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Effect of Mercury on the Histopathological Architecture and Hormonal Changes on the Ovary of Wistar Rats

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Abstract
The study was conducted to assess the effect of mercuric chloride (HgCl2) on the ovary and the hormonal changes associated with it. Sixteen (16) Wistar rats weighing between 180 g – 280 g and of both genders were separated randomly into 4 groups of four animals each after copulation has been confirmed. Groups B, C and D, received 4mg/kg, 6mg/kg and 8mg/kg respectively body weight of mercuric chloride solution for 28 days, while Group A was the control rats received distilled water as contained in the experimental doses. The rats were anesthetized using chloroform, dissected and blood samples for hormonal assay were collected by retro orbital process. The ovaries were removed and fixed in 10% neutral buffered formalin. They were weighed and processed for histological studies using H & E staining techniques. There was insignificant reduction ($p>0.05$) in weight (213.0 ± 3.52g) when compared to the control group. There was significant reduction ($p<0.05$) in LH (2.3 ± 0.55mlU/ml) and FSH (0.167 ± 0.005mlU/ml) when compared to the control group. There was insignificant reduction ($p>0.05$) in EH (0.090 pg/mL). The histology of the ovaries reveals degenerative changes with increasing formation of fibrosis and hyalinized stroma.

Keywords: Luteinizing hormone, Follicle stimulating hormone, Estrogen, Mercuric chloride

Introduction

Mercury exposure can cause various health problems in humans and is known to affect child growth in pregnancy or early life development. Hg has been labeled by the WHO as one of the ten key chemicals with potential public health concerns (WHO, 2017). Mercury (Hg) has harmful effects on human health, and exposure to this metal has been on an increasing level by industrial and anthropogenic activities. Contamination of water and air by toxic metals is an environmental concern and millions of people are being affected around the world. Food contamination with Hg is another concern for human and health (Luo *et al*., 2020).

The environmental pollution from mercury (Hg) and its effects on human health is currently a global challenge (O' Connor *et al*., 2019). The biosphere is contaminated by Hg mainly due to anthropologenic factors (human factors) such as coal combustion, mining, cement production and chemical industry (Wu *et al*., 2018). Mercury (Hg) is a non-essential metal that is naturally produced via vulcanization and earth crust erosion, and anthropologically as a byproduct of fuel combustion, cement production, metal smelting, and silver and gold extraction released into the environment. Apart from this, the uncontrollable use and discard of Hg-containing products (lamps, batteries, paints, fungicides, and medical instruments) have enhanced the environmental Hg levels (Horowitz *et al.,* 2014). Humans can be exposed to Hg occupationally (Dacharat *et al*., 2014), or through the intake of contaminated water (WHO, 2007). Adding to this, mercury has been used in a wide range of products ranging from seed treatment, consumer application, dental fillings and preservatives in

vaccines. Thus, humans are exposed to mercury in some form and at some concentration. The development of industries especially in developing economy where there is a potential release of mercury will continue to be a challenge to both humans and the environment. In addition to dental amalgam, mercury has been of considerable use in laboratory instruments, which during the last decade have been replaced by other technologies (Ajibade *et al.*, 2019).

Some clinical signs that have been noticed in children and adults exposed to mercury are neurobehavioral deficiency and some few other clinical signs. Cytogenetic impairment, changes in immune function, and cardiovascular toxicity owing to exposure from mercury have been recorded (Passos and Mergler, 2008). Acute inhalation and exposure to mercury, at high concentration, may induce respiratory distress including dyspnea. Chronic exposure may induce symptoms from the central nervous system (CNS) including tremors, delusions, memory loss and neurocognitive disorders (Barnardes *et al.*, 2003).

The organic and inorganic forms of Hg chemicals cause toxic effects on the haematology, hepatic, cardiovascular, and reproductive systems (Syversen and Kaur, 2012). The central nervous system is more susceptible to organic Hg exposure (Nogara *et al.*, 2019) and the renal system is more susceptible to inorganic exposure (Oliveria *et al.*, 2016).

Recently, large populations worldwide are exposed to Hg, especially via the use of pesticides in agriculture and fluorescent light bulbs. Hg exists in a wide variety of physical and chemical states, each of which has specific characteristics for target organs. Exposure to Hg vapor as well as to organic Hg compounds specifically affects the central nervous system and kidney, liver, and gastrointestinal tract are mainly targeted by inorganic Hg compounds (Schurz *et al.*, 2000).

Despite the effort to reduce the use and release of Hg to the environment, there is still emission of Hg in high concentration. Due to the bio-magnification and bioaccumulation of Hg in the food chain, the main source of Hg exposure is found in the consumption of contaminated food, with fish and aquatic invertebrates which are a major source of methyl Hg exposure. Another group of special concerns are individuals working or having contact dental amalgam (Rowland *et al.*, 1994).

In humans, there have been demonstrated menstrual cycle abnormalities, including changes in bleeding patterns and cycle length among women occupationally exposed to Hg (Davis *et al.*, 2001). Hg compounds also affect pregnancy outcome. It has been found that the metal level in maternal blood and infant hair is inversely associated with birth weight (Lee *et al.*, 2010)

Pregnancy loss is the end point most frequently used to monitor effects of metals on female reproductive function, starting from early losses, which contain a large proportion of chromosomal abnormalities and may represent 35– 40 % of human pregnancies. The remaining 10–15 % later abortions are clinically manifested, and some have been linked to environmental factors (Sengupta, 2012).

One crucial aspect of health affected by the exposure to mercury is the reproductive system since the reproductive system is needed for procreation and the survival of an individual. There is therefore a need to understand the effect of these metals on the reproductive system. This will be done by assessing the effect of these metals (Hg) on the histopathological architecture of Wister rat ovaries. Additionally, hormonal assay Follicle stimulation hormone (FSH), luteinizing hormone (LH) and Estrogen hormone (EH) were carried out.

Materials and Methods

Study Design

The research included a total of sixteen (16) adult wistar rats weighing 200 – 210g that were sacrificed after 28 days of mercury administration. After the administration, the rats were put under light chloroform anaesthesia and the ovaries were harvested for histologic processing. The ovaries were obtained from the adult rats and fixed in 10% neutral buffered formalin for 24 hours. The samples were cut at 3 mm thickness and the selected tissues were

transferred into a tissue cassette, carefully labeled and processed histologically.

Animal Grouping and Administration
The rats were separated randomly into 4 groups of 4 animals each. Group B, C and D, received 4g/kg, 6g/kg and 8g/kg body weight of mercury chloride solution respectively while Group A serves as the control; receiving distilled water as contained in the experimental doses, throughout the research work.

Animal Welfare and Ethical Clearance
This study was carried out in accordance with current National Academy of Science (NIH,1985) guideline for animal welfare. The protocol was reviewed by the Institutional Animal Care. The Ethical clearance for this study was obtained from the Center for Research and Development (CERAD), Federal University of Technology Akure (FUTA/ETH/24/134).

Experimental Animals/Housing Condition
Adult albino rats, 16 in number, were maintained under conditions of controlled temperature and 12- hour light and dark cycle and were given access to food and water.

The rats were fed with grower mash and water was provided adlibitum. The animals were maintained and utilized in accordance with the standard guide for and use of Laboratory animals (NIH,1985).

Blood Sample collection
At the day 28th day, blood was collected using retro orbital blood puncture into sterile containers. The serum was collected and assayed for follicle stimulating Hormone (FSH), Estrogen and Luteinizing Hormone (LH).

Determination of FSH and LH
The concentrations of FSH and LH were determined using ELISA in-vitro diagnostic kit (IBL International, Hamburg, Germany). Each of the standards (0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative), control and samples were dispensed (25 µL) in appropriate well, 100 µL of the enzyme conjugate (0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative) was dispensed into each well and thoroughly mixed for 10 seconds. The mixture was incubated for 30 min at room temperature. The wells were rinsed 5 times with aqua dest (400 µL per well). Substrate solution (tetramethylbenzidine; TMB) of 100 µL was added to each well and incubated for 10 min at room temperature. The enzymatic reaction was terminated by adding 50 µL of the stop solution (0.5 M H_2SO_4) to each well. The absorbance was determined at 450 nm with a microtiter plate reader. The absorbance was extrapolated on a standard curve expressed in mIU/mL.

Determination of Estrogen Hormones
The sera of the control and sample were analyzed for estrogen hormone level and expressed in pg/ml with AccuLite master CLIA VAST Enabled kit.

Tissue Processing and Staining
An incision was made on the chest wall anteriorly through the abdomen; samples of the ovaries were excised and fixed in 10% formalin on the last day of the experiment. They were processed for the paraffin wax technique and stained with H&E.

Statistical analysis
A statistical analysis was performed on the collected data with SPSS version 25 using ANOVA (Scheffe) at 95% confidence level, the test groups' values were compared to those of the control group.

Result
The administration of Hg after 28 days caused histopathological damage to tissue morphology such as the formation of fibrotic and hyalinized stroma in the ovaries of the test Wistar rats.

Table 1: Showing the Effect of Hg on the Body Weight of the Rats

Weight (control group)	Weight (Exp. group)	t-value	p-value
215.0 ± 7.91	213.0 ± 3.52	0.231	0.635

Key: p<0.05 = Significant; >0.05 = Not Significant
Note: The collated values were expressed in mean ± SD. P- value of less than 0.05 was considered significant. The average weight of animal after 28days of Hg administration shows insignificant reduction (p>0.05) when compared with the control group.

Table 2: The Effect of Hg on Hormonal Parameters

Parameter	Control	Experimental group	t-value	p-value
FSH	0.190 ± 0.001	0.167 ± 0.005	4.459	0.047*
LH	0.441 ± 0.001	2.3 ± 0.55	5.033	0.002*
EH	63.00. ± 0.05	53.19 ± 3.16	4.876	0.090

Keys: p<0.05 = Significant; p>0.05 = Not Significant
Note: The collated values were expressed in mean ± SD. P- value of less than 0.05 was considered significant. The LH value of the experimental group was significantly reduced (p<0.05) when compared to the control. There were no significant changes in the EH of the experimental group when compared with control. There was significant reduction in FSH (p<0.05) in the test group in comparison with the control group.

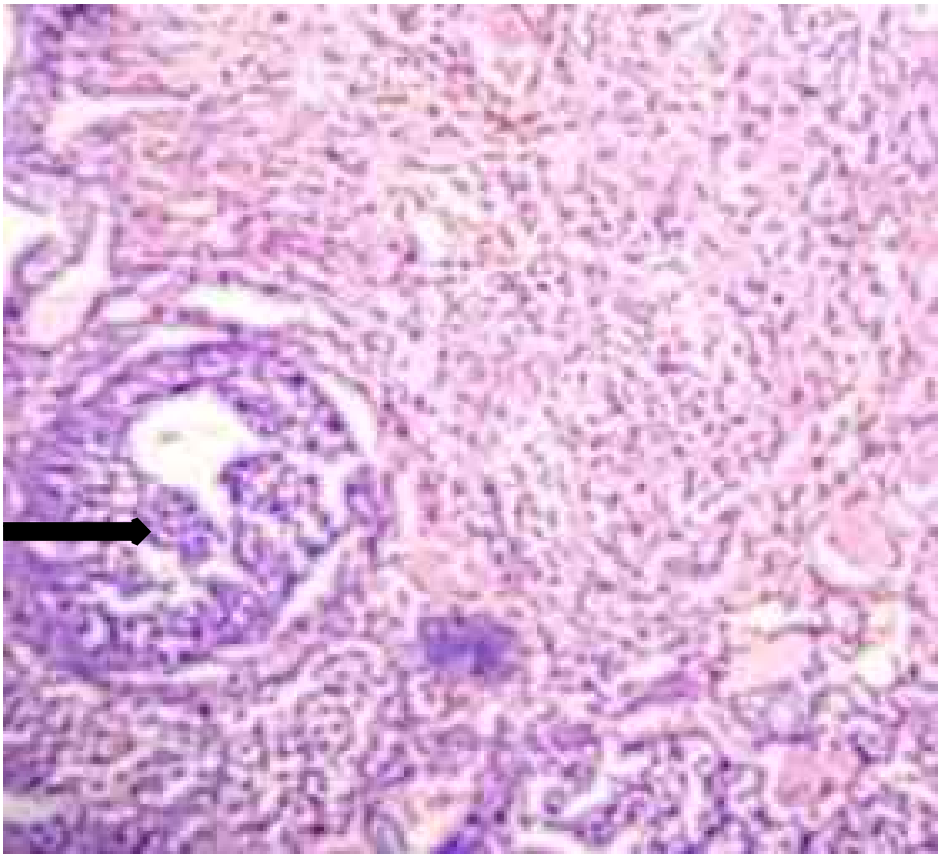

Plate 1: Photomicrograph of a normal section of ovary of the control rat (Group A) showing the graffiti follicle (Black arrow) in a background of ovarian stroma; haematoxylin and eosin staining, X 400.

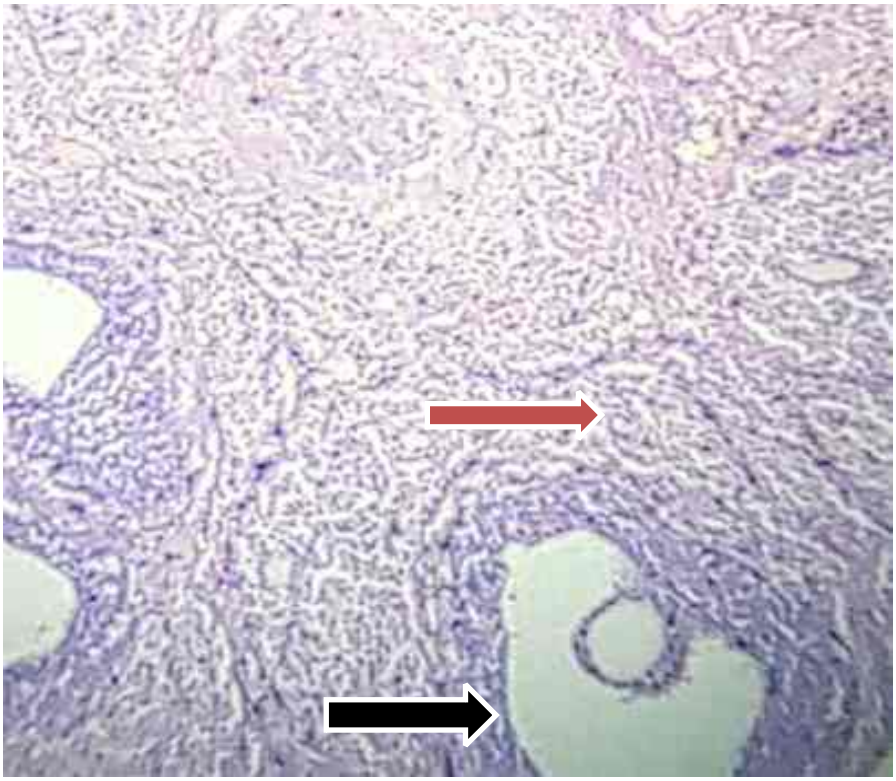

Plate 2: A photomicrograph section of the ovary (Group B) which were given 4mg/kg of $HgCl_2$ throughout the experiment showing graffian follicle (black arrow) besides a corpus luteum and a slightly fibrotic and hyalinized stroma (Red arrow); haematoxylin and eosin staining, X400.

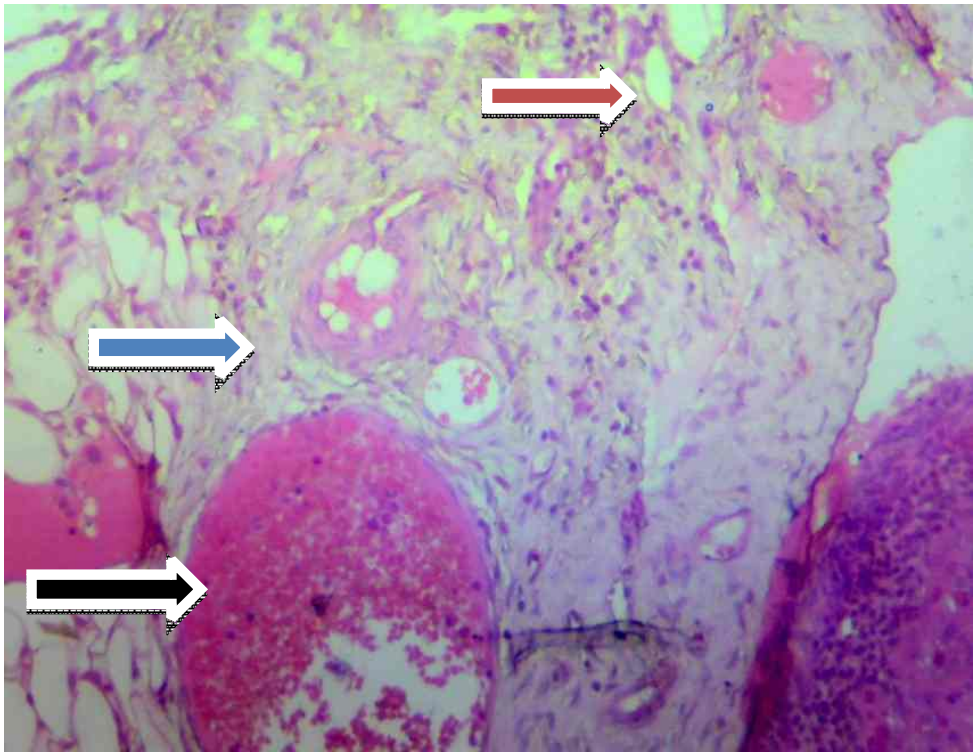

Plate 3: A photomicrograph section of the ovary (Group C) which were given 6mg/kg of $HgCl_2$ throughout the experiment showing showing graffian follicle (Black arrow) surrounded by hyalinized stroma (Blue arrow) and fibrotic ovarian stroma (Red arrow); haematoxylin and eosin staining, X40.

Plate 4: A photomicrograph section of the ovary (Group D) which were given 8mg/kg of $HgCl_2$ throughout the experiment showing graffian follicle surrounded by extensively hyalinized stroma (Black arrow) and fibrotic ovarian stroma (Red arrow); haematoxylin and eosin staining, X400.

Discussion

Once mercury is absorbed, it is distributed in all tissues of the body. Low concentration has been seen to affect the central nervous system and the placenta. This study therefore seeks to examine the effect of mercury on the ovaries, as well as the hormonal changes that occur.

Our study revealed that the continuous oral administration of Hg for 28 days have adverse effect on specific hormones. Administration of mercuric chloride did not have effects on the weight of the ovary.

Three hormones, luteinizing hormone (LH), follicle stimulating hormone (FSH), and Progesterone were studied in this experiment. There was a significant difference in the level of FSH between the control group and administered group. There was also a significant difference in the level of LH between the control group and the administered group. In females, mercury has been shown to have an inhibitory effect on the release of LH and FSH. This finding is in line with Chen and Davis (2006) who indicated that mercury inhibits the effect of LH and FSH from the anterior pituitary (Davis *et al*., 2001).

There was an increase in the level of progesterone in the administered group. This is also as a result of the effect of Hg. According to Maeda *et al*., (2019), infertility in women is usually influenced by an imbalance of the female hormone due to exposure from Hg. The progesterone/oestrogen ratio changes in favour of oestrogen growth, which inhibits the release of LH and FSH (Maeda *et al*., 2019).

Exposure to Hg causes changes in ovarian cells and arrest oocyte development, apoptosis of

ovarian granulosa cells and the hyalinization of stroma cells. From the ovary sections of plate 2 - 4, there was a progressive degenerative morphological change of the ovarian tissue, and fibrolytic and hyalinized stroma.

Conclusion

Exposure of Wistar rats to mercury chloride resulted in a dose – dependant histopathological changes with alteration of some hormonal parameters. These may impair reproductive functions of the ovary.

Conflict of Interest: The researcher has no conflict of interest of any kind.

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