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A Comparative Study of the Effect of Lead Nitrate and Sodium Arsenite on Rat Blood Cells

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ABSTRACT: *During the present study, we have studied the Lead (Pb) and Arsenic (As) effects on the hematopoietic system of rat. Rats were divided into seven groups, each of which having four members. The first group was considered as the control and the ulterior six groups were given different concentrations of lead (0.75, 1.5 and 3 mg/L) and arsenic (50, 100 and 200 mg/L) via drinking water. At the end of two months of treatment, the blood-sampling was done and laboratory tests were conducted on the blood cells. Results showed that blood cells in lead and arsenic-treated rats, especially at high concentration, demonstrated significant changes in comparison to the control group. According to the results, both lead and arsenic are able to decrease the generation of red blood cells and debilitate the immune system as well. The reasons of these changes may be related to the effects of these elements on ancestral cells. Lead and arsenic can also affect the life and activity of blood cells.*

Keywords*: Lead, arsenic, blood cells, rat*

1. INTRODUCTION

As naturally occurring elements, lead and arsenic are ubiquitous environmental contaminant and belong to the group of the most toxic heavy metals in the atmosphere. Because of the stability of these elements in nutritional chain, their poisoning is long and fatal. Pollution of these elements is a serious environmental problem [13, 15]. Heavy metal stability of environment causes a gradual accumulation of pollutants in the plants and animal-bodies, which also affects the humans adversely (1, 2). Inorganic arsenic and the consequence of geogenic origin exist in underground water. In several area of world, such as; Bangladesh and Taiwan, the people use these waters for drinking purpose (6). Also, the mortality of dolphins in the Gulf of Mexico and the metabolic activation of mutagenic and potentially carcinogenic substances from south Atlantic Spanish littoral fishes are related to the aquatic level of lead pollution (17). Apart from the natural sources, these elements are used in medicine and pharmacy for the treatment of some diseases [13, 20]. Lead and arsenic are chiefly absorbed through the digestive and respiratory tracts.A little arsenic is also absorbed through the skin [14, 18]. The natural excretion of these elements is usually through the urinary and digestive system. There are some reports regarding the existence of other ways of excretion such as; the sweat and hair effusion [11, 17].

Lead and arsenic poisoning is responsible for a variety of pathological states both in children and adults. These metals cause damage to different tissues such as the immune, nervous, excretory, cardiovascular and reproductive systems [1, 8]. Hematopoietic system is the most sensitive of all to the lead and arsenic poisoning [5, 19]. Poisonous effects of these elements tothe bone marrow cause decreased red and white blood-cell production, which in turn creates the anemia and debilitated immune system [3, 20]. The mechanisms responsible for the influence of As and Pb on the hematopoietic system have not been fully elucidated but it was found that the arsenic substitutes phosphorous in some biochemical reactions [8]. Therefore, after the influence of arsenic on red blood cells, the amount of ATP decreases and the signs of metal poisoning appear [19]. Lead and arsenic can also decrease the quantity of iron in the body through different ways [13]. Hence,

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such poisoning increases the quantity of microcytes in the body [10 and 19]. Inspite of the existence of many warnings about lead and arsenic toxicity, these elements are used in medicine and pharmacy, and even added to industrial merchandise.

Because of the superabundant sensitivity of hematopoietic system to heavy metal poisoning, the present study was designed to evaluate the effects of Pb and As exposure to the hematopoietic system.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemical, used in this study, including lead nitrate and sodium arsenite were purchased by Merck (Germany).

2.2. Animals

Twenty eight adult male Wister rats were randomly selected and transferred to polyethylene animal house, under standard conditions, at a temperature of 22-25 and 30-40% humidity. Rat feed included 20% protein, 50% starch, 10% cellulose, 15% fat and some vitamin-containing additives. Two weeks after adaptation to the new environment, these animals were divided into seven groups, each of which containing four members. First group was considered as the control group and the other six groups were given different concentrations of Pb (0.7, 1.5 and 3 mg/lPb) and arsenic (50, 100 and 200 mg/l) alongwith the drinking water for two months. It is notable that all of the rats, within different gropus, were fed with the same food but the quantity of Pb and As was different among their drinking water, except for the control group, which was not exposed to the experimental metals-containing water (Table 1).

Sodium arsenite and lead nitrate are concoctions of lead and arsenic that are more water-soluble and cause the swinging pollution- of drinking water. Therefore, we have used these compounds during our work.

2.3. Preparation of Lead and Arsenic Solution

For making lead and arsenic mother solutions, 40g Pb and 4g As were separately dissolved in 1L water. Then, by the utilization of these solutions, the disposability concentration of each group was provided (Table 2). Drinking water was replaced after every 24 hours and the experimental mice were weighed to record any changes in their weights.

Table 1
Experiment Design of the Control and Experimental Groups, Administered with Different Concentrations of Pb and As.

Experimental term		*Treatment groups*					
	control	*1*	*2*	*3*	*4*	*5*	*6*
Two months	control	0.75 g/1 Pb	1.5 g/1 Pb	3 g/1 Pb	50 mg/ 1As	100 mg/ 1As	200 mg/ 1As

Table 2
Preparation of Drinking Water for Control and Treatment Groups

	disposability deal of mother solutions for preparation of needful concentration of each group in term of treatment
Treatment groups	
1	19 ml of mother liquor with a final volume of 1 liter.
2	37.5 ml of mother liquor with a final volume of 1 liter.
3	75 ml of mother liquors with a final volume of 1 liter.
4	12.5 ml of mother liquors with a final volume of 1 liter.
5	25 ml of mother liquors with a final volume of 1 liter.
6	59 ml of mother liquors with a final volume of 1 liter.
Control	Water only.

2.4. Statistical Analyses

All of the statistical analyses were performed by using SAS (Statistical Analysis Software 6.09, Cary, NC). The data were subjected to One Way Analysis of Variance (ANOVA). The data groups, showing a significant treatment effects ($p < 0.05$), were further analyzed via a multiple comparison procedure (Tukey's HSD test). The statistical comparisons of the regional metal-content were performed by the means of Student's *t*-test. Differences among the groups were considered to be significant at the *p*-values < 0.05. The results were expressed as means ± SD.

2.5. Blood factor Analysis

Blood sampling was done after the growth phase two months and a cell counter was used to analyze hematological indices including hematocrit, hemoglobin level, mean corpuscular volume (MCV), red blood cell (RBC), white blood cell (WBC) counts and *et*. Blood smears were also prepared from the blood specimens for manual differential cell count.

3. RESULTS

In Vivo Influence of Lead and Arsenic Treatment on the Hematological Indices

Results are expressed as means ± SD.

*: Significantly different from control, $p < 0.05$.

Disposability concentrations of each group in treatment term are according to table 1.

Comparative results have shown that As in comparison with Pb, caused extreme decrease in the number of lymphocyte and eosinophil means. With respect to other blood cells, these elements showed similar behavior (it is notable that in this work, disposability concentrations of Lead versus Arsenic were the highest). When body contains slight lead and arsenic poisoning, it induces the generation of redress of goner with blood cells. Furthermore, lymph node inflammation succors the increase of blood cells. The advancement of heavy metal poisoning remarkably decreases the number of blood cells, which is partly related to the lymph gland atrophy. However, both Pb and As are able to develop significant decrease in the number of blood cells (in high concentration).

Enumeration of blood cells and the investigation of morphological changes was done, for the collected blood sample, to evaluate the influence of lead nitrate and sodium arsenite on the blood cells. According to Fig. 1 no significant differences, in average of white blood cells, were found between the control and the treatment groups with lead and Arsenic (p>0.05). At a high concentration of sodium arsenite (200mg/l), a significant decrease in the means of experimental groups' lymphocytes (6020/ µl to 4010/ µl) was seen as compared to the control group where p = 0.045, (Fig. 2). The existence of significant differences was also seen among all of the sodium arsenite-treated experimental groups, numbered 5, 6 and 7 (p = 0.0014). In contrast, Pb (even at a very high concentration of 3 g/l) did not create a significant level of decrease in the above stated parameter as compared to the control group (Fig. 2 and p>0.05). As shown in the Fig. 4, no significant changes were

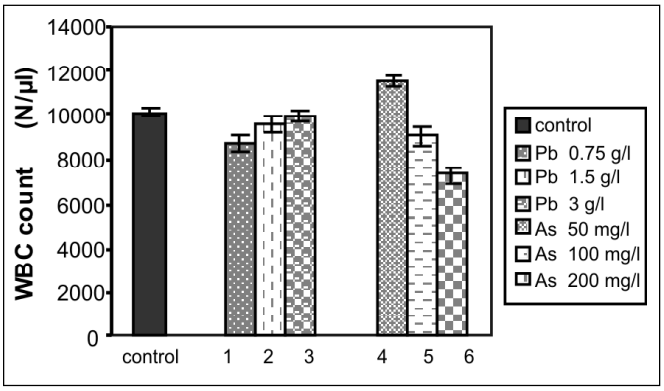

Figure 1: WBC Count in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

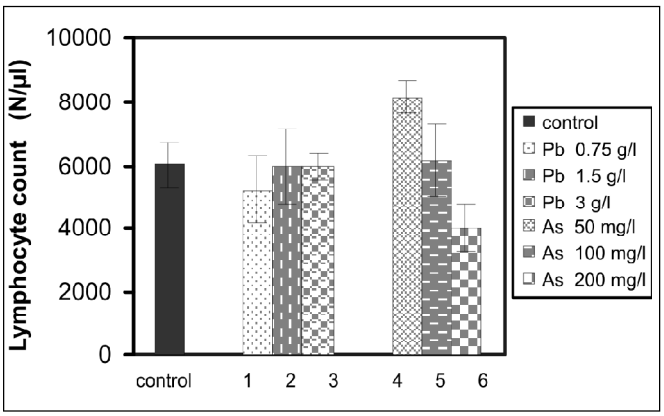

Figure 2: Lymphocyte Count in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

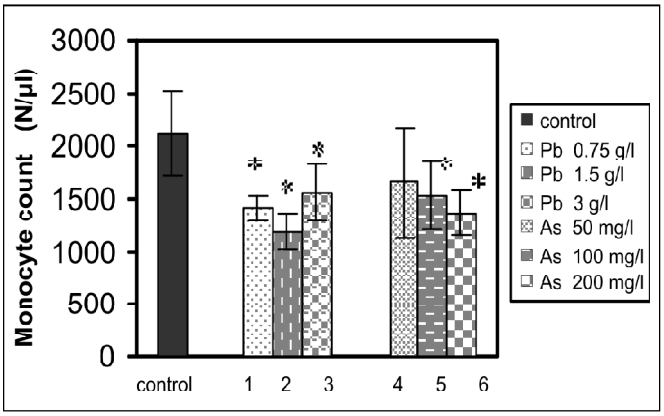

Figure 3: Monocyte Count in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

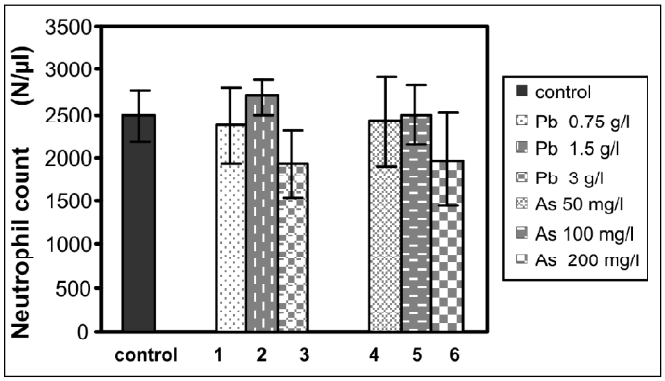

Figure 4: Neutrophil Count in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

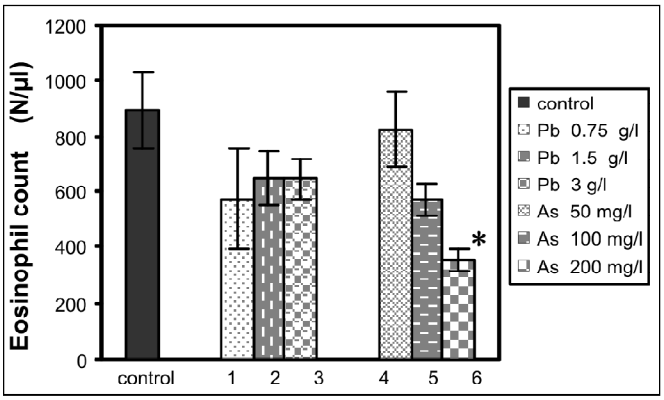

Figure 6: Eosinophil Count in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

observed in the average of neutrophil number among all of the experimental groups when compared to the control with no metal-addition (p>0.05). Following the treatment with lead and arsenic, a decrease in the means of monocyte number was distinctly observed between the control and all of experimental groups, which was statistically significant among all of the groups treated with lead and even at higher concentrations used these metals (Fig. 3 and p = 0.001). As shown in the Figures 5 and 6, a significant decrease in the means of basophil and eosinophil numbers was observed as compared to the control group (p = 0.028), when administered with higher concentration of arsenic. On the contrary, these blood cells count means were not found to be significant over those of the control groups after lead administration (p >0.05). Arsenic caused a significant level of decrease in thehemoglobin amount and the percent averages of hematocrit when compared with the control group, which was wholly significant at highest concentration of As (200 mg/l, HGP = 0.045, HCTP = 0.033). Pb showed similar effect, when administered at highest concentration (3 g/l, Figures 8 and 9). Pb and As-treated groups had disorderly changes in the means of red blood cells, which were non-significant in comparison with the control group (Fig. 7, p>0.05). During our experiment, the highest concentrations of Pb and As (3 g/l Pb and 200 mg/lAs) showed a significant decrease in the MCV, which was the next concordant hematological change in the experimental groups. This status signifies the increase of microcyte counts (Fig. 10, p = 0.048).

4. DISCUSSION

Lead and arsenic are considered as the toxic air pollutants and environmental contaminants. It has been reported that the exposure to Pb and As leads to a variety of toxic effects on the cells and tissues

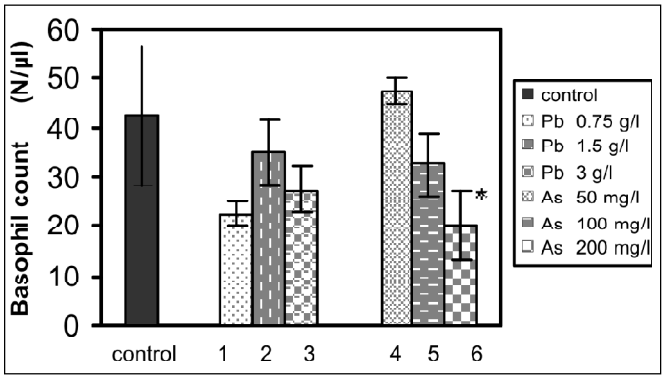

Figure 5: Basophil Count in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

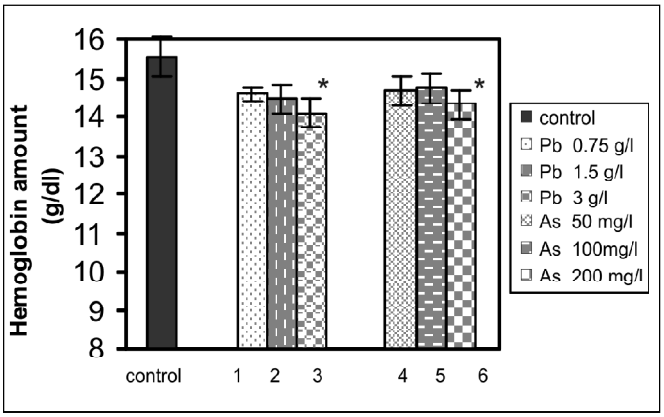

Figure 7: Amount of Hemoglobin in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

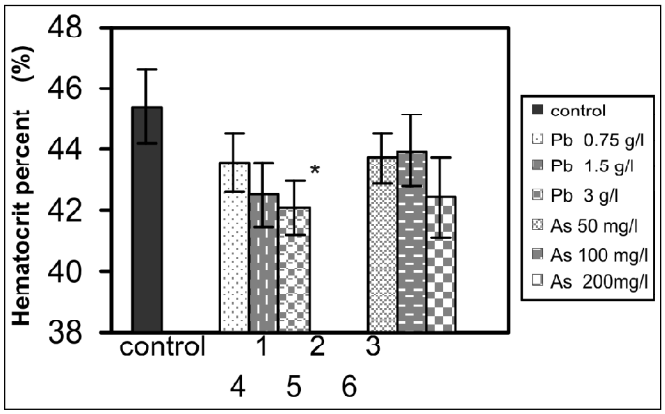

Figure 8: Hematocrit Percentage in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

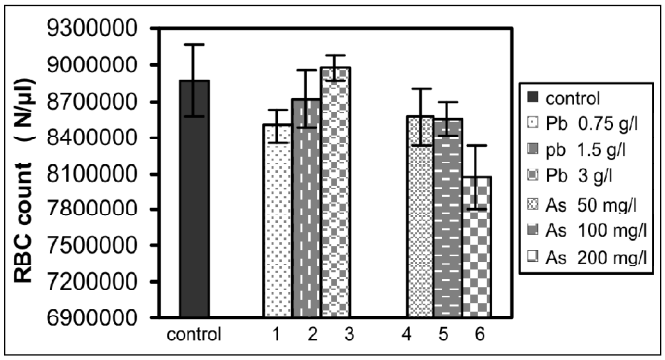

Figure 9: RBC Count in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

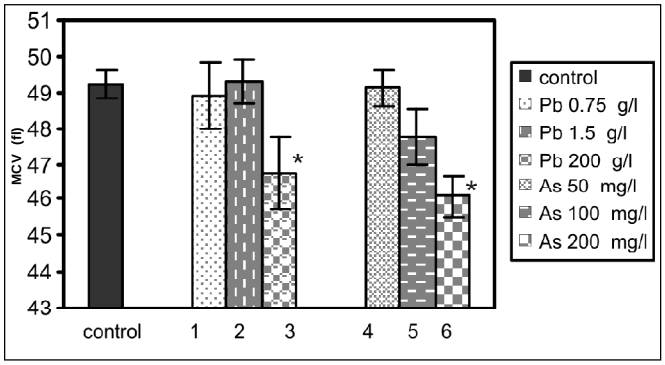

Figure 10: MCV in Control and 6 Experimental Groups when Administered with Different Concentrations of Pb and As

(1, 8). The hematopoietic system is one of the main targets of Pb and As accumulation. The effects of these elements on hematopoietic system have received little attention. The results of our study demonstrate that various doses of As are able to decrease white blood cell (WBC) count, which was recorded to be significantat high concentration (200 mg/l, $p<0.05$). These data are in agreement with the report of Steven *et al.*, (2001) (16). While, the experiments with Pb administration showed that still higher dose/concentration was required to weaken the immune system, as compared to the As-treated groups. Nevertheless, it has been reported that Pb is able to promote the apoptosis in other cells such as the Cerebellar neurons and such phenomenon occurs at low Pb concentration (1µm) (4). Comparative results have shown that in contrast with Pb, the As caused an extreme decrease in the means of lymphocyt and eosinophil numbers. It is notable from our experiment that Pb showed higher disposability concentrations as compared to the As, which shows that these elements had similar behaviors in respect to the other blood cells. As exerts deleterious effects on the activity of B- and helpful-T-lymphocytes within the immune system. It also causes decreased generation of antibodies and some essential supplements for humoral immune system (7). At lower doses of As, lymph node inflammation is increased and concurrently, the transference of G1→S stage of cell division is also increased in lymphocytes (20). At the extreme levels of As-poisoning, the activity of thymus gland, which participates in the immunological evolution of T-cells, is decreased with anincrease of immune cell necrosis. (15). Similarly, Pb reduces the amount of active blood cells by suppressing the cell's activity (3), mitotic repercussion (17), participating in the oxidation-reduction reaction and finally, by reducing antioxidant level within the cell (3 and 21). When the body suffers from slight Pb and As poisoning, it induces the generation of redress of goner with blood cells. Furthermore, lymph node inflammation helps in increasing the blood cells (12, 20). With the advancement of poisoning, remarkable decrease in the blood cell number was observed, which partly rletes to the lymph gland atrophy (15). As showed a higher induction of blood cell necrosis as compared to Pb, which is related to its being more toxic as compared to the later one. These findings are in agreement with the use of As in the treatment of acute promyelocytic leukemia (6). The results of our study show that the means of RBCs number and MCV evaluation had demonstrated an increased amount of microcytes. Furthermore, a concentration-dependent reduction, obtained for the amount of hemoglobin and hematocrit percentage during all experimental

groups was statistically significant only at higher doses (p<0.05). These data are in agreement with the reports of Jacob *et al.*; (2000) and Noori *et al.*; (2003) (5, 10). Several experimental studies have reported that Pb interferes with the biosynthetic pathway of heme at various steps, mainly through disturbing the activity of three major enzymes. It also affects the formation and function of RBCs (5, 21). Moreover, Pb interferes the iron utilization, for heme synthesis within the mitochondria, and incorporation into theRBCs (5, 10, 13). However, the precise mechanism of action, with which Pb interacts with the cellular metabolism, remains unknown.

Our result for RBCs number, hematocrit, hemoglobin and MCV were similar between both the Pb and As-treated groups. Nevertheless, because of the more toxic nature of As , the changes in above mentioned parameters began at lower concentration when compared with the Pb. Alike Pb, As affects the cellular metabolism by altering the enzyme activity (20). Interaction of As with heme biosynthesis has been related to the inhibition of cytoplasmic and mitochondrial enzymes. A decreased activity of the main biosynthetic enzymes is reported to be due to the defects in iron metabolism (8, 19). Furthermore, As can replece itself with phosphorus during some biochemical reaction (16). Hence, following the poisonous influence of As on the RBCs, the reduction in ATP amount was observed (19).

5. CONCLUSION

Our results have proven that both Pb and As developed significant decrease in the number of blood cells, (when administered at high concentrations). Designation and provision of the health programs to limit the causal exposure to these toxic elements is highly important for our health.

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