

Age-Related Effects of Lead Poisoning on Some Haematological Parameters in Adult Wistar Rats

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Summary: 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 evaluate sub-chronic effects of lead poisoning on haematological parameters and some sex hormones, as well as age-related changes on Wistar Rats. Thirty (30) of 3-, 5-, and 7-months old male Wistar rats, were divided into experimental (lead fed) and control (distil water) groups. Haematological parameters were determined, while blood lead concentration was determined using the method of Atomic Absorption Spectrophotometer. There was a significant ($P < 0.05$) increase (46.00, 46.75, 50.75 vs 14.56, 18.00, 17.60) in blood lead concentration with insignificant ($P > 0.05$) increase in the concentration of WBC counts (12.433, 13.000, 12.250 Vs 12.400, 10.000, 11.250) between the experimental and control groups. Significant decrease in Body Weight (77.43, 107.88, 134.35 Vs 130.66, 150.60, 165.62), RBC counts (5.333, 7.000, 6.250 Vs 7.000, 7.500, 7.250), PCV (22.667, 40.00, 35.25 Vs 37.600, 45.5, 43.25), Hb (10.000, 12.000, 10.75 Vs 13.200, 13.250, 12.50), MCV (45.333, 54.500, 55.750 Vs 55.400, 59.500, 58.250), MCH levels (15.000, 16.250, 16.500 Vs 18.400, 17.750, 17.000), as well as insignificant decrease in platelet counts (410, 373, 341 Vs 437, 313, 384), and MCHC (29.67, 29.75, 30.00 Vs 32.800, 30.25, 29.250). The effect of lead (Pb) on these parameters was observed to be more pronounced in younger animals ($P \leq 0.05$). It was concluded that, ingestion of lead acetate produces more physiological derangement in young Wistar Rats.

Keywords: Hematological, Lead acetate, Wistar Rats.

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INTRODUCTION

Lead is a soft, malleable, and heavy metal with a bluish-white color which tarnishes to a dull grayish on exposure to air (Olade, 1987). Over the last three decades, there has been increasing global concern over the public health impacts attributed to direct and indirect environmental lead 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, 2014). The health of 200 million people in low-income countries is at risk from 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).

Sources of occupational exposure to lead include 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).

Lead 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 of varying degrees of concentration; and lead poisoning usually occurs from repeated exposure to small amounts (Agency For Toxic Substances and Disease Registry (ATSDR) 1999). 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 duration (Freeman, 1970).

Countries in the sub region of Africa, with the exception of the Republic of South Africa, have not implemented lead reduction programmes, and lead pollution has continued to pose health hazards in animal and man in Nigeria and many other parts of the world (Ajayi, Adeniyi and Babayemi, 2009). Leaded petrol, as one of the metal's sources in the environment, its usage levels is a good indicator of environmental lead exposure (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, but very few researchers tried to correlate haemato-biochemical alterations of lead acetate in laboratory animals especially in rats (Suradkar, *et al*, 2009). There are many reports on the effects of lead poisoning on various body systems, organs and blood parameters with limited studies on age- related effects of the heavy metal in relation to some hematological variables.

The purpose of the study was to evaluate the age-related sub-chronic effects of lead poisoning on haematological parameters in Wistar Rats.

MATERIALS AND METHODS

Materials used:

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

Methodology

Thirty (30) Wistar Rats of different ages of 3-, 5-, and 7-months old were divided into two groups of experimental and control groups respectively. The experimental (n=15), animals were fed orally with aqueous lead acetate solution at 250mg/kg body weight per day (Ambali *et al.*, 2011) for 22 days, while the control (n=15) received distilled water.

Both control and experimental animals were acclimatized for 7 days prior to commencement of experiment; animals were housed in metallic cages and given free access to laboratory chow and water. After intervention, rats were anesthetized by intravenous injection of 0.5ml of 0.4% solution of sodium thiopental (Greene, 2002) and afterwards decapitated. Blood samples were collected through a glass funnel into two test-tubes for each rat: first sample collected in heparinised test-tubes was used for RBC, WBC and platelet counts as well as determination of PCV, MCHC, MCV values, and Hb concentration; Second sample collected in EDTA test-tubes was used for spectrophotometric analysis of blood lead concentration in the blood samples, using Atomic Absorption Spectrophotometer (BUCK Scientific; model: 210 VGP, USA).

Statistical Analysis

Data was collected and analyzed using student independent T-test to compare difference between

experimental and control groups, while ANOVA was used to compare significant difference between experimental animals between the three age groups. All analysis were performed using Statistical Package for Social Science (SPSS) (Windows Evaluation Version 20, LEAD Technologies, USA) at p -value ≤ 0.05 .

RESULTS

Effect of lead poisoning on weight gain

The initial and final body weights of the animals are shown table 1 while table 2 shows the weight(g) changes in the animals after experiment in different age groups of animals. Significant difference ($p \leq 0.05$) was recorded between experimental and control animals among all 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 ($p \leq 0.05$) weights changes in 5-months old animals were also found with weight gain in normal controls (42.5g). However, a loss in weight was recorded in experimental group (28.25g). Mean weight gain of 16.25g was observed in normal control of 7-month old animals, though a loss was recorded in experimental animals (16.00g). There was no significant ($P > 0.05$) difference in weight loss between the three age groups.

Table 1:

Weight of Animals Before Experiment

Ages of Animals (months)	Weight(g) Group	
	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

Table 2:

Weight Changes in Animals After Experiment

Ages of Animals (month)	Weight(g) Group	
	Experimental	Control
3-month old	77.43±3.38* _a	130.66±28.80
5-month old	107.88±12.80* _a	150.60±6.65
7-month old	134.35±8.97* _a	165.62±17.80

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

Table 3:

Blood Lead Levels of Animals After Experiment

Ages of Animals (months)	Lead Levels(µg/dl) Group	
	Experimental	Control
3-month old	46.00±6.00* _a	14.56±7.65
5-month old	46.75±18.95* _a	18.00±3.65
7-month old	50.75±12.65* _a	17.60± 4.50

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

Effect of exposure on the plasma lead level

Table 3 represents values of Blood lead concentration in different animal age groups. Significant differences ($P \leq 0.05$) were recorded in experimental compared to control groups. While no significant ($P > 0.05$) difference in Blood Lead levels between all age groups among experimental animals.

Effect of lead poisoning on haematological variables after exposure

The effect of lead exposure on the various measured haematological variables are shown Table 4.

Platelet counts: Platelet count was not significantly ($P > 0.05$) different between the experimental and control animals among all age groups. Similarly, no significant ($P > 0.05$) difference was observed between all age groups experimental animals.

Red blood cell (RBC): There was a significant ($P \leq 0.05$) reduction in RBCs was recorded in experimental animals of both 3- and 7-months old compared to control animals. While no significant ($P > 0.05$) difference in RBC Counts was observed between all age groups in the experimental animals.

White blood cell (WBC): There was no significant ($P > 0.05$) difference in the WBC between the experimental and control animals among all age groups.

Packed cell volume (PCV): Significant reduction ($p < 0.05$) was recorded between the experimental and control animals among all age groups. Also, the 3-months old animals had a significant ($P < 0.05$) decrease in PCV levels compared to 5- and 7- months old experimental animals.

Haemoglobin concentration: Significant differences ($p \leq 0.05$) were recorded in experimental animals of 3- and 7-months old animals compared to their respective control groups. Also, a significant ($P < 0.05$) decrease in Haemoglobin levels between 3-months old compared to 5-months old experimental animals was recorded with more decrease among the 3-months old animals.

Mean Capsular Volume (MCV): A significant ($p \leq 0.05$) difference was recorded in experimental animals of 3- and 5-months old compared to control animals. While no significant ($P > 0.05$) difference in MCV between all age groups among experimental animals.

Mean Capsular Haemoglobin Concentration (MCHC): Significant ($p \leq 0.05$) reduction in MCHC was recorded in 3-months old experimental animals compared to the control. While no significant ($P > 0.05$) difference in MCHC levels between all age groups among experimental animals.

Table 4.

Haematological parameters of the animals after the Experiment

Haematological variables	Ages of Animals (months)	Group		P-Value
		Experimental	Control	
Platelet Counts ($\times 10^3/\mu\text{l}$)	3-month old	410 \pm 151.12 _a	437 \pm 99.19	0.739
	5-month old	373 \pm 152.11 _a	313 \pm 125.52	0.625
	7-month old	341 \pm 124.89 _a	384 \pm 24.23	0.464
RBC Counts ($\times 10^6/\mu\text{l}$)	3-month old	5.333 \pm 1.52* _a	7.000 \pm 0.70	0.027
	5-month old	7.000 \pm 0.58 _a	7.500 \pm 0.58	0.900
	7-month old	6.250 \pm 0.50* _a	7.250 \pm 0.50	0.035
WBC Counts ($\times 10^3/\mu\text{l}$)	3-month old	12.433 \pm 2.30 _a	12.400 \pm 0.55	0.944
	5-month old	13.000 \pm 0.82 _a	10.000 \pm 4.08	0.313
	7-month old	12.250 \pm 5.19 _a	11.250 \pm 1.89	0.757
PCV Levels (%)	3-month old	22.667 \pm 5.21* _a	37.600 \pm 5.46	0.024
	5-month old	40.00 \pm 1.41* _b	45.5 \pm 2.65	0.040
	7-month old	35.25 \pm 2.87* _b	43.25 \pm 0.96	0.045
Hb Concentration (g/dl)	3-month old	10.000 \pm 1.00* _{ac}	13.200 \pm 2.28	0.034
	5-month old	12.000 \pm 0.82 _b	13.250 \pm 1.70	0.397
	7-month old	10.75 \pm 0.95* _{bc}	12.50 \pm 1.00	0.044
Mean Capsular Volume, MCV (fl)	3-month old	45.333 \pm 6.86* _a	55.400 \pm 4.22	0.019
	5-month old	54.500 \pm 1.00* _a	59.500 \pm 1.91	0.023
	7-month old	55.750 \pm 3.59 _a	58.250 \pm 2.21	0.192
Mean Capsular Haemoglobin Concentration (MCHC) (g/dl)	3-month old	29.67 \pm 1.15* _a	32.800 \pm 0.84	0.001
	5-month old	29.75 \pm 0.96 _a	30.25 \pm 2.87	0.709
	7-month old	30.00 \pm 1.41 _a	29.250 \pm 2.21	0.510
Mean Capsular Haemoglobin, MCH (pg)	3-month old	15.000 \pm 2.65* _a	18.400 \pm 1.51	0.027
	5-month old	16.250 \pm 0.50* _{ac}	17.750 \pm 1.26	0.031
	7-month old	16.500 \pm 0.57 _{bc}	17.000 \pm 2.00	0.571

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

Mean Capsular Haemoglobin (MCH).

A significant ($p \leq 0.05$) reduction in MCH was recorded in 3- and 5-months old experimental animals as compared to control group. There was a significant ($P < 0.05$) decrease in MCH levels between 3-months old compared to 7-months old experimental animals was recorded with more decrease among 3-months old animals.

DISCUSSION

Result of this study showed that, sub-chronic lead poisoning led to significant loss of weight in all lead-treated animals. It was previously reported that, the action of lead in causing weight loss may be attributed to loss of appetite and gastrointestinal disturbances (Cezard and Haguenoer, 1992); 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's activity in weight loss was relatively more pronounced in young 3-months old animals.

Lead showed no significant ($P > 0.05$) effect on platelet count among experimental animals compared to the control groups and between age groups. However, on relative comparison, lead-treated groups showed lower platelet count compared to control groups; this is possibly due to platelet high susceptibility to oxidative stress (Mcmurphy *et al.*, 1995; Ohyashiki, Kobayashi, and Mashi, 1991). Other workers documented a contrary view that lead intoxication causes a considerable increase in Platelet Count compared to the control (Saeed, 2015 and Suradkar, 2009), which may be due to thrombocytopenia (Sudakova *et al.*, 1983) followed by thrombocytosis (Sudakova *et al.*, 1983; Yagminas *et al.*, 1990).

Among both 3- and 7-month age groups, lead showed significant decrease ($P \leq 0.05$) in RBC count, PCV and Haemoglobin Concentrations compared to control groups. This agrees with previous studies reporting effect of lead in reducing RBC count (Helmy *et al.*, 2000; Klassen, 2001; Alexa *et al.*, 2002; Mugahi., *et al.*, 2003; Noori *et al.*, 2003; Othman *et al.*, 2004; Teijon *et al.*, 2006; Suradkar., *et al.*, 2009; USEPA, 2009; Wahab, 2010; Toplan *et al.*, 2004; Hanan, and Riham, 2012; Ibrahim., *et al.*, 2012; and Diefy, Sharkawy, Sayed, and Shehata, 2014).

Reduction in RBC counts is due to fact that, erythrocyte membrane is vulnerable to lipid peroxidation with limited capacity to repair its damaged components due to oxidative stress (Flora, Pande, Kannan and Mehta, 2004).

Such haematological alteration on erythrocyte are attributed to effect of lead on some erythrocyte enzymes (Calderon-Salinas *et al.*, 1993) in addition to its effect on cell metabolism, interaction with some reactions where calcium is their secondary mediator

and inhibition of some enzymatic activities like amino-levulinic acid dehydrase (ALAD) (Klassen, 2001).

Lead shortened life span of erythrocytes due to increased fragility of their cell membrane while the reduced haemoglobin production is due to decreased levels of enzymes involved in heme synthesis (Guidotti *et al.*, 2008). Nabil, (2012) reported elevation of plasma bilirubin level in lead exposure which is probably due to red blood cells destruction mediated by heme oxygenase.

Contrary to this, Golalipour, *et al.* (2007) reported increased RBC count in adult albino Wistar rats after lead acetate exposure, which they suggested to be due to reduced oxygen transfer and tissue hypoxia. They attributed that the bone marrow could overcome the lead toxicity because of subchronic exposure of low dose levels. No significant ($P > 0.05$) difference in RBC Counts between age groups in experimental animals was recorded.

Lead showed no significant effect on plasma WBC count in all experimental groups, nor between age groups. However, on comparison with controls, lead-treated animals showed higher WBC counts compared to control groups. Similarly, repeated lead exposure has been demonstrated to increase WBC count (Mcmurphy., *et al.*, 1995; Mugahi., *et al.*, 2003; Okedran, 2010; Ibrahim., *et al.*, 2012). This contradicts a report by Suradkaret., *et al.*, 2009 that lead reduces WBC count.

Leukocytosis in lead acetate administered rats has been attributed to the lead-induced inflammation (Yagminas *et al.*, 1990) and direct toxic action of lead on leucopoiesis in lymphoid organs (Mugahi *et al.*, 2003).

While there was no significant difference in lead's action on WBC counts between age groups, there was a significant age-related decrease in PVC. It was also observed that, age-related effect of lead poisoning led to decrease in Hb concentration in 3-, 5- and 7-months old animals respectively. The effect of which, was found to be more pronounced in the lower-aged animals.

On contrary Ajayi, *et al.* (2009) reported that lead to significantly increase PCV, of which he possibly attributed to short duration of lead treatment (100mg/kg bwt intraperitoneally for 7days). This suggest an initial increase in PCV followed by a decrease, during lead exposure.

Lead decreases heme biosynthesis by inhibiting amino-levulinic acid dehydrase (ALAD) and Ferrochelataase activity (USEPA, 2009). In continuous exposure, lead affects heme biosynthesis through inhibition of cytoplasmic and mitochondrial enzymes (ATSDR, 1993). The presence of reduced PCV, RBC and Hb in lead-treated groups suggests that sub-chronic lead poisoning leads to the development of anaemia. The anaemia may be caused by reduced haemoglobin synthesis, haemolysis of mature and immature erythrocytes with reduced life span. Lead poisoning is

also being linked to the interference of hematopoietic progenitor development, copper metabolism and production of erythropoietin (Klauder and Petering, 1977; Osterode, Barnas and Geissle, 1991).

Lead also reduces MCV, MCHC and MCH (Golalipour, et al. 2007; Wahab, 2010; Diefy, Sharkawy, Sayed, and Shehata, 2014; Yagminas et al., 1990; Helmy et al., 2000; Teijon et al., 2006; Durgut et al. 2008; USEPA, 2009; Nabil et al. 2012 and Nuran, Gurer-orhan, and Nukhet, 2001. The effect of lead poisoning on MCH were found to be more in younger (3-months old) animals.

The presence of reduced MCV and MCHC in the present study indicates microcytic hypochromic anaemia in lead-treated rats; this agrees with earlier studies (Noori., et al, 2003, Suradkar., et al, 2009; Mugahi et al., 2007; Suradkar et al., 2009; Klassen, 2001; Reichelmayr-Lais, and Kirchgessner, 1984). The decreased MCHC and MCH is attributed to effect of lead on haemoglobin synthesis. But Ambali et al 2012; Sherif, 2014 reported increased MCV in lead exposure in their studies, and also reported macrocytic hypochromic anaemia in lead treated animals.

Lead increases WBC Counts and significantly reduces RBC related indices in the experimental group and these effects were more pronounced in younger age group.

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