ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 5 No. 1b June 2019*

Lead Poisoning and it's Age-Related effects on Male Sex Hormones

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Abstract
Nearly a quarter of deaths in developing countries, including Nigeria and Ghana are linked to pollution (MSN News, 2013). The aim of this study was to determine age-related effects of lead poisoning on male sex hormones. Thirty (30) male Wistar rats of 3- months, 5- months, and 7-months old age-groups were divided intoc5-experimental (lead fed) and 5-control (distil water fed) group according to age-group. Sex hormones plasma levels were measured using ELISA, while for the blood lead concentration, Atomic Absorption Spectrophotometer was used. Result showed significant (P<0.05) increase in blood lead concentration, while ther was no significant (P>0.05) increase in Leutinizing Hormone (LH) (5.000, 1.500, 1.000 Vs 3.400, 1.000, 1.750) between experimental groups compared to control groups. Significant (P<0.05) decrease in body weight (77.43, 107.88, 134.35 Vs 130.66, 150.60, 165.62), testosterone levels (0.110, 1.310, 0.523 Vs 0.656, 4.200, 4.235), Follicle Stimulating Hormone (FSH) (0.333, 2.750, 0.0325 Vs 3.800, 0.250, 0.100) were observed between experimental groups compared to control groups. The effect of lead (Pb) on testosterone levels was more pronounced in older animals. It was concluded that, ingestion of lead acetate has an age related effect on male sex hormones are more pronounced in older rats.

Keywords: Age-related, Lead Poisoning, Male, Sex Hormones, Wistar Rats.

INTRODUCTION
The health of 200 million people in low-income countries is at risk due to toxins such as lead or mercury, more than from AIDS, tuberculosis and malaria combined (C-Dynamics, 2014); nearly a quarter of deaths in developing countries, including Nigeria and Ghana are linked to pollution (MSN News, 2013).

The metal is a soft, malleable, and heavy post-translation; it has a bluish-white color after being freshly cut but it soon tarnishes to a dull grayish color when exposed to air. Lead has a shiny chrome-silver luster when it is melted into a liquid. It is also the heaviest non-radioactive element (Olade, 1987).

Lead poisoning has been documented from ancient Rome, Greece, and China. Lead poisoning usually occurs from repeated exposure to small amounts, through ingestion or inhalation and, to a lesser extent, dermal absorption. Human exposure can be estimated directly through body

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burden measurements (in blood, teeth or bone) or indirectly, by measuring its levels in the environment (air, dust, food or water) (Agency for Toxic Substances and Disease Registry [ATSDR], 1999). Lead is a dangerous heavy metal and harmful even in small amounts as its health effects may occur at blood-lead concentrations as low as 0–1mg/dl (Bersenyi', 2003).

Lead is used in building construction, lead-acid batteries, bullets, and part of solders, pewter, fusible alloys, and as a radiation shield. Lead is also used in production prints, gasoline, battries, water pipes, cosmotic products, pottery glazing, tank linings, brass faucets, toys, and others (Harbison, 1998).

Soil, industrial polluted air, agricultural sources such as lead-containing pesticides, phosphate fertilizers and food processes are sources contaminating food and feed. The metal exists in food depending on its natural state in the soil. There is therefore, practically no lead-free food. The lead contained in of stable foods like meat, milk, bread, fruits and vegetables is in small amount about 0.02-3mg/kg w/w (Bersenyi', 2003).

Sources of occupational exposure include lead mining, refining, smelting, construction work, paint removal, demolition, maintenance of bridges and water towers, car repair, ammunition, batteries, solder, X-ray shields and recycling (Pizent *et. al*. 2012).

Leaded petrol, as one of the metal's many sources in the environment (through automobile exhausts), is a good indicator of reduction in exposure to lead (Landrigan *et. al.* 2000). It has been shown that, decreases in blood-lead concentrations correlate well with the removal of the heavy metal from petrol; multiple studies have shown reductions in blood-lead concentrations in parallel with decreases in levels of lead in petrol (Thomas *et. al.* 1999).

The major sources of heavy metal pollution in urban areas of Africa are anthropogenic sources associated with fossil fuel, coal combustion, industrial effluents, solid waste disposal, fertilizers, mining and metal processing; confined mostly to the urban centres with large populations, high traffic density and consumer-oriented industries, while rural contaminations are from natural sources including weathering of mineral deposits, bush burning and windblown dusts (Olade, 1987). Other sources include emissions from electric generators used in both homes and industries.

The World Health Organization (WHO, 2000) recommended lead limit in drinking water to be 100µg/L, the concentrations in reality are often higher due to increasing environmental pollution especially areas close to emission source such as automobile, industrial pollution; about 50-70% of lead compound are emitted with the exhaust fumes (Bersenyi', 2003). Despite the global preference for the use of unleaded petrol and the ban of lead-based paint and lead solder in food cans, these products are still predominant in Nigeria market (Jegede *et. al.* 2013). Terace metals such as Cadmium (Cd), Lead (Pb), and Mercury (Hg) are known to be environmental pollutants; they affect both aquatic and terrestrial ecosystems (Demichele 1984). Human activities are responsible for large discharge of trace elements into the environment (Bersenyi', 2003). Such contaminations in air and water are made available for human absorption through incorporation into plant and animals used for food, also more than 70% of body heavy metals burden are derived from food, drinking water and ambient air, which all contributed to daily lead intake (Demichele 1984).

Lead is toxic to the male reproductive system (Sallmen, 2001). Lead disrupt testicular enzymology which is characterized through steroidogenesis process, by oxidative stress mechanisms (Biwas and Ghosh, 2004). It exerts it testicular effects through inhibiting the

testicular steroidogenic enzymes (Liu *et al.,* 2008). Exposure to lead results in suppressed spermatogenesis and testosterone levels even-though without significant changes in luteinizing hormone (El-tohamy and El-Nattat 2010).

Human exposure can be estimated directly, through body burden measurements (in blood, teeth or bone) or indirectly, by measuring its levels in the environment (air, dust, food or water).

There are reports on lead toxicity in animals,its pharmacokinetics and genotoxicity, but few researchers correlate haemato-biochemical alterations of lead acetate in laboratory animals (Suradkar., *et al*, 2009). Similarly, several studies had reported on the effects of lead poisoning on various body systems, organs, and blood parameters, however studies on age- related effects of the heavy metals are limited. This work intend to study the age-related sub-chronic effects of lead poisoning on male sex hormones.

NULL HYPOTHESIS:
Lead exposure has no age-related effects on male sex hormones.

MATERIALS AND METHOD

Experimental Animals

Male Wistar rats were obtained from animal house of Biological Science Department, Bayero University, Kano, acclimatized for 7 days befoere the experiment. Thirty (30) Wistar rats that are 3- months, 5- months, and 7-months old were divided into 5-experimental groups, lead fed at 250mg/kg body weight per day (Ambali *et al*., 2011) and 5-control (distil water fed) group per age-group, over a period of 22 days, then on the 23rd day animals were injected intravenously with 0.5cm^3 of 0.4% solution of sodium thiopental (Greene, 2002) later decapitated.

Animal weights were measured before and after the 22 days of experiment.Two blood samples were collected from each Wistar rat. One sample centrifuged for 10 minutes at 2500 revolution per minute, plasma aspirated, and plasma levels of FSH, LH, and testosterone concentrations were measured using ELISA tests kit. Second blood sample was used for measuring blood lead concentration using Atomic Absorption Spectrophotometer (BUCK Scientific; model: 210 VGP, USA).

Test for FSH, LH and Testosterone Immunoassay

All reagents, serum references and samples were brought to room temperature. Microplates were formated for each reagent, serum references and sample for clear identification). Then 0.050ml of serum was pipetted into apprpprate platform, 0.100ml of enzyme reagent was pipetted into apprpprate platform.
Microplate was gently swirt to mix serum and reagent for 30 minutes and later incubated for 60 minutes. At room temperature. Content of each platform was discarded by aspiration, 0.050ml of wash buffer was added into each platform and 0.100ml of working substrate solution was added to all platform and later incubated for 15 minutes at room temperature then 0.050ml of stop solution was added into each platform, mixed for 20 minutes.

Absorbance in each platform was measures at 450nm in the microplate reader, absorption against concentration graph was plotted for standard values; point of intersection between the sample reading and the standard curve indicated the hormone content in the blood sample.

Results collected were tabulated in mean±SD and analyzed using independent T-test to compare between experimental and control aniamals, and ANOVA between the three age groups. All analysis were performed using SPSS (Version 20) at p-value ≤ 0.05.

RESULT

Table 1: Weight Change in Wistar rats(g)

Age of Animals (months)	Weight (g) Groups		
	Experimental	Control	P-Value
3 month old	77.43±3.38* ˣ	130.66±28.80	0.000
5 month old	107.88±12.80* ˣ	150.60±6.65	0.001
7 month old	134.35±8.97* ˣ	165.62±17.80	0.040

Data with same superscript letter are not significantly (P > 0.05) different *P < 0.05 compared to control animals

There was a significant difference (p < 0.05) in body weight before and after experiment, between experimental and control animals among all three age groups. While no significant (P > 0.05) weight loss among experimental animals within the three age groups.

Table 2: Blood Lead Levels

Age of Animals (months)	Lead Levels (µg/dl) Groups		
	Experimental	Control	P-Value
3 month old	46.00±6.00* ˣ	14.56±7.65	0.000
5 month old	46.75±18.95* ˣ	18.00±3.65	0.039
7 month old	50.75±12.65* ˣ	17.60± 4.50	0.002

Data with same superscript letter are not significantly (P > 0.05) different *P < 0.05 compared to control animals

There was significant differences (P ≤ 0.05) in Blood Lead levels between experimental and controlanimals with no (P > 0.05) difference between age groups of experimental animals.

Table 3: Levels of Luteinizing Hormone

Age of Animals (months)	LH Levels (mIU/ml) Groups		
	Experimental	Control	P-Value
3 month old	5.000±4.66 x	3.400±2.03	0.405
5 month old	1.500±0.58 y	1.000±0.56	0.143
7 month old	1.000±0.18 y	1.750±0.96	0.135

Data with same superscript letter are not significantly (P > 0.05) different animals *P <0.05 compared to control

No significant (P > 0.05) difference recorded in plasma levels of luteinizing hormone between experimental and control groups. While significant increase among 3-months old animals incomparism to 5- and 7- months old experimental animals was recorded.

Table 4: Levels of Follicle Stimulating Hormone

Age of Animals (months)	FSH Levels (mIU/ml) Group		
	Experimental	Control	P-Value
3 month old	0.333±0.58* x	3.800±2.76	0.035
5 month old	2.750± 0.50x	0.250± 0.50	0.200
7 month old	0.0325±0.39 x	0.100±0.82	0.157

Data with same superscript letter are not significantly (P > 0.05) different animals *P < 0.05 compared to control

There was significant (P < 0.05) FSH reduction between experimental group compared to control animals in 3-months old animals.

Table 5: Testesterone Levels of rats

Age of Animals (months)	Testesterone Levels (ng/ml) Groups		
	Experimental	Control	P-Value
3-month old	0.110±0.03x	0.656±0.87	0.265
5-month old	1.310±1.53* xz	4.200±3.77	0.045
7-month old	0.523±0.26* yz	4.235±5.45	0.040

Data with same superscript letter are not significantly (P > 0.05) different*P < 0.05 compared to control animals

Significant ($P < 0.05$) decrease in Testesterone Levels was observed between 5- and 7-months old experimental animals compared to control rats. In addition significant ($P < 0.05$) decrease between 3- and 7- months old experimental animals was obeserved.

DISCUSSION

Lead pollution has continued to pose health hazards in animal and man in Nigeria and many other parts of the world (Ajayi *et. al.* 2009). A study on some physico-chemical characteristics and heavy metal profiles of 72 Southern Nigerian rivers, streams, and waterways, conducted in their water samples showed that, about 34 (47%) of them contained some levels of lead (Asonye *et. al.*2007). Exposure to lead mainly occurs through the respiratory and gastrointestinal systems. Absorbed lead (whether inhaled or ingested) is stored in soft tissues (Adikwu *et. al.*2013).

The study showed that, sub-chronic lead poisoning led to significant weight loss in lead-treated animals of all age groups, such effect may be attributed to appetite loss, gastrointestinal disturbances (Cezard and Haguenoer, 1992) metabolism and absorption (Marchlewicz., *et al*, 2006).

In this study lead showed no effect on LH levels in conformity with El-tohamy, 2010; Fatima., *et al,* 2011 and Sokol., *et al*, 1987, while significant increase in LH level was observed in 3-month old among the experimental groups, indicating a higher level of LH among lower aged animals. Others reported lead to decrease LH concentration (Mukherjee, and Mukhopadhyay, 2009; Taiwo, *et al.* 2010).

Significant increase of FSH level was observed in 3-month old experimental animals in this study, indicating stimulation of anterior pituitary gland for FSH among lower aged group. Such increase in FSH, has also been reported following lead exposure in rats (Petrusz , *et al.* 1979). Even though Pinon-Lataillade *et al.* (1995) reported unchage FSH levels. Such diverse reports of lead on FSH are possibly due to differences in lead doses and/or the duration of exposure (Mohsen, *et.al* 2011).

Wadi and Ahmad (1999) supported that lead targets the spermatogenesis and sperms within the epididymus by producing reproductive toxicity rather than acting within the hypothalamic-pituitary-testicular axis. Also the gonadotoxic effects of lead are on the intra-testicular sites with minimal effects on hormonal levels and no effect on extra testicular sites (Taiwo *et al.* 2010).

Lead inhibits or mimics the action of calcium, interacts with proteins and binds with virtually every available functional group, including sulfhydryl, amine, phosphate, and carboxyl groups, with sulfhydryl having the highest affinity (Guidotti, and Ragain 2008). Many studies implicate transition metals to act as catalysts in biological macromolecule reactions, and their toxicities to be due to oxidative stress causing tissue damages.

Redox-active metals such as iron, copper and chromium undergo redox cycling, while redox-inactive metals such as lead, mercury, cadmium, and others deplete cell major anti-oxidants, especially thiol-containing anti-oxidants and enzymes. Such metals cause increase in reactive oxygen (ROS) species production, such as hydroxyl radical (HO^-), superoxide radical (O_2^-) or hydrogen peroxide (H_2O_2) leading to oxidative stress, consequently causing protein, lipid, and DNA lesions (Nran *et. al.* 2001). Fatty acids containing up to 2 double bonds are more resistant to oxidative stress than are the polyunsaturated fatty acids (Bradberry and Vale, 2009). Most of the hazardous effects of lead poisoning is based on the negative role of lead produced ROS (Lightfoot and Yeager, 2008).

Leydig cells secrete testesterone in response to pituitary LH secretion. This study suggests lead to exerts its effect more at the testicular level, as the result reveal in-siginificant change in LH levels with significant decrease in testosterone level, and such decrease more pronounced among the older age group. This also indicates enzyme inhibition in the biosynthesis pathway by lead (Mukherjee and Mukhopadhyay, 2009).

Jegede *et al.* (2013) established the toxicity of lead on male reproductive function by disruption in spermogram (sperm motility and morphology), deleterious effects on seminiferous tubules, with degeneration of interstitial spaces and narrowing of lumen.

Edrees *et. al.* (2013) also sugest direct toxic action of lead on the testes, anti- androgenic effect of lead, decrease testicular sensitivity to gonadotrophic actions(Okamura *et al*. 2002). and also gonadotoxicity (Taiwo, *et. al*. 2010).

CONCLUSION

Lead in-significantly increases LH levels more pronounced in lower age group, significantly decrease FSH, testesterone levels, with lower testesterone reduction in older animals.

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