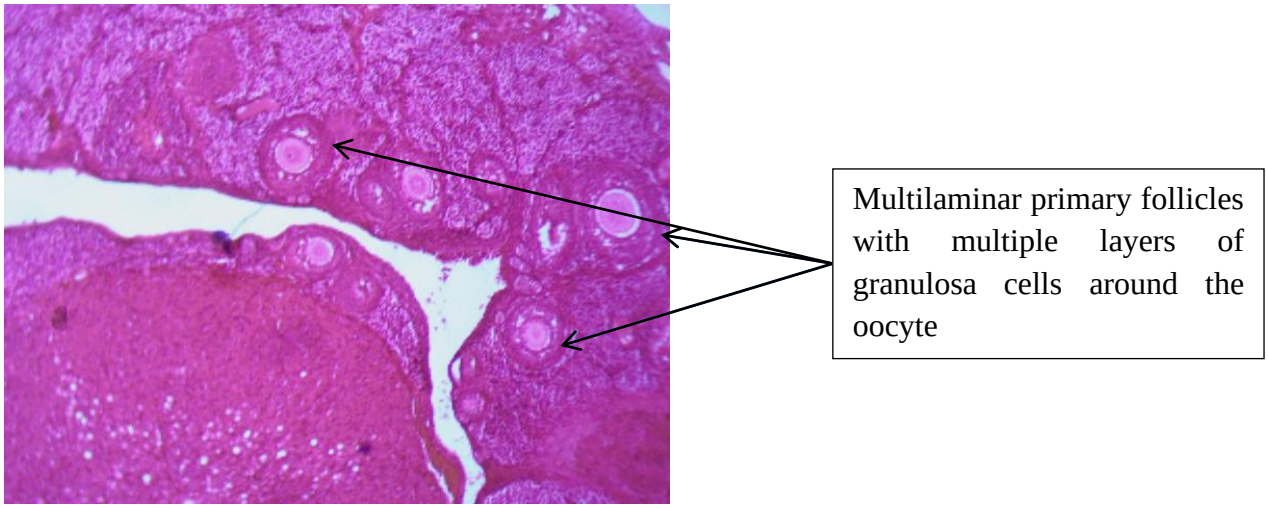

Plate 1: Photomicrograph of section of ovary from Cd and Fe-free rat (H and E x100).

Ovary of Cd Exposed Rat

Examination of the photomicrograph (Plate 2) of ovary obtained from rat exposed to Cd revealed the presence of atretic follicles as well as disintegrating follicles.

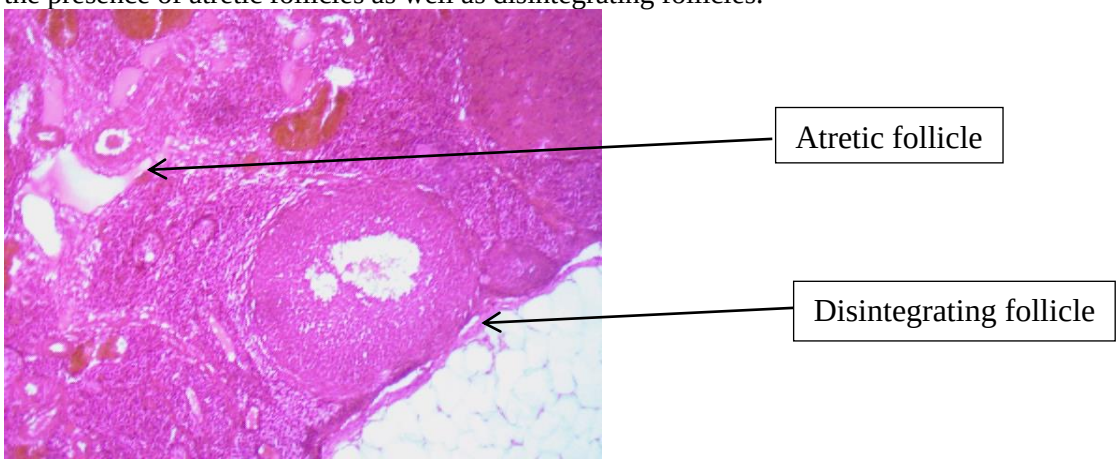

Plate 2: Photomicrograph of section of ovary from Cd exposed rat (H and E x100).

Ovary of Fe Exposed Rat

Examination of the photomicrograph (Plate 3) of ovary obtained from rat exposed to Fe revealed the presence of normal follicles.

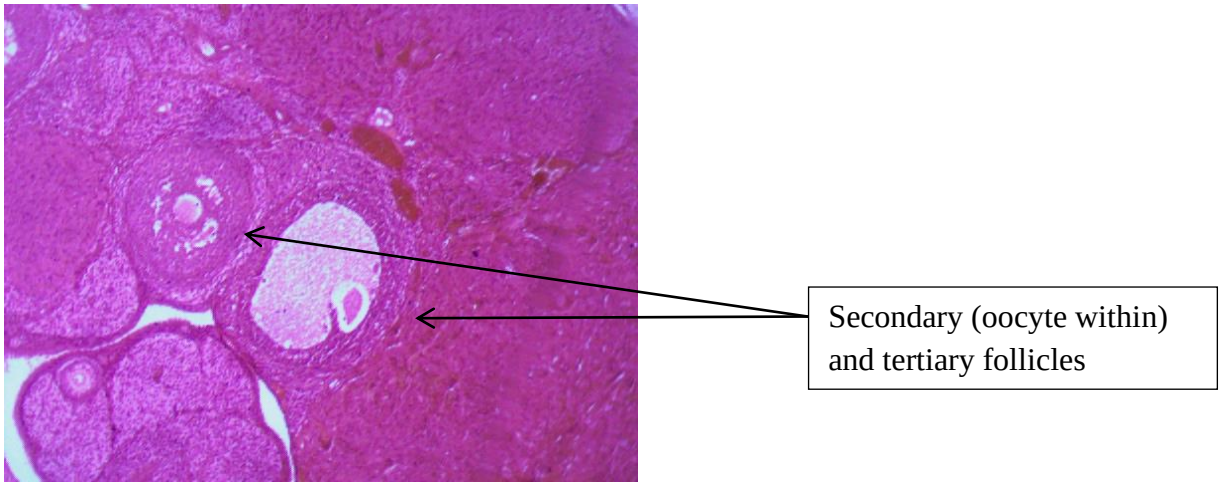

Plate 3: Photomicrograph of section of ovary from Fe exposed rat (H and E x100).

Ovary of Cd and Fe Exposed Rat

Examination of the photomicrograph (Plate 4) of ovary obtained from rat exposed to Cd and Fe revealed the presence of pronounced follicle disintegration.

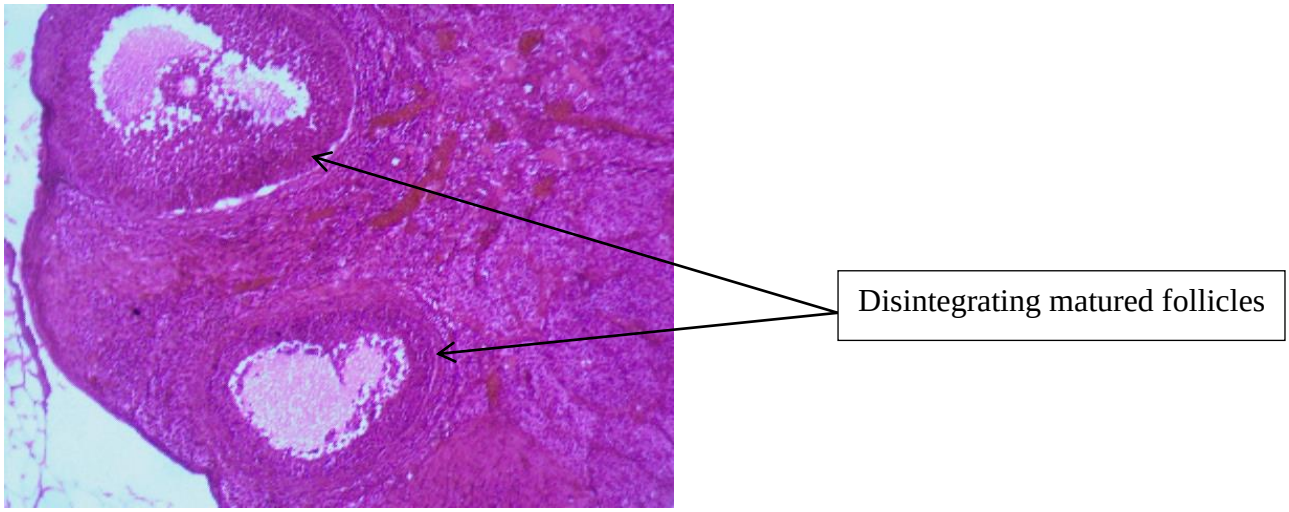

Plate 4: Photomicrograph of section of ovary from Cd and Fe exposed rat (H and E x100).

Discussion

Several controlled studies on the biochemical effects of cadmium using different animal models have been conducted (Liu *et al*. 1995; Gur *et al*. 1995; Horiguchi *et al*. 1996; Sidhu *et al*. 1997; Asagba and Obi, 2005; Asagba, 2009; Nawal *et al*., 2015). In this study, the toxicological effects of cadmium and iron are evaluated to elucidate their individual and combined effects on reproductive organs when exposed to the animals orally via tainted drinking water.

Increase in MDA level in the ovary of rat exposed to Fe observed in this study reveal the ability of the metal to induce oxidative stress in the ovary of the rat. Antioxidant enzymes play essential part in the cellular defense against free radical-mediated tissue or cellular damage. The involvement of oxidative stress in Cd induced cellular toxicity was known (Nampoothiri *et al.*, 2007). Data from the present study on antioxidant and free radicals clearly suggest the onset of oxidative stress in the ovary of Fe treated rats (Table 3).

In the present study, the observed increase in MDA level in the ovary of rat exposed to iron and the attendant increase in CAT activities in the Cd-, Fe- and Cd+Fe tainted water exposed groups indicates the development of oxidative stress. This appears not to agree with the earlier report of Bu *et al* (2011) and Akunna (2017) who observed decrease in SOD and CAT activities in rat testis after cadmium chloride exposure intraperitoneally for 8 weeks. Cadmium has been reported to induce oxidative stress in ovarian rats by increasing the CAT activity, SOD activity and MDA level (Tribowo *et al.*, 2014; Iwan *et al.*, 2015).

The toxic mechanisms of cadmium are not well understood, but it is known to act intracellularly, mainly via free radical-induced damage, particularly to the lungs, kidneys, heart, bone, central nervous system and reproductive organs (Jornova and Valko, 2011). It has also been reported that Cd may induce oxidative damage in a variety tissue enchancing peroxidation of membrane lipids due to inhibition of antioxidant enzyme. Other authors have noted that Cd exposure might lead to lipid peroxidation, causing an increase in antioxidant enzyme activities. The alteration of antioxidant enzyme activities may depend on several factors such as, Cd dose, Cd exposure times, type of Cd administration (Sandalio *et al.*, 2001) and even the presence of other metals.

Serum and ovary cholesterol level was also used as an index for stress (Kumar et al. 2010; Obi et al. 2014; Ogunbiyi, 2017) and the possible precursors for steroid hormone biosynthesis in this study. The decrease in serum cholesterol levels of combined metals appears to suggest that concurrent

exposure to cadmium and iron may perturb cholesterol metabolism. Cd alone may exhibit its toxicity over time by down-regulating the concentration of cholesterol in the plasma. Cd has been shown to down-regulate the plasma and erythrocyte concentrations of cholesterol in rats following Cd exposure via drinking water for six weeks (Ogunriola, 2015).

Results obtained from this study also show increase in serum level of LH and FSH in the rats after exposure to cadmium and iron for 4 weeks via tainted water. This observation was similar to the one observed by De Souza Predes *et al*. (1999) and Nawal *et al*. (2015) in rat testis after oral cadmium administration. The increase level of FSH could be as a result of decrease level of cholesterol in the serum or decrease rate in the cholesterol de novo process and accelerated rate of cholesterol degradation. Variation in the level of LH observed in this study appears to be as a result of the modification activity of these metals in the pituitary gland. Cd has been considered as an important environmental endocrine disruptor (Li and Wu, 2002; Nawal *et al*., 2015), this may lead to variations in plasma LH levels, thus indicating that the metal may act at the hypothalamic level, modifying the activity of the endogenous clock, changing the mean concentration of LH secreted daily by the pituitary gland (Shirama *et al*. 1982; Nawal *et al*., 2015).

The decrease in serum estradiol observed in the cadmium and iron exposed rats may reflect direct effect of the metals on the ovary as these metals accumulate in this tissue (López-Artíguez *et al*. 1993; Nawal *et al*., 2015). Hence, the observed decrease in the level of estradiol in Cd-treated rats and Cd+Fe treated rats may be due to impaired gonadotropin levels reported in a previous study (Priya *et al.*, 2004). This observation was similar to data obtained by Jawahar (2011) on estradiol level of rat exposed to cadmium via drinking water.

Consistent with the *in vitro* observation of increased testosterone in the presence of Cd, chronic Cd oral exposure increased plasma testosterone in rats (Zeng *et al.*, 2003). The increase in plasma testosterone was not evident until after more than 1-month exposure to Cd in the drinking water (Dyer, 2007). In contrast, Cd given by subcutaneous injection to adult rats caused a decrease in plasma testosterone (Lafuente *et al.*, 2000). Discrepancies between these studies suggest that the route of exposure to Cd affects whether it stimulates or inhibits ovarian androgen production. Human Cd exposure through ingestion or occupationally also is associated with increased testosterone and estradiol (Zeng *et al.*, 2002; Jurasovic *et al.*, 2004). The mechanism for Cd-induced increase in human testosterone is unknown (Dyer, 2007). Hence, the observed decrease in the level of estradiol in Cd-treated rats and Cd-Fe treated rats may be due to impaired gonadotropin levels reported in the previous study (Priya *et al.*, 2004). Further, enzymes required for the biosynthesis of ovarian steroid hormones have been shown to be affected (Nampoothiri *et al.*, 2007). Hence, the decrease in the levels of ovarian hormones might be mediated through impaired gonadotropin levels or steroidogenic enzymes (Dyer, 2007).

Histological examination of the ovary of experimental rats (Plate 2 and Plate 4) revealed disintegrated follicular architecture in the ovary of rats. Ovaries obtained from rats exposed to Cd-tainted water and Cd-Fe tainted water, showed gross follicle disintegration.

Cd treatment-induced disruption of ovarian histoarchitecture was reported (Massanyi *et al.*, 2000). In previous work, Gurel *et al*. (2007) reported ovarian follicular cell damage in Cd-treated female rats. The present study showed that Cd exposed via tainted water caused histoarchitectural changes in follicular cells and oocytes, increase number of atretic follicles and unrecognized oocytes. This observation was in agreement with Massanyi, *et al.* (2005). Thus, the present study revealed the gonadotoxic nature of Cd and its possible effects in presence of Fe.

Conclusion

Findings from this study suggest that iron toxicity on female rat gonads (ovaries) is apparently raised as evidenced by elevated status of stress indices in rats exposed. In conclusion, it appears that cadmium in the presence of iron can still exhibit its gonadal toxicity without antagonism or synergism with iron. Further investigation is needed to clarify the interaction of Cd with other metals and downstream consequences.

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