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Age-related effects of lead poisoning on sex hormones in adult male Wistar rats

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The World Health Organization (WHO) estimates that, about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution, and that most of these environment-related diseases are however, not easily detected and may be acquired during childhood and manifested later in adulthood. The aim of this work was to determine the sub-chronic effects of lead poisoning on and some sex hormones, as well as age-related changes on Wistar Rats. Thirty (30) of 3-, 5-, and 7-months old age-groups male Wistar rats were selected; each age group was divided into experimental (lead fed) and control (distil water fed) groups. The levels of sex hormones (LH, FSH and Testestrone) were measured using ELISA method, while blood lead concentration was determined using the method of Atomic Absorption Spectrophotometer. There was a significant (P<0.05) increase between experimental and control groups in blood lead concentration and insignificant (P>0.05) increase in LH in both experimental and control groups. Significant (P<0.05) decrease in body weight, testosterone levels, FSH was observed between experimental and 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 in older rats.

Key words: Sex hormones, lead, male, Wistar rats.

INTRODUCTION

Over the last three decades, there has been increasing global concern over public health impacts attributed to direct and indirect environmental pollution, in particular, the global burden of disease. The World Health Organization (WHO) estimates that, about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution (WHO, 2000). Most of these environment-related diseases are however, not easily detected and may be acquired during childhood and manifested later in adulthood (CCNM (Calgary Centre for Naturopathic Medicine), 2014).

Lead metal may be found in dirt, dust, house hold utensils, dishes, furniture, leaded petrol, paints, ceramics, food cans, make-ups, traditional remedies, batteries, soil and water in varying degrees of concentration; and lead poisoning usually occurs from repeated exposure to small amounts (Agency For Toxic Substances and Disease Registry (ATSDR), 1999) The three major sites

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Table 1. Weight of rats before experiment.

Age of animals (months)	Weight (g)	
	Groups	
	Experimental	Control
3 month old	107.20±13.70	96.66±28.80
5 month old	136.20±17.99	110.98±18.62
7 month old	150.37±14.07	149.45±18.27

Weight Changes in rats after experiment

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

of lead intoxication are kidney, blood, and the nervous system (Bersenyi', 2003), it is also a significant immuno-toxin (Koller, 1990). Leaded petrol, as one of the metal's many sources in the environment, is a good indicator of reduction in exposure to lead (Landrigan et al., 2000).

There are many reports on lead toxicity and its deleterious effects in various species of animals as well studies on its pharmacokinetics and genotoxicity (Suradkar et al., 2009), but very few researchers try to correlate haemato-biochemical alterations of lead acetate in laboratory animals especially in rats.

Sources of food and feed contamination are soil, industrial polluted air and agricultural sources such as lead-containing pesticides, phosphate fertilizers and food processes(Bersenyi', 2003). Lead has become a regulatory concern and subject of much interest because of its widespread distribution in environment due to its continuous emission from industrial sources, automobile exhaust and its pharmacological behaviour to remain bound to mammalian tissues for a long time (Freeman, 1970). Human exposure has changed in origin but probably not in amount over the last half century; the quantity of lead used in the 20th century far exceeds the total consumed in all previous years (Fatima, et al. 2011). Such heavy uses have caused both local and global contamination of the air, dust, and soil (Elgohary, 2009).

There are reports on the effects of lead poisoning on various body systems, organs, and blood parameters but studies are limited on age- related effects of the heavy metal. The purpose of the study was to determine the age- related sub-chronic effects of lead poisoning on male sex hormones.

MATERIALS AND METHODS

Materials and chemicals

1. Accubind Elisa Microwells, Monobind Inc Lake Forest; CA 92630. USA Product Code: 625-300) were used to estimate LH concentration in blood samples
2. Accubind Elisa Microwells, Monobind Inc Lake Forest, CA 92630. USA; Product Code: 425-300) were used to estimate FSH concentration in blood samples
3. Accubind Elisa Microwells, Monobind Inc Lake Forest, CA 92630. USA; Product Code: 3725-300) were used to estimate Testosterone concentration in the blood samples
4. Atomic Absorption Spectrophotometer (BUCK Scientific; model: 210 VGP, USA) were used to estimate blood lead concentration in blood samples.

Procedure

Thirty (30) male Wistar rats were selected of three age-groups, of 3-, 5-, and 7-months old; each age-group was divided into experimental and control groups respectively. The experimental (n=15) animals were given lead acetate solution at 250 mg/kg body weight per day (Ambali et al., 2011) for 22 days, while the control (n=15) were placed on distiled water only. Both experimental and control animals were acclimatized for 7 days prior to commencement of experiment; animals were housed in metallic cages and given free access to chow and water. After the last day of lead treatment, rats were lulled to sleep by intravenous injection of 0.5cm^3 of 0.4% solution of sodium thiopental (Greene, 2002) and afterwards decapitated. Blood samples were collected and separated into two: first sample collected portion was centrifuged to get plasma which was used for the estimation of plasma FSH, LH, and testosterone concentrations using ELISA tests; the second blood sample was used for spectrophotometric analysis of blood lead concentration in blood samples, using Atomic Absorption Spectrophotometer (BUCK Scientific; model: 210 VGP, USA); weight of rats was measured before and after the experiment.

Data were collected and presented in tables of mean±SD and analyzed using student independent T-test to compare difference between experimental and control groups, while ANOVA was used to compare significant difference in experimental animals between the three age groups. All analyses were performed using Statistical Package for Social Science (SPSS) (Windows Evaluation Version 20, LEAD Technologies, USA) at p-value ≤ 0.05 (Table 1).

RESULTS

Data with similar letter are not significantly (P > 0.05)

Table 2. Blood lead levels of rats after experiment.

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

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

Table 3. Levels of luteinizing hormone after experiment in rats**.**

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

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

Table 4. Levels of follicle stimulating hormone after experiment in rats.

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

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

different *P < 0.05 compared to control animals. Table 1 represents values of weight (g) changes in animals after experiment in different age groups. Significant difference (p < 0.05) was recorded between experimental and control animals among all three age groups.

Significant changes in weights were recorded in all age groups: weight gain of 34.6g in normal control, while experimental had mean weight loss of 29.66g. Also significant (p ≤ 0.05) weights changes in 5-months old animals were also found with weight gain in normal controls (42.5 g). However, a loss in weight was recorded in experimental group (28.25 g).

Mean weight gain of 16.25 g was observed in normal control of 7-month old animals; though a loss was recorded in experimental animals (16.00 g). There was no significant (P > 0.05) difference in weight loss between the three age groups.

Table 2 represents values of Blood lead concentration in different animal age groups. Significant differences (P ≤ 0.05) were recorded in experimental compared to control groups.

While there was no significant (P > 0.05) difference in Blood Lead levels between all age groups among experimental animals.

Table 3 represents values for levels of luteinizing hormone in different animal age groups. Ther was no significant (P > 0.05) difference between experimental and control groups. A significant (P < 0.05) difference in LH level between 3-months old compared to 5- and 7-months old experimental animals was recorded with more level among 3-months old animals

Table 4 shows levels of Follicle Stimulating Hormone in different animal age groups. There was significant (P < 0.05) reduction in experimental group compared to control animals in 3-months old animals. No significant (P > 0.05) difference in FSH levels between all age groups in experimental animals was observed.

Table 5 represents values of testosterone levels in

Table 5. Testesterone levels of rats after experiment.

Age of Animals (months)	Testesterone levels (ng/ml)		
	Groups		
	Experimental	Control	P-value
3-month old	0.110±0.03[a]	0.656±0.87	0.265
5-month old	1.310±1.53*[ac]	4.200±3.77	0.045
7-month old	0.523±0.26*[bc]	4.235±5.45	0.040

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

different animal age groups. Significant (P < 0.05) reduction in Testesterone Levels was recorded in experimental animals of 5- and 7-months old compared to control rats.

Also a significant (P < 0.05) decrease in testesterone levels between 3-months old compared to 7- months old experimental animals was recorded with more decrease among 7-months old animals.

DISCUSSION

This study supports other workers (Cezard and Haguenoer, 1992) who suggested that the weight loss may be attributed to loss of appetite and gastrointestinal disturbances; as well as to interruption in absorption and overall metabolism of feed nutrients (Marchlewicz et al, 2006). Even though there was no significant (P > 0.05) difference between the three age groups, lead effect on weight loss was observed to be more pronounced in the younger 3-months old rats compared to the older ones.

Lead effect on reduction of body weight has been reported by other workers (Szymezak et al., 1983; Aseth et al., 1995; Teijon et al., 2006; Jegede et al., 2013). Such loss of body weight might also be due to interruption of absorption and metabolism of essential feed nutrients by lead acetate (Marija et al., 2004).

Absorption of lead presumably took place in all lead-treated groups across all ages, as all control groups had plasma lead level not more than 18 µg/dl. This shows the absence of contamination of animals in these control groups. After absorption into the blood, 95% of lead is bound to erythrocytes due to their high affinity for the metal. These cells contain the majority of lead found in blood stream, and the remaining part stays in plasma to be carried to other tissues; lead acetate and lead nitrate administration have been shown to increase blood lead level in experimental animals (Sokol, 1987; Ferguson et al., 1998; Sharma et al*.,* 2011; Monira, et al. 2012;). Also there was no significant (P > 0.05) difference in blood lead levels between age groups among experimental animals, suggesting same manner of lead absorption between the age groups.

There is no significant difference in LH levels between the experimental and control rats. This is in accordance to Sokol (1987) and Fatima et al. (2011) that lead showed no significant effect on LH level,but contrary to findings of Camoratto (1990), Mukherjee and Mukhopadhyay (2009) and Taiwo et al. (2010), who reported that lead decreases LH concentration in experimental animals. While, an age-related difference among experimental animals was recorded with higher increase in LH among 3-months old animals.

Studies in rats have also demonstrated direct effect of lead on the testes and interference in the hypothalamic-pituitary axis (Saxena et al., 1989); disruption of the hypothalamic control of pituitary hormone secretion and in turn, spermatogenesis (Sokol, 1987); also gonadotoxic (Taiwo, et al. 2010).

Lead caused significant reduction in FSH level among 3-months old age group in this study. This is in conformity with Sokol (1987), but contrary to Raule and Morra (1952) and Petrusz (1979), who reported that lead increased FSH concentration. Increased concentration of FSH has also been reported following lead exposure in rats (Petrusz et al., 1979). Even though unchanged FSH concentrations in lead acetate treated mice were also reported (Pinon-Lataillade, et al. 1995),such differences in FSH secretion levels might relate to different lead levels and/or the duration of exposure among subjects (Mohsen et al., 2011).

Lead poisoning caused age-related reduction of testosterone level in this study with decrease more pronounced among 7-months old animals. This significant (P ≤ 0.05) reduction in testesterone levels is also in conformity with reports of Cullen et al. (1983), Sokol (1987), Camoratto (1990), Mukherjee and Mukhopadhyay (2009), Fatima et al. (2011) and Taiwo et al. (2010), who reported similar effects. The study by Fatima et al. (2011) supports the hypothesis that lead exerts its toxic effects on the hypothalamic or supra-hypothalamic sites, leaving an intact pituitary-testicular axis intact. In this study lead showed no siginificant effect on the LH levels, but significant decrease in testosterone. This suggests that lead exerts its effect more at the testicular level. This study also supports the finding of Sokol (1987), Wadi (1999) and Taiwo et al. (2010) who aserted that lead targets the spermatogenesis and

sperms within the epididymis by producing reproductive toxicity rather than acting within the hypothalamic-pituitary-testicular (H-P-T) axis. They also sugested that the gonadotoxic effects of lead are on the intra-testicular sites with minimal effects on hormonal levels and no effect on extra testicular sites.Lead induced imbalances in the HPT hormonal axis, which causes pituitary cells to release inappropriate levels of LH and change the steroid negative feedback loop (Ronis, et al*.* 1996), usually at the hypothalamus level (Grattan, et al*.* 1996).

Conclusion

Lead increases LH levels and significantly reduces FSH, testesterone levels in the experimental group and this effect on LH was more pronounced in younger age group, while the older age groups showed lower testesterone levels.

Conflict of interest

The authors have not declared any conflict of interest

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