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Leukocytic Response and Spleen Morphology of Albino Rats Exposed to Graded Levels of Lead Acetate

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Key words: Lead acetate; leukocytic response; differential leukocyte count; spleen morphology; rats.

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Abstract

Aim. The study investigated the leukocytic response and spleen morphology of albino rats exposed to graded dose levels of lead acetate.

Material and Methods. Four groups of 5 rats received lead acetate treatment per os for 14 days, as follows: group A (0.25 mg/kg body weight), group B (0.50 mg/kg body weight), group C (1.00 mg/kg body weight) and group D (no lead acetate treatment-control). Thereafter, total leukocyte count (TLC), differential leukocyte count (DLC) and histomorphology of the spleen were assessed. Total leukocyte count, differential leukocyte count and histomorphology of rats that received the lead acetate treatment were compared to control rats.

Results. Results have shown that the administration of lead acetate to rats led to a significant ($p < 0.05$) increase in TLC with an increase in the number of lymphocytes ($p < 0.05$). The number of absolute monocytes and neutrophils in the lead acetate exposed rats were significantly ($p < 0.05$) low. The microscopic changes from the spleen sections of the lead acetate treated rats suggest immune alteration and splenic damage.

Conclusion. Therefore the study confirms the risk of experiencing immunosuppression for humans and other species that may be exposed to lead.

Introduction

There are so many materials and industrial products in our environment that contain lead and as a result of this; lead accessibility to biological systems via different routes appears very common. Lead has been used in shotgun pellets, bullets, fishing sinkers, paints, batteries, gasoline, solder, water pipes, and other industrial products [1]. From these sources, lead and other myriad toxic substances are released into our environment on daily basis. Inappropriate sitting of lead manufacturing plants can result in accidental release of lead into groundwater, surface water, soil and air thereby posing great health risk to human beings and animals living in such areas [1].

The adverse effects of chronic exposure to lead have received enormous attention [2]. Lead has been known as a toxicant that can affect normal functions of many systems in animals and humans, among which are the cardiovascular [3-5], cerebrovascular [6], renal [7, 8], and the reproductive system [9,10]. People in certain occupations such as newspaper printers, bus drivers, auto-electricians, battery factory workers and lead smelters are more prone to lead poisoning [11-13], but also

animals that inhabit such industrial areas are vulnerable from accidental lead exposure. The WHO [14] published a report that both occupational and environmental chronic lead exposure can damage the central nervous, renal, cardiovascular, reproductive, and haematological systems.

The leukocytes are critical in immune and allergic responses of the body. Leukocytic profile (total leukocyte count and differential leukocyte counts) provide information or clue in clinical diagnosis of most suspected cases of heavy metal poisoning in humans and animals. Experimental work on the effect of lead salt exposure on leukocytic profiles and spleen morpholgy in animals is lacking.

This study was therefore designed to investigate the leukocytic response and spleen morphology of the albino rats exposed to graded dose levels of lead acetate.

Materials and Methods

Experimental animals

Twenty male albino rats weighing 225.8 – 314.5 grams were used for this study. These animals were housed in aluminum wire mesh cages with screen top. They were fed with commercially prepared diet and allowed access to drinking water *ad libitum* throughout the period of experimentation. Before the commencement of the experiment, the rats were allowed a free adjustment period of 14 days. Later, they were randomly assigned to 4 groups (n = 5) (Groups A, B, C and D). Rats of the test groups (A, B and C) received graded levels of lead acetate solution per os, (A-0.25 mg/kg, B-0.50 mg/kg and C-1.00 mg/kg), every other day for a period of 14 days. The chosen dose levels for this study fell within the range of doses used by other researchers [15] who had investigated on the effects of chronic lead treatment in cerebral microvessels of mice. Group D rats served as the control and received no lead acetate treatment.

Handling, management and use of animals for experimentation were in conformity with Laboratory Animal Rights Regulation of the University of Nigeria, Nsukka.

Sample collection

Before sacrificing the rats in the 4 groups on day 14-end of study period, blood samples were collected from retrobulbar plexus of the median canthus of the eye after a mild ether anesthesia, using a capillary tube. The blood samples were transferred into bijour bottles containing EDTA-an anticoagulating agent and used to assess the total leukocyte count (TLC) and differential leukocyte counts (DLC). On sacrificing the rats in the four groups, the spleen from the lead acetate exposed rats and the control were carefully dissected out, trimmed free of extraneous tissues and preserved in clean bottles containing 10% formosaline, for histopathological studies.

Total leukocyte count (TLC)

Total leukocyte count was determined by diluting 0.02 ml of blood sample with 0.38 ml of leukocyte diluting fluid [16]. Thereafter, the Neubauer chamber of the haemocytometer was charged with a drop of the diluted blood sample and the leukocytes were counted under a light microscope using a tally counter. The figure obtained was multiplied by a factor of 50 to give the total number of leukocyte per microlitre of blood.

Differential leukocyte count (DLC)

This was done using the standard method of Coles, [16]. With the aid of a micropipette, a drop of the blood sample was placed on a clean microscope slide. The end of a second slide was placed against the surface of the first slide at a 30° angle, and drawn back into the drop of blood. This action made the drop of blood to spread along most of the width of the spreader slide (2nd slide). The spreader slide was then pushed forward with a steady even rapid motion to make a thin blood smear (film). The prepared smear was air dried and subsequently stained with Leishman's stain. The stained slides were later observed under the microscope using the oil immersion objective (x 100). The differential leukocyte counter machine was used to count a total of a hundred different leukocyte cells. Each cell type was recorded as a percentage of the total. The different percentages of the cells were converted to absolute number of cells per microlitre of blood using the formula below

$$\frac{Percentage\,number\,of\,cell\,type}{100} \times \frac{TLC}{1}$$

Histopathology

Tissue sections of the spleen from the lead acetate exposed and control rats were fixed in 10% formosaline and dehydrated in ascending grades of ethanol. Thereafter, the samples were cleared in chloroform overnight, infiltrated and embedded in molten paraffin wax. The blocks were later trimmed and sectioned at 5-6 microns. The sections were deparafinized in xylene, taken to water, and subsequently stained with haematoxylin and Eosin (H

and E) for light microscopy [17].

Statistical analysis

Means and standard errors for the total leukocyte count and differential leukocyte counts were calculated for each group and the control. The data were statistically analyzed using ANOVA (MS-Excel 2001: MS USA).

Results

Table 1 shows the comparison of total leukocyte count (TLC) and differential leukocyte counts (DLC) of lead acetate exposed male rats and controls.

Table 1: Comparison of leukocyte profile of lead acetate exposed male rats and controls.

Leukocytic profile ($\times 10^3$ µl)	Rat groups (mean ±SD)			
	Group A	Group B	Group C	Group D
Total leukocyte count (TLC)	15.69±1.09^{b}	12.06±1.32^{a}	16.65±1.11^{b}	12.18±1.55^{a}
Absolute neutrophil count	4.46±0.42^{b}	2.79±0.65^{a}	5.23±0.39^{b}	4.02±0.45^{ab}
Absolute lymphocyte count	10.68±0.66^{b}	8.99±0.83^{ab}	10.13±0.59^{b}	7.820.97^{a}
Absolute monocyte count	0.34±0.10^{b}	0.00±0.00^{c}	0.80±0.14^{a}	0.81±0.09^{a}
Absolute eosinophil count	0.18±0.04^{a}	0.28±0.13^{a}	0.39±0.14^{a}	0.44±0.11^{a}
Absolute basophil count	0.04±0.02^{ab}	0.00±0.00^{a}	0.10±0.01^{b}	0.06±0.04^{ab}

Values within the same row with different superscripts (a, b, and c) are significantly different ($p < 0.05$).

The effect of exposing male rats to increasing dose levels of lead acetate on TLC and DLC is presented in Table 1. Both low (group A) and high (group C) doses of lead acetate significantly ($p < 0.05$) increased the TLC relative to the control. There was no significant ($p > 0.05$) difference between the mean TLC value of the medium (group B) dose lead acetate exposed rats and the control. Mean TLC values in low and medium dose groups did not significantly ($p > 0.05$) differ from each other. The mean absolute neutrophil value for the medium dose lead acetate exposed rats was significantly ($p < 0.05$) low when compared with the mean values for groups A (low dose), C (high dose) and D (control). There was no significant ($p > 0.05$) difference in the mean absolute neutrophil value of group A rats when compared with group C and the control rats. Absolute lymphocyte counts were significantly ($p < 0.05$) increased in groups A (low dose) and C (high dose) rats relative to the recorded low mean values in group B (medium dose) and the control (group D). The recorded mean absolute monocyte counts in groups A and B rats were significantly ($p < 0.05$) reduced relative to the mean values in groups C and D (control) rats. The mean absolute eosinophil counts for the three treatment groups did not differ significantly ($p > 0.05$) from the mean value of the control.

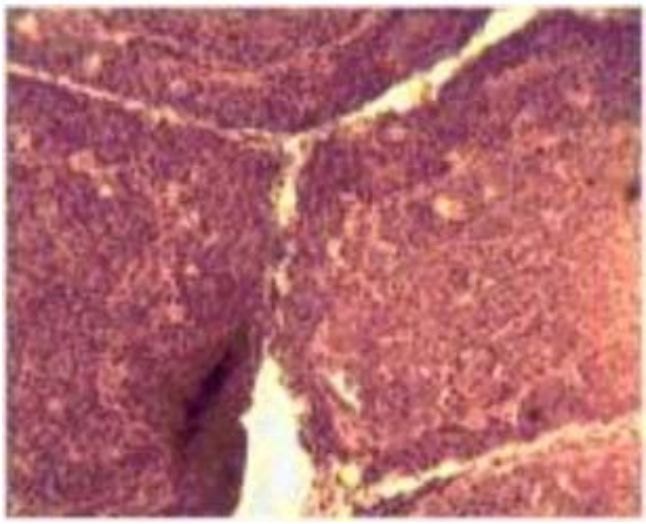

Figure 1: Histological section of spleen of rat treated with 0.25 mg/kg body weight of lead acetate showing perivascular cuffing. Haematoylin and eosin. X200.

Histopathology: Samples of the spleen from the lead acetate treated rats showed basophilic granular degeneration of erythrocytes, necrosis, lymphocytic depletion and perivascular cuffing. These pathological lesions increased in magnitude with increase in the dose of lead salt. The high dose (1.00 mg/kg body weight) showed distinct areas of basophilic granular degeneration of eryth-

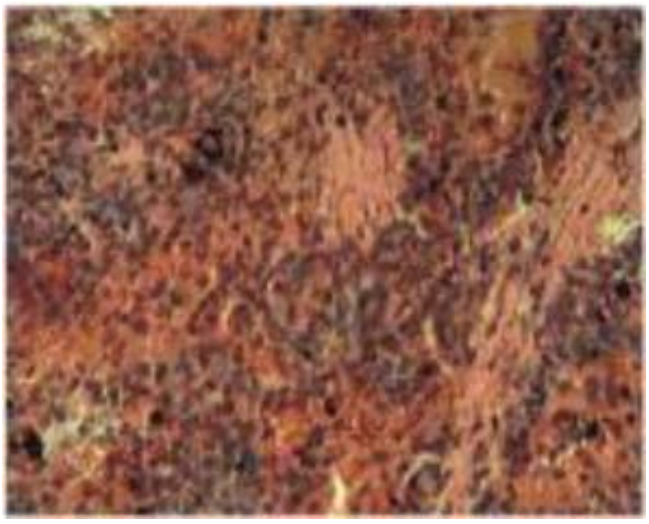

Figure 2: Histological section of spleen of rat treated with 0.5 mg/kg body weight of lead acetate showing basophilic granular degeneration of the erythrocytes and necrosis. Haematoxylin and eosin. X200.

rocytes, necrosis and lymphocytic depletion (Fig. 3). Spleen section of the control show normal sinuses with evenly distributed lymphocytes.

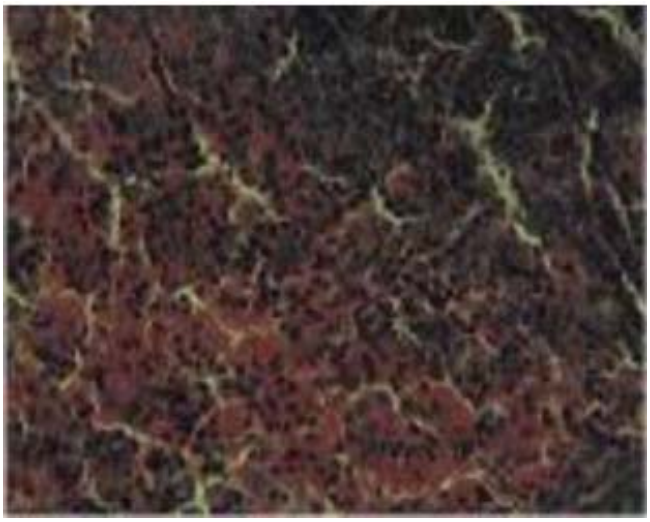

Figure 3. Histological section of spleen of rat treated with 1 mg/kg body weight of lead acetate showing basophilic granular degeneration of the erythrocytes, necrosis and lymphocytic depletion. Haematoxylin and eosin. X200.

Discussion

Lead is an important environmental pollutant exhibiting various forms of toxicity in man and animals [18, 19]. Occupational and environmental chronic lead exposure can damage the central nervous, renal, cardiovascular, reproductive and haematological systems [14]. In this study, lead acetate exposed rats in groups A and C (low and high dose groups) recorded a significant ($p < 0.05$) increase in their mean TLC values relative to the control. The increase in mean TLC values of these lead exposed rats is most likely as a result of stress imposed in the body of these rats following their exposure to lead acetate. The pathophysiology of this observation can be explained from the point of view of proliferative response of the rats' immune cells (immunostimulation). The immune system is synonymous with circulating leucocytes, all of which derive from a single precursor, the pluripotential haemopoietic stem cell [20]. Mature and immature neutrophils, lymphocytes, monocytes, eosinophils and basophils make up the leukocytes (WBC) found in the blood. Leukocytosis is a usual body response to an underlying pathophsiological condition [21]. The leukocytosis observed in this study possibly points to proliferative response by the immune cells due to the presence of the lead salt in the blood stream of the lead acetate treated rats.

The differential leukocyte count of the lead acetate exposed rats showed that the lymphocytes predominated in the observed leukocytosis as evidenced from the increase in the number of absolute lymphocyte counts in treatment groups A and C rats. In other words, the leukocytosis observed in the lead acetate exposed rats was more lymphocytic which suggests immune alterations. The histomorphologic changes in the spleen sections (Figs. 1, 2 and 3) of rats exposed to lead acetate tend to suggest that exposure of rats to lead acetate; especially at the highest dose level (1.00 mg/kg body weight) used in this study stimulated the spleen to become reactive. The impact on human and animal health from an immune perspective, following environmental lead exposure is of great health implication.

The increase in absolute lymphocyte number is usually a natural reaction of the body to stress, especially involving antigens. The lymphocytes being antibody producers defend the body against stress or debilitation imposed by foreign agents (antigens) [22]. In conditions of stress, the glucocorticoid level rises [23, 24]. If the effect persists, a depression of the lymphoid organs and tissues with subsequent fall in the production of neutrophil occurs. In this study, the absolute neutrophil count value for rats in group B was found to be significantly ($p < 0.05$) low, when compared with the values for rats in groups A, C and D. The absolute monocyte count values for groups A and B rats were significantly ($p < 0.05$) reduced relative to the recorded values for group C and the control group D rats. This difference in absolute monocyte reading for the three treatment groups could be attributed to some experimental errors during the counting process. There were no observable differences in the absolute basophil and eosinophil count values of rats in treatment groups A, B and C relative to the value of the control group D rats. Functionally, eosinophils modulate allergic inflammatory reactions and destroy antigen-antibody complexes.

This study has established that exposing male rats to graded levels of lead acetate can lead to immune alterations and splenic damage. Further study is being advocated to enable us understand the pathophysiology of these two major findings of this study.

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