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(RESEARCH ARTICLE)

Perinatal lead administration alters markers of antioxidant levels and reproductive hormone function in F1 offspring of exposed Wistar rats

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Abstract

Objectives: This study investigated an angle of lead intoxication via maternal inorganic lead exposure during gestation and lactation, and the outcome on some physiological indices in the first (F1) generation of the exposed female Wistar rats.

Methods: It was a multi-phased study comprising of the animal acquisition, acclimatization, mating, grouping, treatment and post-treatment/terminal protocols. Treatment was per os and on alternate days, lasting from conception, evidenced by the presence of a vaginal plug, until post-lactation in the given groups. Thus, group I served as the untreated control. Groups II, III, and IV were administered 100mg/kg, 50mg/kg, and 25mg/kg lead acetate respectively throughout gestation which lasted for 21 days. Groups V, VI, and VII received same doses but this time, up to the lactation phase; lasting for 42 days. Groups VIII, IX, and X equally received same doses respectively, but this time, during lactation only. Terminal protocols were humanely carried out upon sexual maturation of the offspring. Blood lead levels were ascertained using Varian AA240 Atomic Absorption Spectrophometer (AAS). The reproductive hormones - oestradiol (E2), Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH), were assayed using competitive ELISA Elabscience test kits. Serum levels of the oxidative stress markers - malondialdehyde (MDA), catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) - were measured using literature-derived protocols. Data obtained was expressed as mean+/-SEM (standard error of mean). Comparative analysis among groups was done using ANOVA tool of the SPSS version 25 software. $P<0.05$ was considered statistically significant.

RESULTS: There was a statistically significant decrease between the BLL (ppm) of the control animals in contrast to the rest of the groups. For the reproductive hormone findings, the F1 progeny of the 100mg/kg treated rats in groups II and V showed markedly reduced serum oestradiol and FSH concentrations in comparison to the control animals and others in their subgroups. Also, these same animals showed statistically significant SOD and GSH decreases in comparison to the control and other groups ($P<0.05$ in each case).

CONCLUSION: Our findings suggest that the antioxidant and hormone profiles of the F1 offspring can result in antioxidant and reproductive dysfunction especially in the *in utero* cum lactationally lead-exposed 100mg/kg treated groups. This suggests a dose-dependent response pattern as well as an oxidative stress-mediated distortion of the two key hormones affected - oestradiol and FSH.

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Keywords: F1 offspring; Oestradiol; Malondialdehyde; FSH; *In utero*

1. Introduction

Despite its ubiquitous nature, lead has no known biological functions; unlike other heavy metals such as iron, copper and manganese [1]. Exposure usually occurs via the respiratory and gastrointestinal tracts [2]; with its rate of absorption hugely dependent on the nutritional status and age of the individual.

Flora *et al.*, [3] reported that Pb toxicity occurs through its affinity for proteins and its capacity to simulate calcium and iron channels, leading to an induced increase in reactive oxygen species [4]. Thus, oxidative stress is generated as a result of an imbalance between the production of the free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [3]. This culminates in the Pb-induced depletion of the antioxidant reserves and the hampering of the ability of the biological system to reverse the resultant effects which include lipid peroxidation, disruption of cell membrane, protein oxidation and oxidation of nucleic acids [5].

Al-Saleh *et al*. [6] reported a direct influence of lead during fetal development especially in the gestational period, while Dart *et al.* [7] equally reported the transfer of lead through lactation thereby making the blood lead levels of the mothers and infants usually similar.

Studies on the endocrine and reproductive toxicity due to lead exposure do not appear as detailed as those on other systems. The profiles of the major reproductive hormones - follicle stimulating hormone, luteinizing hormone, and oestradiol - which subserve reproduction have not been adequately reported, despite the reports of Dart *et al*. [7], Al-Saleh *et al*. [6], and Flora *et al.* [3] which focused on birth outcomes and gestational impact of lead exposure without showing a direct link between the aforementioned hormones on their observations/findings.

Previous experimental and epidemiological studies suggested that exposure to environmental toxicants during pre- and post-natal period of development is associated with health outcomes throughout the lifetime of an individual [8,9]. These evidences have increased the debate on the concept of epigenetic programming; where it is hypothesized that a given genotype can give rise to several phenotypes depending on the early environment.

This study, subsequent to a maternal dietary Pb exposure (F0), aims to investigate the mechanism of lead-induced perturbations of the hormonal systems of interest in F1 offspring of the exposed mothers.

2. Materials and methods

2.1. Procurement of study animals

This research study was carried out at the Animal House Unit of the Department of Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University Nnewi Campus, Nnewi North Local Governement Area, Anambra State, Nigeria. The male and female Wistar rats weighing between 180-200 grams were recruited from in-bred ones from the aforementioned animal house; while the lead acetate was obtained from the Pharmacognosy unit of the Faculty of Pharmacy, Nnamdi Azikiwe University Agulu Campus, Nigeria.

2.2. Dosage calculation

The average weights of the animals in each group were calculated to get the dose to be administered to each rat. The required volume to be administered was calculated thus:

$$\text{Required volume} = \frac{\text{Dosage (mg/kg)} \times \text{weight of the animal (kg)}}{\text{Stock concentration (mg/ml)}}$$

To get the volume, the stock concentration was first obtained:

For the lead acetate, the stock concentration was obtained by dissolving 1 gm of Pb acetate in 20 ml of normal saline and this gave a stock concentration of 50mg/ml.

2.2.1. Acclimatization and mating protocols

Thirty (30) nulliparous and non-pregnant adult female Wistar rats weighing between 180-200gm and ten (10) adult male Wistar rats were housed separately for a period of fourteen (14) days to acclimatize them to their environment. They were exposed to 12-hour light-dark cycles and given water and feed ad libitum. At the end of the acclimatization period, a male rat was introduced into a cage containing three (3) female rats. On the first day, it was isolated from the female rats via a partition in the same cage (so as to bring them to oestrous) before it was finally allowed to mix and mate freely for the remaining three days.

Presence of a vaginal plug was considered as a confirmation of conception, and the males were withdrawn from the females.

2.2.2. Grouping and treatment

The rats were split into ten (10) groups. Control representing individually caged untreated pregnant rats, groups II-IV representing individually-caged pregnant rats who were treated with lead acetate throughout their gestational period only (G/Ge groups), groups V-VII representing individually-caged pregnant rats who were treated with lead acetate throughout gestation as well as lactation (i.e., during weaning) (gestation/postnatal – G/PN groups), i.e., throughout the lactation period only, and groups VIII-X representing individually caged pregnant rats administered lead acetate during lactation ONLY (Postnatal – PN groups)

Treatment stage for the pregnant rats of groups II, III and IV lasted throughout gestation (21 days). They were given orally at sub lethal doses of lead acetate at 1/10, 1/20, and 1/40 of the oral LD, respectively, for three weeks. One dose was ingested every two days [10]. Following parturition, administration of lead acetate ceased.

The fifth, sixth and seventh groups received same doses as those of the second, third and fourth groups. However, their treatment extended from the onset of gestation till the cessation of breastfeeding.

Groups VIII, IX and X also received the same doses of lead as the previous groups: however, their treatment commenced following parturition and equally lasted for three weeks. One dose was administered every two days according to the design of the protocol. Offspring were individually housed with their mothers until weaning at 21 days of age. At weaning, offspring from each litter were group-housed together based on sex. Only F0 rats received treatment.

2.2.3. Terminal protocols

At 60 days, post-birth, each animal was humanely anaesthetized following Sotoudeh and Namavar [11] ketamine and xylazine (100–2 mg/kg of K-X) optimization dose study, and then an incision was carefully made via the linear alba down to the thoracic cavity to expose the heart for sample collection. With the heart exposed, a 5ml syringe was used to make a puncture at the apex of the heart to collect the blood sample. The blood samples were transferred into EDTA (for BLL assay) and plain tube containers. Plasma was obtained after centrifugation at 3500 revolutions per minute for 15 minutes. The plasma was collected into a plain tube and stored at -20 degree Celsius until the analysis.

Malondialdehyde was measured as an index of lipid peroxidation using the assay of thiobarbituric reacting substances (TBARs) by the method of Nagababu *et al.* [12]. Serum catalase activity was determined according to the method of Beer and Sizer [13]. The procedure to estimate the reduced glutathione (GSH) level followed the method described by Ellman [14]. The level of SOD activity was adapted from Misra and Fridovich [15]. Elabscience ELISA kits were used for the hormone assays. The AAS method was performed to determine rat blood lead level (BLL).

2.3. Data analysis

The descriptive data was expressed as mean ± SEM (standard error of mean). Comparative analysis amongst groups was done using Analysis of Variance (ANOVA) tool of the SPSS (version 25) software. Further comparative analysis was done using Post Hoc Bonferroni multiple comparative test. $P<0.05$ was considered statistically significant.

3. Results

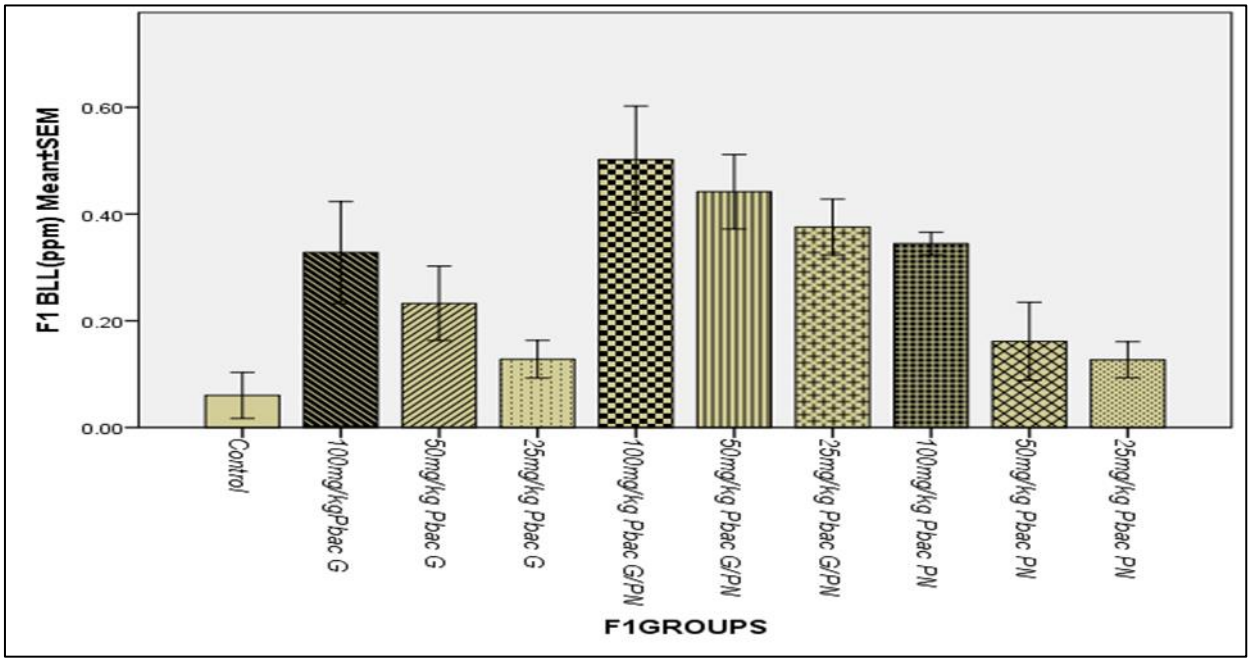

Figure 1 Graph showing the mean±SEM BLL concentration (ppm) of the F1 Offspring of the lead treated F0 rats**.**

Table 1 The mean±SEM Oestradiol, Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH) serum levels in F1 offspring of perinatally exposed Wistar rats

GROUPS	**F1 GENERATION Mean±SEM N = 5**		
	Oestradiol (pg/mL) Mean±SEM	**FSH (mIU/mL) Mean±SEM**	**LH (mIU/mL) Mean±SEM**
I (CONTROL)	342.87±14.53	22.82±1.72	41.14±2.01
II (100mg/kg Pb G)	195.56±4.20	13.97±0.56	31.24±1.05
III (50mg/Kg Pb G)	279.56±8.18	19.48±1.58	37.74±1.41
IV (25mg/kg Pb G)	293.15±7.54	24.10±2.45	38.23±2.83
V (100mg/kg Pb G/PN)	193.55±12.32	13.07±0.44	39.11±2.86
VI (50mg/kg Pb G/PN)	254.70±9.48	14.61±0.44	39.70±3.21
VII (25mg/kg Pb G/PN)	276.56±7.13	16.15±1.60	40.52±1.42
VIII (100mg/kg Pb PN)	220.90±11.24	12.15±1.54	33.96±1.00
IX (50mg/kg Pb PN)	247.31±13.64	24.09±3.11	37.25±0.75
X (25mg/kg Pb PN)	319.89±27.78	25.51±1.91	42.48±0.59
	F = 14.351	F = 7.439	F = 3.043
	P = 0.000	P = 0.000	P = 0.018
POSTHOC (BONFERRONI) MULTIPLE COMPARISON	p<0.05 for: 1/2, 1/5, 1/6, 1/8, 1/9 2/3, 2/4, 2/7, 2/10	P<0.05 for: 1/5; 2/4, 2/9, 2/10; 4/5, 4/6; 5/9, 5/10; 6/9, 6/10.	*P<0.05* 2/10

	3/5; 4/8; 5/10; 8/10; 9/10.		

Table 2 The mean±SEM serum Superoxide dismutase (SOD), Catalase (CAT), Malonedialdehyde (MDA) and reduced glutathione (GSH) levels in F1 offspring of perinatally exposed Wistar rats

GROUPS	**F1 GENERATION Mean±SEM N = 5**			
	SOD (U/mg protein)	**CATALASE (U/mg protein)**	**MDA (mg/l)**	**GSH (mg/l)**
I (CONTROL)	265.49±8.37	748.99±17.96	1.04±0.06	7.58±0.49
II (100mg/kg Pb G)	122.35±1.46	556.32±41.38	1.85±0.12	3.49±0.38
III (50mg/Kg Pb G)	165.82±16.34	559.01±46.62	1.71±0.07	4.01±0.31
IV (25mg/kg Pb G)	173.96±14.22	669.26±41.19	2.06±0.10	6.51±0.52
V (100mg/kg Pb G/PN)	94.13±4.76	506.82±28.64	1.89±0.13	1.77±0.23
VI (50mg/kg Pb G/PN)	131.50±8.93	637.55±30.86	1.65±0.02	2.76±0.58
VII (25mg/kg Pb G/PN)	176.76±11.03	692.07±13.21	1.50±0.14	5.14±0.69
VIII (100mg/kg Pb PN)	157.53±8.30	657.16±29.73	1.71±0.07	4.99±0.27
IX (50mg/kg Pb PN)	171.06±18.35	671.29±56.47	1.76±0.05	7.29±0.42
X (25mg/kg Pb PN)	276.88±3.76	650.74±31.18	1.24±0.03	6.93±0.68
	F = 28.277	F = 3.726	F = 11.870	F = 17.465
	P = 0.000	P = 0.007	P = 0.000	P = 0.000
POSTHOC (BONFERRONI) MULTIPLE COMPARISON	*p*<0.05 for: *1/2, 1/3, 1/4, 1/5, 1/6, 1/7, 1/8, 1/9. *2/10; *3/5, *3/10. *4/5, 4/10; *5/7, 5/8, 5/9, 5/10; *6/10; *8/10; *9/10	*P*<0.05 for: *1/5.	*P<0.05 for:* *1/2, 1/3, 1/4, 1/5, 1/6, 1/8, 1/9. *2/10; *4/7, 4/10; *5/10; *9/10.	*P<0.05 for:* *1/2, 1/3, 1/5, 1/6. *2/4, 2/9, 2/10. *3/9, 3/10 *4/5, 4/6. *5/7, 5/8, 5/9, 5/10 *6/9, 6/10.

4. Discussion

The adverse outcomes of maternal inorganic lead exposure in gestationally and lactationally exposed animals – with the Wistar rat as our experimental animal of choice; and our parameters of interest (majorly informed by facilities at our disposal) i.e., oxidative stress, and reproductive hormone indices, were undertaken to further highlight the impact of lead, as an environmental toxicant, on the above mentioned parameters on the F1 offspring of the exposed animals. This is quite poignant since various studies have reported the link between environmental exposures and the reproductive capabilities of the exposed [16,17]. Also, developmental exposure to lead (Pb) remains a significant risk factor for physiological outcomes in exposed offspring [18,19].

4.1. Blood lead levels

In this group of animals, all the high-dosed groups - II, V, and VIII – showed significantly higher blood lead levels in comparison to other groups within their sub-groupings that were exposed to lead (the sub-groupings used here mean time of exposure – whether in utero only, gestational/lactation, and postnatal only). That is, the Group II animals that were in utero exposed to 100mg/kg Pb showed significantly higher BLLs than groups III and IV animals who were

exposed to 50mg/kg Pb and 25mg/kg Pb respectively, *in utero*. It was the same scenario with group V vs groups VI and VII; and group VIII vs groups IX and X.

Nonetheless, group V rats that were prenatally and postnatally exposed to 100mg/kg Pb showed the highest BLL values. Again, the positions of Anas *et al.* [20] and Venturelli *et al.* [21] on the potency of dose-effect outcome in developmental studies come to mind.

4.2. Oxidative stress markers:

SOD: All the lead-exposed rats –besides the 25mg/kg lactationally exposed group i.e. group X – showed reduced SOD levels compared to the control rats. A dose-dependent and time-dependent response was also observed in the 100mg/kg gestationally and lactationally exposed group V who had the lowest mean±SEM serum SOD concentration. This observation suggests that the generation of hydrogen peroxide which is the by-product of the reaction catalyzed by SOD, was impaired in these animals.

This is in tandem with the multigenerational study done by Trujillo-Vasquez *et al.* [22] where they linked oxidative stress to altered reproductive markers in offspring of F0 Pb-exposed animals. Although conception was achieved in these animals up to F2, it is important to mention that the animal model employed in their study was mice and it was a low-dose Pb study.

Equally, in another study by Kovacevic *et al.* [23], F1 was shown to be the most sensitive and most affected with marked oxidative stress and inability to reproduce in Springtails (*Folsomia candida*).

CATALASE: There was a statistically significant difference ($P<0.05$) between the mean±SEM serum (U/mg protein) value of the control rats (group I) when weighed against that of the group V perinatally Pb-exposed rats. Given its important role in the disposal of hydrogen peroxide generated by SOD, decreased catalase levels was associated with lead exposure in a research study by Sirivarasai *et al*. [24].

MDA: There was marked lipid peroxidation in all the lead-exposed rats compared to the control. This is in line with the plethora of evidence on the effect of lead intoxication on lipid peroxidation and this has been shown to persist from generation to generation [22,23].

GSH: The reported association between lower GSH levels with increased blood lead levels in Vacchi-Suzzi *et al.* [25] study on occupationally Pb-exposed workers, agrees with this study, whereby gestationally and lactationally 100mg/kg Pb-exposed group V rats had the lowest mean±SEM GSH concentration. Also, other equally dosed groups - i.e. groups II and VIII - who were administered 100mg/kg Pb acetate *per os* showed decreased mean±SEM serum GSH levels compared to the lower dosed group IV (25mg/kg gestationally lead exposed), group IX (50mg/kg lactationally Pb exposed), and group X (25mg/kg lactationally lead exposed) rats.

The Control (group I rats) showed higher mean±SEM serum GSH levels than the rest of the experimental animals and this was statistically significant when compared to groups II, III, V, and VI rats.

4.3. The reproductive hormones

4.3.1. Oestradiol (E2) levels

All the Pb-treated groups showed lower mean±SEM serum E2 levels in comparison to the untreated litters (i.e., control animals). The *in utero* exposed high dose groups - II and V - showed significantly decreased serum oestradiol levels compared to the rest of the groups. This finding agrees with Tchernitchin *et al.* [26] who reported that prenatal exposure to lead suppresses oestrogen action in prepubertal rats.

Also, several other studies examined the importance of the exposure period in lead toxicity experiments. Epstein *et al.* [27] in comparing premating, prenatal, and postnatal conditions in lead-treated mice reported more adverse outcomes in the prenatal and postnatal groups. In the same vein, Ronis and his teams's [28] investigation of rats exposed to lead acetate in drinking water in utero, prepubertally, and postpubertally showed the most severe effects to be observed in the *in utero* group; with delayed vaginal opening and disrupted estrus cycling: suggesting Pb action on the hypothalamo-pituitary axis and gonadal seteroidogenesis.

In one of the earliest reprotoxicity studies involving lead, McGivern *et al.* [29] in administering lead acetate in drinking water to Sprague Dawley rat dams reported that the female offspring from these Pb-treated dams had significantly

delyaed vaginal opening, and 50% of them exhibited prolonged and irregular periods of diestrus, accompanied by an absence of observable corpora lutea. Alterations in pubertal progression and hypothalamic-pituitary-ovarian-uterus functions have been confirmed in female monkeys prenatally or postnatally exposed to Pb [30].

4.3.2. FSH and LH

Absent or low pituitary secretion of FSH and LH, as occurs in hypothalamic/pituitary hypogonadism, leads to anovulation, amenorrhea and absent ovarian follicular development [31]. In this study, there was a marked suppression of the mean±SEM serum FSH levels of the *in utero* (+ lactationally) exposed offspring.

This decrease in mean±SEM serum FSH (mIU/mL) concentration of the group V rats was statistically pronounced in comparison with the offspring of the 50mg/kg and 25mg/kg postnatally treated rats of group IX and X respectively ($P<0.05$, in each case). In the same vein, the group II rats showed statistically significant lower mean±SEM serum FSH (mIU/mL) levels when compared with those of groups IV, IX, and X ($P<0.05$ in each case).

Regarding LH levels, group II rats showed a statistically significant lower mean±SEM serum LH (mIU/mL) concentration when compared to the mean±SEM serum LH (mIU/mL) concentration of the group X rats ($P<0.05$). The rest of the comparisons between the groups did not detect any significant difference ($P>0.05$).

McGivern *et al*. [29] fingered irregular patterns of both follicle-stimulating hormone (FSH) luteinizing hormone (LH) secretion in their investigation of female offspring of lead-treated Sprague-Dawley rats.

However, our current study points to Pb having an effect on oestradiol and FSH secretion while the circulating LH levels were not significant. Nonetheless, this study suggests that the different levels of the hypothalamo-pituitary gonadal axis can be affected by exposure to lead, mainly when structures are undergoing rapid proliferation. Equally, it is important to reiterate the possible link between oxidative stress and impaired reproductive function in this study, as the *in utero* and lactationally 100mg/kg Pb-treated group presented a disadvantaged antioxidant profile as well as decreased E2 and FSH levels: this suggests a possible link between these two systems.

5. Conclusion

This study stands out on three levels:

- The route of Pb administration was via oral gavage, with only the F0 mothers exposed to lead intoxication.
- The presence of three subgroups receiving graded doses of Pb acetate in utero, and during lactation.
- The animal model employed in this research was the Wistar strain of Rattus novergicus (Wistar rat).

This is in contrast with other multigenerational and epigenetic studies that employed mice and invertebrate animal models. Also, in many of such studies, lead acetate was mixed with the drinking water of the animals.

Thus, our findings showed that the biochemical and reproductive profiles of the F1 offspring who were exposed to different doses of oral lead acetate perinatally, showed antioxidant and reproductive dysfunction, with the F1 in utero and lactationally lead-exposed group worst hit.

In summary, lead acetate administration - through a dose-dependent pattern - impaired reproductive hormone (especially oestradiol and FSH) secretion and expression, possibly through an oxidative stress-mediated pathway.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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