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Effect of Oral Administration of Lead Acetate on Hematological Indices of Male and Female Adult Wistar Rats

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Abstract

Occupational and environmental exposure to lead (Pb^{2+}) remains a serious problem in many developing and industrialized countries. However, little information is available on the influence of sex on lead toxicity. This study evaluated the hematological effects of lead acetate at different durations and dose in male and female Wistar rats. Thirty-Six adult Wistar rats were divided into six groups containing 3 male and 3 female rats each. Group 1 served as control for Groups 2 and 3. Groups 2 and 3 were administered 30mg/kg and 60 mg/kg of lead acetate for 14 days respectively. Group 4 served as control for Groups 5 and 6. Groups 5 and 6 were administered 30mg/kg and 60 mg/kg of lead acetate for 28 days respectively. All administrations were done orally. At the end of the experiment, the rats were sacrificed and blood samples were collected by cardiac puncture and stored in EDTA bottles for hematological analysis. Group 2 female Wistar rats had a significant decrease ($P < 0.05$) in red blood cell count when compared to Group 2 male Wistar rats.Comparison of Neutrophil count of Groups 5 and 6 male Wistar rats to Groups 5 and 6 female Wistar rats showed a significant difference ($P < 0.05$).Also, comparison of Eosinophil count of Groups 2 and 5 male Wistar rats to Groups 2 and 5 female Wistar rats showed a significant difference ($P < 0.05$). A significant increase ($P < 0.05$) in Basophil count was also observed in Group 6 male Wistar rats when compared to Group 6 female Wistar rats. Results from this study suggests that male Wistar rats are more susceptible to the effect of lead acetate on hematological indices. However, further studies using castrated rat models to determine the influence of sex hormones on lead toxicity is recommended.

Keywords: Hematological indices, Lead acetate, Sex differences, Toxicity, Wistar rats

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INTRODUCTION

Lead is a toxic, heavy, bluish-gray metal that occurs naturally in the earth's crust, it is ranked as the second hazardous heavy metal after arsenic in the Agency for Toxic Substances and Disease Registry (ATSDR) 2011 Substance Priority List. Lead has no known beneficial function in the human body unlike some other metals such as zinc and manganese which are required as essential nutrients (Ahmed *et al*., 2007). In addition, it cannot be biodegraded nor can it be detoxified by living organisms [LahDou, 2007; Brajesh *et al*., 2014). Human exposure to lead is common and results from it's usage in plumbing materials, lead alloys, lead acid batteries, cable sheathing, paints, dyes, ceramic glazes, leaded gasoline, ammunition and soldering materials due to its exceptional and unique properties (Gagan *et al*., 2013).

The main routes of exposure to lead are inhalation, ingestion and dermal contact from a range of sources via air, dusts, food and water (WHO, 2019). Inhalation and dermal contact are routes of exposure more typical of occupational settings, whereas the primary route of exposure for the general population is ingestion (WHO, 2019). Lead has many toxic effects, including neurological, behavioural, immunological, renal, hepatic and haematological dysfunctions (Ercal *et al*., 2000; MuGahi *et al*., 2003; Soltaninejad *et al*., 2003; Patra and Swarup, 2004; De Marco *et al*., 2005 and Omotosho *et al*., 2015) and oxidative stress has been identified as the primary contributory agent in the pathogenesis of lead poisoning (Hsu and Guo, 2002). According to WHO, exposure to lead is estimated to account for 143,000 deaths per year with the highest burden in developing regions. In recent times, there has been an increase in reported cases of lead poisoning in Nigeria (Yahaya 2010; Zinggl, 2016).

Blood is a specialized connective tissue that is composed of blood cells and plasma, it has various functions including transport of nutrients, respiratory gases, waste products, hormones etc. Lead is known to directly affect the hematopoietic system by inhibiting the activity of key enzymes aminolevulinic acid synthetase (ALAS), δ- aminolevulinic acid dehydratase (ALAD), and ferrochelatase involved in the heme synthesis pathway (Flora *et al*., 2012; Scinicariello *et al*., 2007). Extensive experimental studies conducted on laboratory animals clearly points to the potential toxic effects lead has on the hematopoietic system which includes leukocytosis, monocytosis, eosinopenia, neutrophilia, thrombocytosis and anemia (Suradkar *et al*., 2009; Kilikdar *et al*., 2011; Ibrahim *et al*., 2012). However, several factors such as Dose, Age, Sex, Route of exposure, Solubility, Retention percentage, duration of exposure, frequency of intake, absorption rate, mechanisms and efficiency of excretion may affect lead toxicity (Ibrahim *et al*., 2012). Schneider *et al*., (2011) reported that the response of the brain to a given lead exposure may vary depending on sex. Also, Roles *et al*., (1979) reported that women are 1.3 to 1.5 times more sensitive to lead induced changes in red cell biochemistry. There is paucity of information on the influence of sex on the toxic effect of lead on hematological indices. Therefore, this study was aimed at evaluating the sex differences in hematological indices following oral administration of lead acetate at different durations and dose in male and female adult Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Thirty-Six adult Wistar rats (18 males and 18 females) were used in this study. The Wistar rats were purchased from the Animal House of Human Anatomy Department, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. They were housed in clean plastic cages with soft wood shavings as their beddings in the Animal House of the Department of Human Anatomy and allowed to acclimatize for two weeks prior to the commencement of the experiment. The animals were fed with pelletized vital feed manufactured by Grand Cereals and Oil Mills Ltd. Plateau State, Nigeria

and allowed access to clean drinking water throughout the experimental period. All procedures were in accordance with the standard guidelines for care and use of laboratory animals.

Chemicals
Analytical grade of Lead-acetate manufactured by Best of Chemical (BOC) Science was purchased from a reputable chemical store in Zaria, Kaduna, Nigeria.; Ketamine manufactured by Cayman Chemical was purchased from a reputable pharmacy in Zaria, Kaduna, Nigeria.

Experimental Protocol
Based on the oral LD_{50} of lead acetate which was 600 mg/kg body weight for Wistar rats (Sujatha *et al*., 2011), 5% (30 mg/kg) and 10% of the LD_{50} (60 mg/kg) were used in this study.

Experimental Design
A total number of 36 Wistar rats (18 male and 18 female) were distributed randomly into six groups with each group containing 3 male and 3 female rats that were kept separately.

Group 1 which served as control for group 2 and 3 were orally administered distilled water for 14 days. Groups 2 and 3 were orally administered 30mg/kg and 60 mg/kg body weight of lead acetate daily for 14 days respectively. Group 4 which served as control for group 5 and 6 were orally administered distilled water for 28 days. Groups 5 and 6were orally administered 30 mg/kg and 60 mg/kg body weight of lead acetate daily for 28 days respectively.

Animal Sacrifice and Tissue Collection
Twenty-four hours after the last administration, the animals were humanely sacrificed under anesthesia with ketamine at a dose of 75mg/kg IP (PARP, 2013). The heart was accessed through a midline incision on the anterior abdominal wall, and blood samples were collected by cardiac puncture. The blood samples were stored in Ethylenediaminetetraacetic acid (EDTA) bottles for hematological analysis.

Determination of Hematological Indices
The blood samples collected into EDTA specimen bottle were analysed within 6 hours of collection using a Sysemex XE-2100 Automated Hematology Blood Analyser in Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Each blood sample was well mixed at room temperature using a blood rotor machine for five minutes. The automated analyser was put on about 30 minutes prior to loading the machine for the system to warm. A 10 µl of blood sample was aspirated through tube of the machine. Hematological indices such as Red Blood Cell count (RBC), Packed Cell Volume (PCV), Hemoglobin concentration (Hb), White Blood Cell count (WBC), Neutrophil count. Eosinophil count and Basophil count were determined.

Data Analysis
All the data were analyzed using the Statistical Package for Social Sciences (SPSS version 23) and were expressed as mean ± SEM (standard error of mean). One-way analysis of variance (ANOVA) was used to compare the mean differences between and within the groups followed by Tukey's post-hoc test. Student t-test was used to compare mean differences between male and female Wistar rats. P-value less than to 0.05 was considered to be significant.

RESULTS
The results of the effect of lead acetate on Red Blood Cell count (RBC), Packed Cell Volume (PVC) and Hemoglobin concentration (Hb)are presented in Tables 1 and 2.

The RBC ($\times 10^{12}$/L) obtained for Group 6 male and female Wistar rats that were treated with 60 mg/kg body weight of lead acetate for 28 days were 6.44±0.27 and 5.62±0.45 respectively. These were significantly decreased ($P < 0.05$) when compared to the control (Table 1).A significant decrease ($P < 0.05$) in RBC count was observed in group 2 female Wistar rats (7.45±0.16) when compared to group 2 male Wistar rats (8.06±0.08) as seen in Table 2.

The PCV (%) obtained for Groups 3, 5 and 6 male Wistar rats were 40.07±1.63, 40.13±0.81 and 40.37±0.87 respectively. These were significantly decreased ($P < 0.05$) when compared to their control (Table 1).

The Hb (g/L) obtained for Groups 3, 5 and 6 male Wistar rats were 12.93±0.73,13.57±0.27 and 12.73±0.15 respectively. These were also significantly decreased ($P < 0.05$) when compared to their control (Table 1).

Table 1: Mean levels of some hematological indices of lead acetate treated Wistar rats.

Parameter	Sex	Group 1 (Control 14 days)	Group 2 (30mg/kg Pb 14 days)	Group 3 (60mg/kg Pb 14 days)	Group 4 (Control 28 days)	Group 5 (30mg/kg Pb 28 days)	Group 6 (60mg/kg Pb28 days)	F	P
RBC ($\times 10^{12}$/L)	Male	8.06±0.15	8.06±0.08	7.04±0.23	8.09±0.37^{a}	7.46±0.18	6.44±0.27^{a}	8.61	0.001
	Female	7.56±0.19	7.45±0.16	7.05±0.45	7.50±0.21^{a}	6.87±0.34	5.62±0.45^{a}	5.13	0.010
PCV (%)	Male	46.00±0.62^{a}	45.67±0.47^{b}	40.07±1.63^{ab}	46.37±0.12^{cd}	40.13±0.81^{bc}	40.37±0.87^{d}	13.07	<0.00 1
	Female	40.10±1.01	39.47±1.15	39.23±1.90	41.77±0.73	40.40±0.15	38.13±0.70	1.29	0.330
Hb (g/L)	Male	15.13±0.12^{a}	15.07±0.15^{b}	12.93±0.73^{ab}	15.40±0.25^{cd}	13.57±0.27^{c}	12.73±0.15^{d}	11.96	<0.00 1
	Female	14.50±0.40	14.33±0.38	13.53±0.65	14.43±0.18	13.53±0.54	12.57±0.70	2.22	0.120

One-way ANOVA test followed by Tukey post hoc test. Results expressed as mean ± SEM. Cells carrying same superscripts on each row are significantly different ($P<0.05$). RBC: Red Blood Cell count; PCV: Packed Cell Volume; Hb: Hemoglobin Concentration

Table 2: Comparison of mean levels of haematological indices between male and female lead acetate treated Wistar rats.

Group	Parameter	Male	Female	T	P
Group 1 (Control 14 days)	RBC ($\times 10^{12}$/L)	8.06±0.15	7.56±0.19	2.08	0.106
	PCV (%)	46.00±0.62	40.10±1.01	4.98	0.008
	Hb (g/L)	15.13±0.12	14.50±0.40	1.50	0.207
Group 2 (30mg/kg Pb 14 days)	RBC ($\times 10^{12}$/L)	8.06±0.08	7.45±0.16	3.40	0.027*
	PCV (%)	45.67±0.47	39.47±1.15	5.01	0.007
	Hb (g/L)	15.07±0.15	14.33±0.38	1.78	0.149
Group 3 (60mg/kg Pb 14 days)	RBC ($\times 10^{12}$/L)	7.04±0.23	7.05±0.45	-0.03	0.975
	PCV (%)	40.07±1.63	39.23±1.90	0.33	0.756
	Hb (g/L)	12.93±0.73	13.53±0.65	-0.62	0.571
Group 4 (Control 28 days)	RBC ($\times 10^{12}$/L)	8.09±0.37	7.50±0.21	1.40	0.233
	PCV (%)	46.37±0.0.12	41.77±0.73	6.21	0.003
	Hb (g/L)	15.40±0.25	14.43±0.18	3.15	0.035
Group 5 (30mg/kg Pb 28 days)	RBC ($\times 10^{12}$/L)	7.46±0.18	6.87±0.34	1.54	0.199
	PCV (%)	40.13±0.81	40.40±0.15	-0.32	0.762
	Hb (g/L)	13.57±0.27	13.53±0.54	0.06	0.958
Group 6 (60mg/kg Pb 28 days)	RBC ($\times 10^{12}$/L)	6.44±0.27	5.62±0.45	1.560	0.194
	PCV (%)	40.37±0.87	38.13±0.70	2.006	0.115
	Hb (g/L)	12.73±0.15	12.57±0.70	0.234	0.826

RBC: Red Blood Cell Count; PCV: Packed Cell Volume; Hb: Hemoglobin Concentration. *: Significantly different

The results of the effect of lead acetate on White Blood Cell count (WBC), Neutrophil count Eosinophil count and Basophil count are presented in Figures 1 to 4 and Table 3.

The WBC ($\times 10^9$/L) obtained for Group 5 female Wistar rats, Group 6 male and female Wistar rats were 11.53±0.20,13.40±0.40 and 12.43±0.24 respectively. These were significantly increased ($P < 0.05$) when compared to the control (Figure 1).

The increase in WBC ($\times 10^9$/L) was dose-dependent as there was a significant increase ($P < 0.05$) in mean values of WBC($\times 10^9$/L) in Group 6 male Wistar rats (13.40±0.40) treated with 60 mg/kg body weight of lead acetate for 28 days when compared to Group 5 male Wistar rats (11.33±0.09) treated with 30 mg/kg body weight of lead acetate for 28 days (Figure 1).

The percentage neutrophil count in male Wistar rats, increased significantly ($P < 0.05$) in Group 5 (23.20±0.64) and Group 6 (24.37±0.12) when compared to the control while percentage neutrophil count in female Wistar rats increased significantly ($P < 0.05$) in Group 2 (17.60±0.15), Group 3 (17.97±0.12), Group 5 (19.13±0.15) and Group 6 (22.67±0.28) when compared to the control (Figure 2). The increase in mean values of percentage neutrophil count was dose and duration dependent as seen in Figure 2. Comparison of percentage neutrophil count of Groups 5 and 6 male Wistar rats to Groups 5 and 6 female Wistar rats respectively showed a significant difference ($P < 0.05$) (Table 3).

The percentage eosinophil count obtained for Group 5 male Wistar rats, Group 6 male and female Wistar rats were 14.53±1.14, 14.27±2.17 and 11.90±2.58 respectively. These were significantly increased ($P < 0.05$) when compared to the control (Figure 3).Comparison of percentage eosinophil count of Groups 2 (7.43±0.44) and 5 (14.53±1.14) male Wistar rats to Groups 2 (5.57±0.29) and 5 (9.60±0.90) female Wistar rats showed a significant difference ($P < 0.05$) as seen in Table 3.

The percentage basophil count obtained for Group 6 male and Group 6 female Wistar rats were 8.87±0.48 and 4.03±0.64 respectively (Figure 4). A significant increase ($P < 0.05$) in percentage basophil count was observed in Group 6 male Wistar rats when compared to Group 6 female Wistar rats (Table 3).

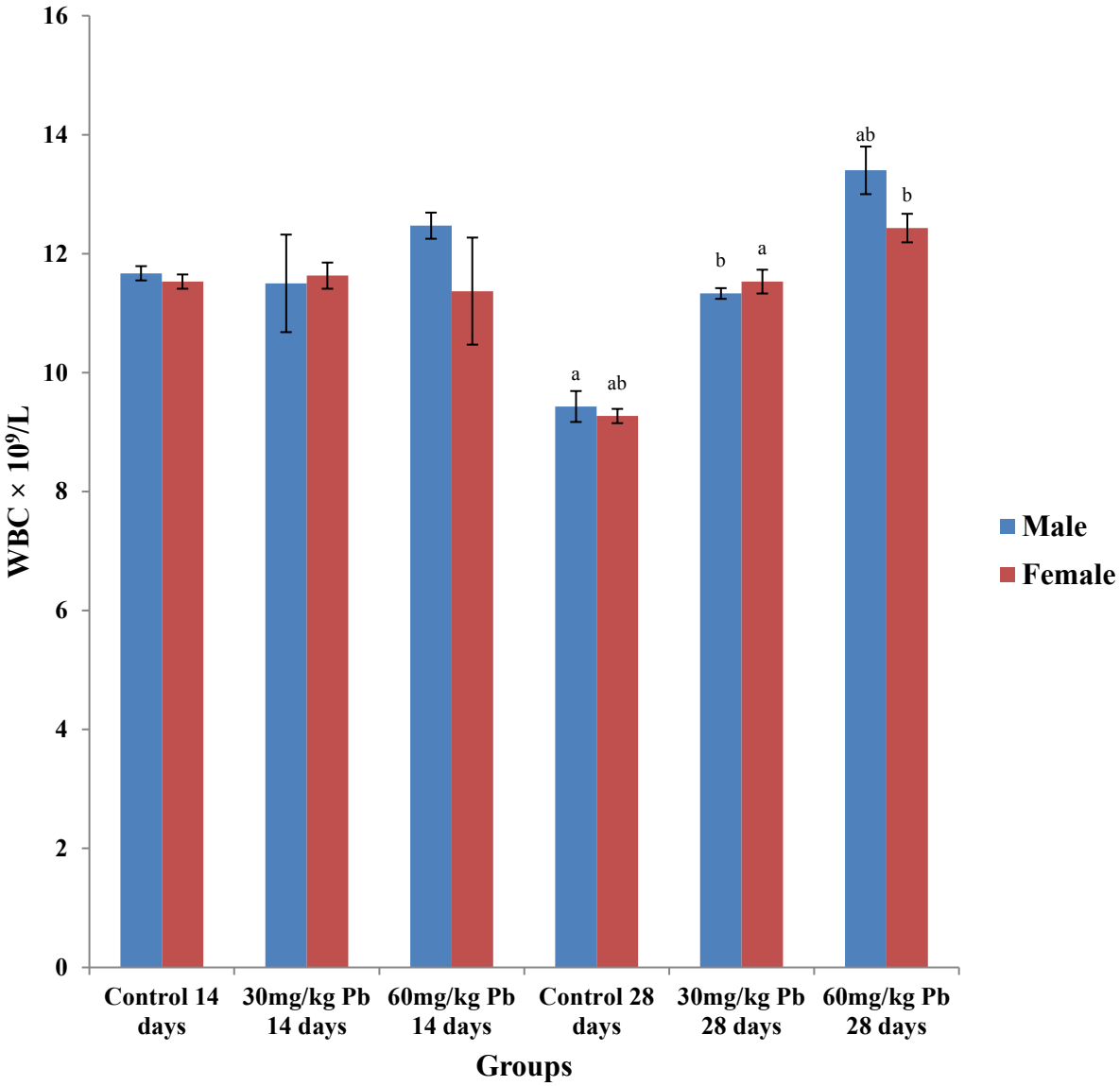

Figure 1: Mean White Blood Cell count (WBC) of lead acetate treated Wistar rats

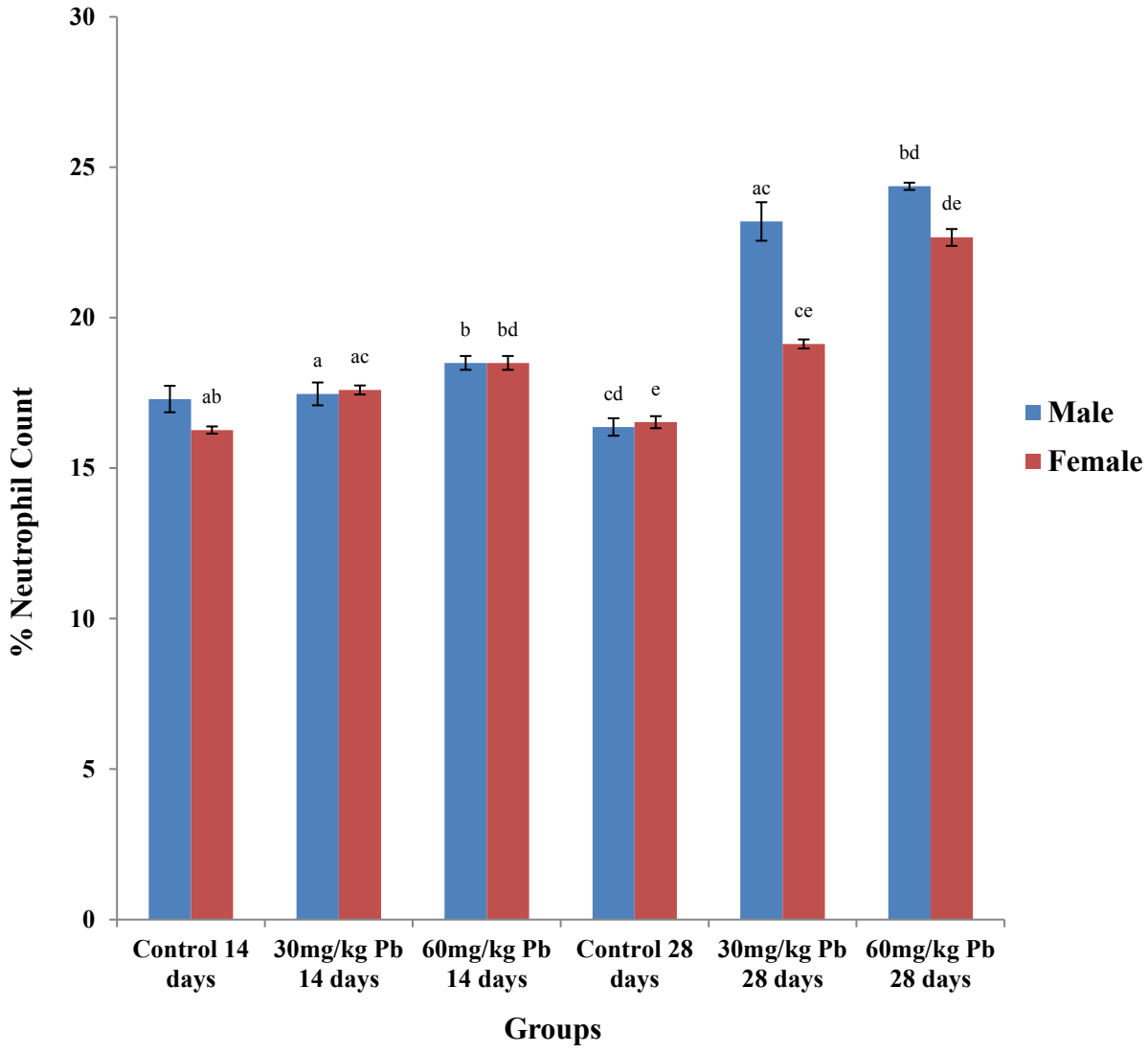

Figure 2: Mean percentage Neutrophil count of lead acetate treated Wistar rats

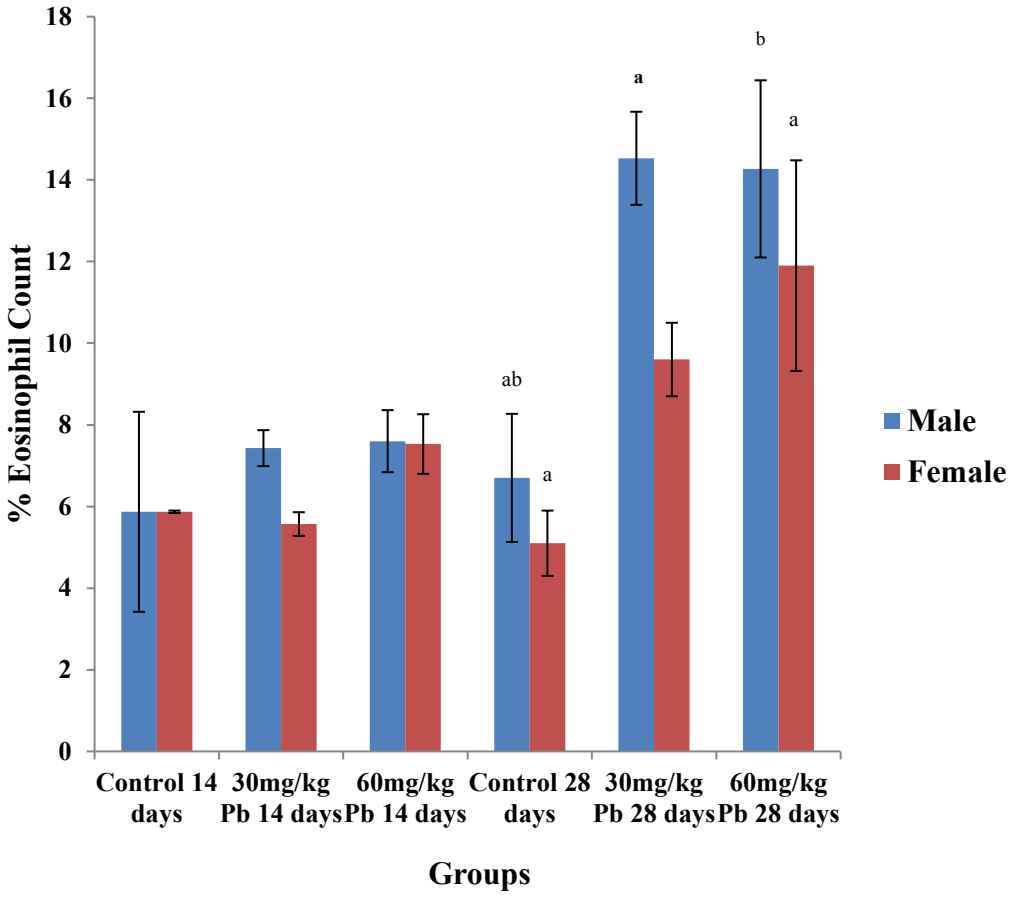

Figure 3: Mean percentage Eosinophil count of lead acetate treated Wistar rats

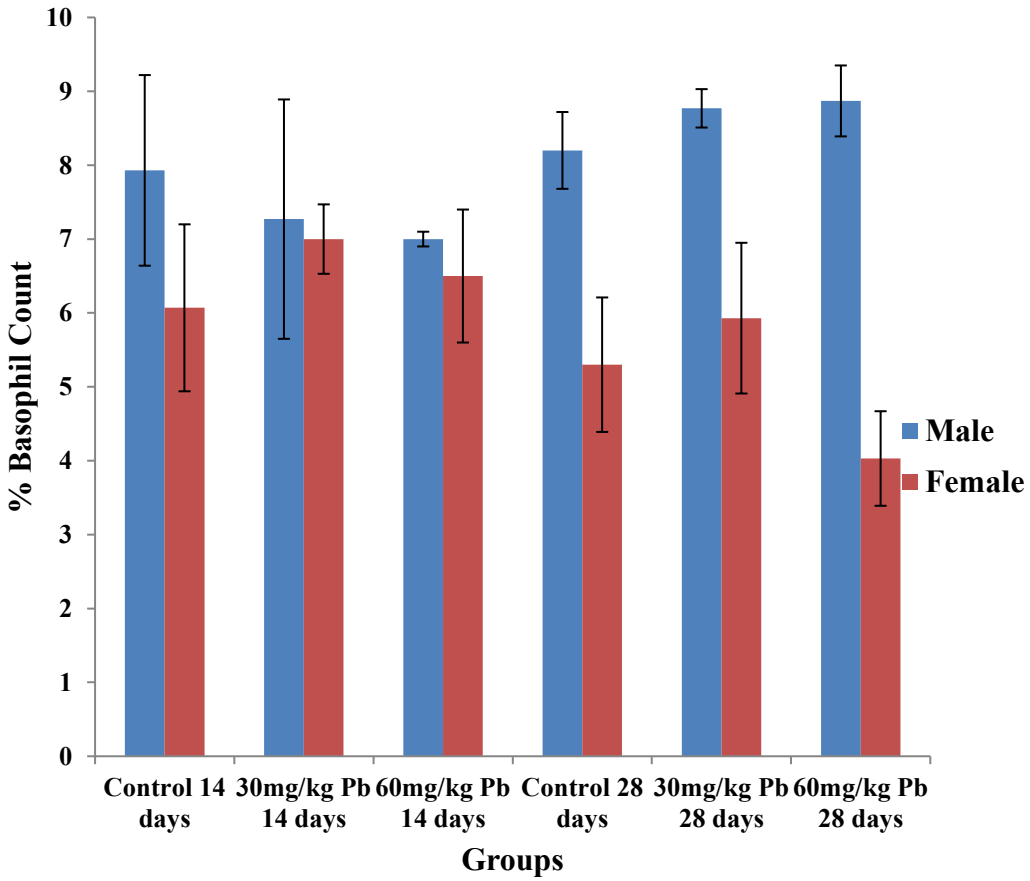

Figure 4: Mean percentage Basophil count of lead acetate treated Wistar rats

Table 3: Comparison of mean levels of haematological indices between male and female lead acetate treated Wistar rats.

Group	Parameter	Male	Female	t	P
Group 1 (Control 14 days)	WBC (×10⁹/L)	11.67±0.12	11.53±0.12	0.78	0.477
	Neut (%)	17.30±0.44	16.27±0.12	2.29	0.084
	Eos (%)	5.87±2.45	5.87±0.03	0.00	1.000
	Baso (%)	7.93±1.29	6.07±1.13	1.09	0.34
Group 2 (30mg/kg Pb 14 days)	WBC (×10⁹/L)	11.50±0.82	11.63±0.22	-0.16	0.883
	Neut (%)	17.47±0.38	17.60±0.15	-0.32	0.763
	Eos (%)	7.43±0.44	5.57±0.29	3.54	0.024*
	Baso (%)	7.27±1.62	7.00±0.47	0.14	0.882
Group 3 (60mg/kg Pb 14 days)	WBC (×10⁹/L)	12.47±0.23	11.37±0.90	1.19	0.300
	Neut (%)	18.50±0.23	17.97±0.12	2.05	0.110
	Eos (%)	7.60±0.76	7.53±0.73	1.30	0.953
	Baso (%)	7.00±0.10	6.50±0.90	0.55	0.611
Group 4 (Control 28 days)	WBC (×10⁹/L)	9.43±00.26	9.27±0.12	0.58	0.592
	Neut (%)	16.37±0.29	16.53±0.20	-0.47	0.663
	Eos (%)	6.70±1.57	5.10±0.80	0.91	0.416
	Baso (%)	8.20±0.52	5.30±0.91	2.78	0.05
Group 5 (30mg/kg Pb 28 days)	WBC (×10⁹/L)	11.33±0.09	11.53±0.20	-0.91	0.417
	Neut (%)	23.20±0.64	19.13±0.15	6.24	0.003*
	Eos (%)	14.53±1.14	9.60±0.90	3.993	0.027*
	Baso (%)	8.77±0.26	5.93±1.02	2.70	0.05
Group 6 (60mg/kg Pb 28 days)	WBC (×10⁹/L)	13.40±0.40	12.43±0.24	2.056	0.109
	Neut (%)	24.37±0.12	22.67±0.28	5.50	0.005*
	Eos (%)	14.27±2.17	11.90±2.58	0.70	0.52
	Baso (%)	8.87±0.48	4.03±0.64	6.00	0.004*

WBC: White Blood Cell Count; Neut (%): percentage Neutrophil Count; Eos (%): percentage Eosinophil Count; Baso (%): percentage Basophil Count. *: Significantly different

DISCUSSION

The haemopoietic system serves as important target for toxic chemicals and is a sensitive index of pathological conditions. In the present study, decrease in RBC, PCV and Hb was observed in Wistar rats in all the groups treated with lead acetate in both sexes. This may be attributed to the fact that lead has an inhibitory effect on key enzymes aminolevulinic acid synthetase ALAS, ALAD δ- aminolevulinic acid dehydratase (ALAD), and ferrochelatase involved in the heme synthesis pathway (Scinicariello *et al*., 2007; Flora *et al*., 2012) and also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes. Significant reduction in RBC, PCV and Hb was observed in groups treated with a higher dose and/or a longer duration. This indicates that the effects of lead acetate is dependent on the dose and duration of exposure which is in accordance with the study by Suradkar *et al*., (2009) where a dose dependent significant reduction in total erythrocyte count, hemoglobin, packed cell volume was observed in Wistar rats treated with 1 PPM, 100 PPM and 1000 PPM lead acetate in drinking water for 28 days and Saeed, (2015) where the levels of hemoglobin (Hb) and packed cell volume (PCV) was also significantly reduced in treated mice relative to the control in both sexes. In another study by Ibrahim *et al*., (2012) lead acetate given orally to female rats at a dose of 10 mg/kg BW also caused significant decrease in Hb concentration, RBC count, and packed cell volume. The significant reduction in RBC count of female Wistar rats in Group 2 when compared to the male Wistar rats shows that the female Wistar rats were more susceptible to the effect of lead acetate on RBC which is in line with the study of Roles *et al*., (1979) who reported that women are 1.3 to 1.5 times more sensitive to lead induced changes in red cell biochemistry.

The increase in WBC count in the groups that received lead acetate for a longer duration (28 days) when compared to their control may be attributed to the toxic action of lead on leukopoiesis in lymphoid organs. This suggests that the increase in WBC is directly related with their increased production from the germinal center of lymphoid organs under the continuous exposure to lead. This result is consistent with that of Saeed, (2015) who observed leukocytosis in higher-dose groups of both sexes of lead treated mice.

Neutrophil count was increased significantly in groups treated with lead acetate with a higher dose (60mg/kg) and/or for a longer duration (28 days) which indicates that the effect of lead acetate is dependent on the duration of exposure and dose. Increased production of neutrophils from bone marrow in response to lead intoxication might account for their rise in animals of both sexes. Also, male Wistar rats had a significantly higher neutrophil count when compared to the female Wistar rats in Groups 5 and 6 respectively which suggests that the male Wistar rats are more susceptible to the effects of lead acetate on neutrophil count. The female Wistar rats being less susceptible to the effects of lead on neutrophil count may be attributed to estrogens which are known to exert anti-inflammatory and anti-oxidative actions by inhibiting the production of inflammatory cells (Carey *et al*., 2007; Huang *et al*., 2008).

Eosinophil count increased significantly in groups treated with lead acetate with a higher dose (60mg/kg) and/or for a longer duration (28 days) which also indicates that the effect of lead acetate is dependent on duration of exposure and dose. Significant increase in Eosinophil count was observed in Groups 2 and 5 male Wistar rats when compared to Groups 2 and 5 female Wistar rats respectively. Also, Group 6 male Wistar rats had a significantly higher basophil count when compared to Group 6 female Wistar which suggests that the male Wistar

rats are more susceptible to the effects of lead acetate on eosinophil and basophil count. The female Wistar rats being less susceptible to the effects of lead on eosinophil and basophil count may be attributed to estrogens which are known to exert anti-inflammatory and anti-oxidative actions by inhibiting the production of inflammatory cells (Carey *et al*., 2007; Huang *et al*., 2008).

CONCLUSION

Results from this study suggests that male Wistar rats are more susceptible to the effect of lead acetate on Neutrophil, Eosinophil and Basophil count due to estrogens which are known to exert anti-inflammatory and anti-oxidative actions by inhibiting the production of inflammatory cells while female Wistar rats are more susceptible to the effect of lead acetate only on red blood cell count. However, further studies using castrated rat models to determine the influence of sex hormones on lead acetate toxicity is recommended.

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