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Alterations in some vital tissues of male Wistar rats exposed individually or simultaneously to lead acetate and sodium arsenite

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ABSTRACT

Lead and arsenic are highly ranked environmental pollutants. In this study, we assessed the effect of lead acetate and sodium arsenite individually or simultaneously administered to male Wistar rats. Twenty male Wistar rats (120-150 g) grouped equally into four and exposed orally and once daily to distilled water (1 ml/kg body weight (b.wt.)), lead acetate (PB) (60 mg/kg b.wt.), sodium arsenite (ARs) (2.5 mg/kg b.wt.) and PB and ARs (60 mg/kg and 2.5mg/kg b.wt. respectively). Experiment lasted 14 days. Relative organs weight, white blood cells and differentials counts, and histological analysis of crucial organs were assessed. Result showed that there was significant reduction ($p<0.05$) in the relative weight of spleen in the co-exposed group as compared to the control. There was significant elevation ($p<0.05$) in the levels of monocytes and neutrophils in the PB and ARs groups comparatively to the control. The increase in total white blood cell in all the groups was not significant as compared to the control. The combined (PB and ARs) group showed significant reduction ($p<0.05$) in platelets count as compared to control. While, no noticeable histological change was observed in the control group, there were alterations in the histology of the lungs, brain, heart, testes and spleen of animals treated with lead and arsenite both individually and simultaneously. In conclusion, PB and ARs were toxic to the tissues assessed. However, the animals simultaneously exposed to both toxicants did not produced significant greater degree of toxicity.

Key words: Blood cells, histology, lead acetate, sodium arsenite, toxicity.

INTRODUCTION

Lead and arsenic have been highly ranked as first and second heavy metal environmental toxicants respectively [1]. These metals are abundantly and ubiquitously present in the environment, hence, the indiscriminate exposure of man and animals to them through drinking water, soil, air and food [2]. Conversely, lead compounds have beneficial properties of malleability, ductility, poor conductibility and resistance to corrosion that have made it useful in the industry while arsenic have also been reported to be useful as components of pesticides, insecticides [3, 4]

However, despite these beneficial properties, both lead and arsenic are posing treat to the environment [5]. It was documented that arsenic altered both haematological and biochemical indices including induction of oxidative stress in a chicken model of arsenic intoxication and has chromosomal-breakage effect [6, 7, 8, 9, 10]. These heavy metals have been reported to cause toxicities individually to vital organs of the body like the liver, kidney, testes [11, 12, 13]. There was a report of disruption in tissue architecture of the liver and kidney of Wistar rats exposed to lead and arsenite [14]. There are also additional findings suggesting toxicities caused by either individual or co-exposure to lead and arsenic [15, 16, 17 18, 19].

Meanwhile, Kim *et al* [20] suggested measurable indices that can be used in the assessment of the changes associated with these organs. Most reports have been in monitoring biomarkers of haemotoxicity, with paucity of information on the assessment of the histology of most vital organs of the body. However, in the present study, we assessed the relevant blood indices and histology of some vital organs of male Wistar rats exposed individually or simultaneously to lead acetate and sodium arsenite.

MATERIALS AND METHODS

Chemicals and reagents

Sodium arsenite (Na_2AsO_2, CAS No: 7784-46-5, Sigma Aldrich) and lead (II) acetate trihydrate ($C_4H_6C_4Pb^*$ $3H_2O$, CAS No: 6080-56-4, Sigma Aldrich) were dissolved in distilled water and administered to the experimental animals at 2.5 mg/kg b.wt. [21] and 60 mg/kg b.wt. [22] corresponding to $1/10^{th}$ of their LD_{50} respectively. All other chemicals and reagents used were of analytical grade and freshly prepared.

Experimental animals

Twenty (20) adult male Wistar rats (120-150 g) were purchased from the animal house of the Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria and housed at the central animal house of Osun State University, Osogbo. The study was approved by the Ethics Committee of the College of Health Sciences, Osun State University and the animals were handled in consonant with the guidelines on humane handling of animal according to US NIH) [23] as adopted in Osun State University. The rats were fed with standard commercial pellets and given water *ad libitum* while they were kept under normal photoperiod of 12 hrs light/12 hrs dark throughout the duration of the experiment.

Experimental design and Sample collection

Animals were assigned equally into four experimental groups (n =5) and acclimatized for one week. The negative control group was administered distilled water orally at 1 ml/kg b.wt daily for 14 days. Lead acetate (PB) group received 1 ml/kg b.wt of distilled water containing 60 mg/kg b.wt. lead acetate and sodium arsenite (ARs) group was also given 1 ml/kg b.wt of distilled water containing 2.5 mg/kg b.wt. However, the co-exposed group was administered 1 ml/kg b.wt of distilled water containing 60 mg/kg b.wt. PB and 2.5 mg/kg b.wt ARs. The doses of sodium arsenite and lead acetate were chosen according to the reports of [21, 22] respectively. The doses correspond to 1/10th of the LD50 reported in these respective studies. All treatments were given orally once daily for 14 days. However, twenty-four hours after the last treatment, blood sample was collected from the retro-orbital sinus and all the rats were sacrificed by cervical dislocation under anaesthetic influence of diethyl ether while the organs of interest were then excised.

Haematology

Twenty-four hours after the last treatment, blood samples were collected with lithium heparinized capillary tube and used for the analysis of total white blood cell counts and its differentials: neutrophils, monocytes, eosinophils, lymphocytes and platelets, as described by [24].

Histological Examination

The tissue sections of the lungs, brain, heart, spleen and testes were excised from the animals after sacrifice by cervical dislocation and stored in 10% formaldehyde solution except the testes

that was stored in bouin solution. These tissues were then embedded in paraffin. 5mm thick paraffin sections were collected with the use of rotary microtome followed by staining with Haematoxylin and Eosin (H&E) dyes. The slides were examined and photographed under light microscope.

STATISTICS

The data were expressed as mean ± standard error of mean (SEM) after analysis by one-way analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) software, Standard version 17.0 (SPSS Inc., Chicago). Differences between mean values of different groups were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Relative organ weights

The relative organ weights of the animals were measured and analysed. It was observed that there was no significant difference ($p>0.05$) among the mean values of the relative organ weights for all organs assessed when compared with the control, except for the mean value for spleen in the co-exposed group D that was significantly reduced ($p<0.05$) as compared to the control (Table 1)

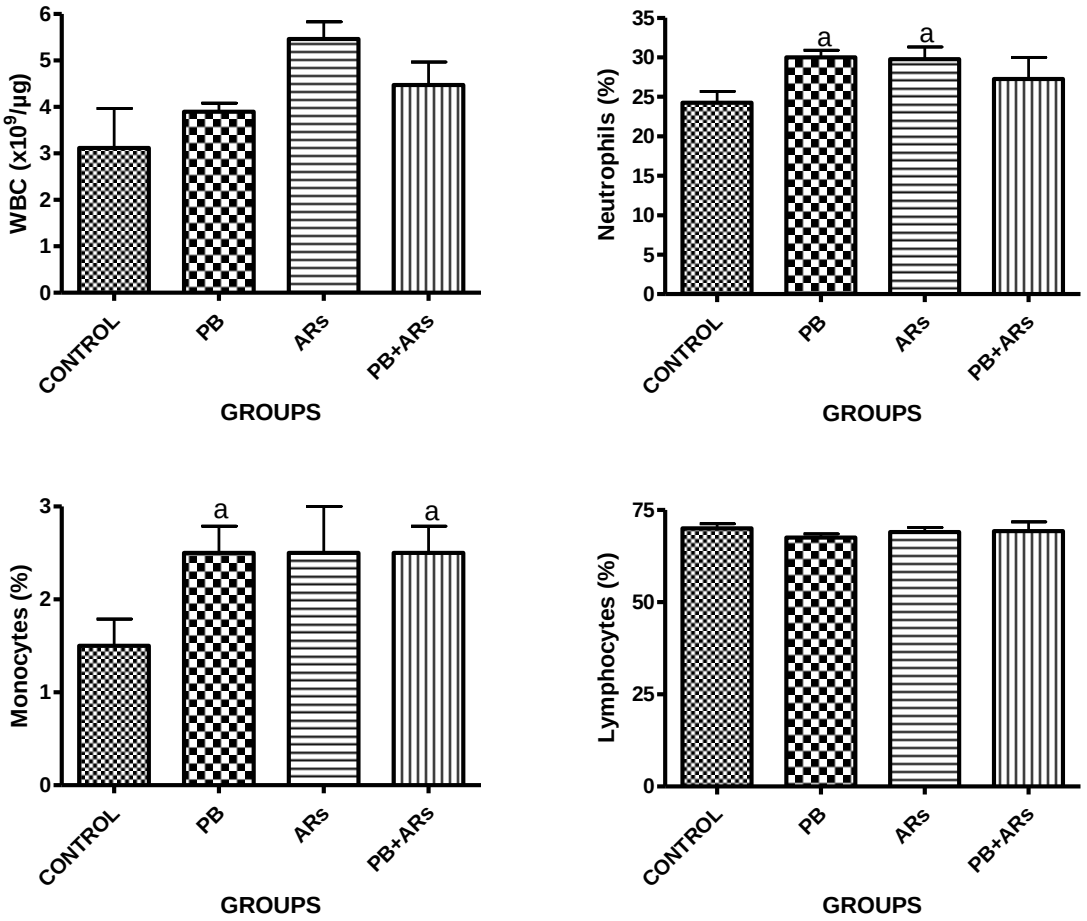
Table 1: Relative organ weights of test animal

Parameters (%)	Group A	Group B	Group C	Group D
Relative lung weight	0.561 ± 0.040	0.650 ± 0.081	0.640 ± 0.102	0.687 ± 0.050
Relative heart weight	0.303 ± 0.033	0.250 ± 0.046	0.273 ± 0.015	0.333 ± 0.061
Relative testes weight	1.040 ± 0.226	1.213 ± 0.327	1.090 ± 0.240	0.557 ± 0.275
Relative brain weight	0.360 ± 0.066	0.290 ± 0.023	0.307 ± 0.019	0.243 ± 0.052
Relative spleen weight	2.037 ± 0.321*	1.230 ± 0.109	1.280 ± 0.065	1.107 ± 0.054*

Values are mean ± SEM. Means with the same superscript* within rows are significantly different ($p<0.05$) from control. Group A (control) received distilled water at 1 ml/kg body weight (b.wt.), Group B; lead acetate (PB) 60 mg/kg b.wt., Group C; sodium arsenite (ARs) 2.5 mg/kg (b.wt.) and Group D; PB and ARs at 60 mg/kg and 2.5 mg/kg (b.wt.) respectively. All administrations were given orally once daily for 14 days. n=5.

Leucocytes and thrombocyte levels

The total white blood cells count and its differentials were monitored and analysed in the present study (Figure 1). Although the mean values for the total white blood cell counts were increased in all the groups as compared to the control, these values were not significant ($p>0.05$). However, there was a significant elevation ($p<0.05$) in the neutrophils values of lead and arsenite treated groups but not in the co-exposed group when compared to the control (Figure 1). The monocytes mean values in the treated groups follow similar pattern (Figure 1). The mean values for eosinophils, and lymphocytes were not significant ($p>0.05$) when compared to the control in all the treated groups patterns (Figure 1). It was discovered that, the group co-exposed to lead and arsenite showed significant reduction ($p<0.05$) in the values for platelets level when compared to the control.



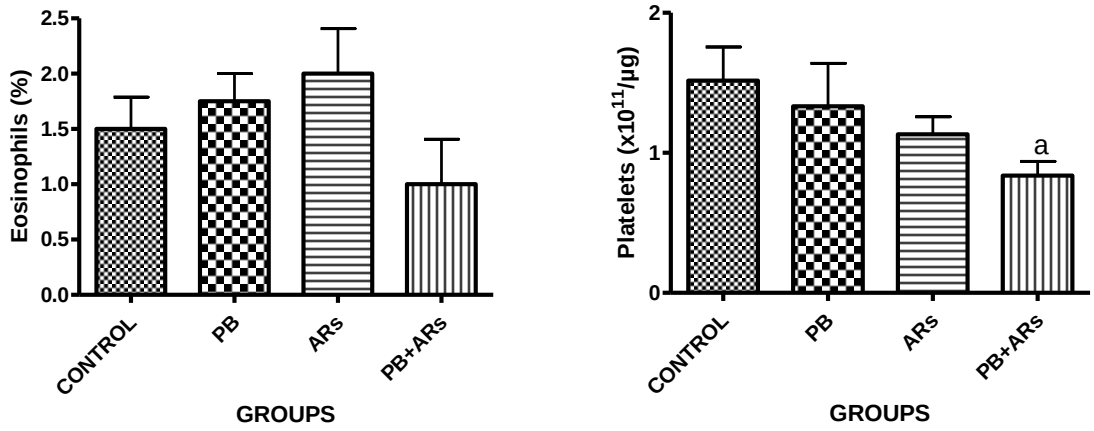


Figure 1: Lead acetate and sodium arsenite increased the levels of white blood cells and its differentials, and decreased the level of platelets in treated rats.

Group A (control) received distilled water at 1 ml/kg body weight (b.wt.), Group B; lead acetate (PB) 60 mg/kg b.wt., Group C; sodium arsenite (ARs) 2.5 mg/kg (b.wt.) and Group D; PB and ARs at 60 mg/kg and 2.5mg/kg (b.wt.) respectively. All administrations were given orally once daily for 14 days. [a] significantly different ($p<0.05$) from control. n=5.

Histological changes in tissues sections of rats exposed to lead and arsenite

The testes were observed for possible histological derangement (Figure 2). However, there was no observed lesion in the control group. The testes of animals in Group B shows a mild scanty sperm precursor (spematogonia) cells (arrowed) while animals in Groups C and D showed erosion of the seminiferous tubules and arrested development of the primary and secondary spermatogonia evidenced by increased number of immature cells (large cytoplasm to nuclear ration) to maturing sperm cells (red arrows) respectively (Figure 2).

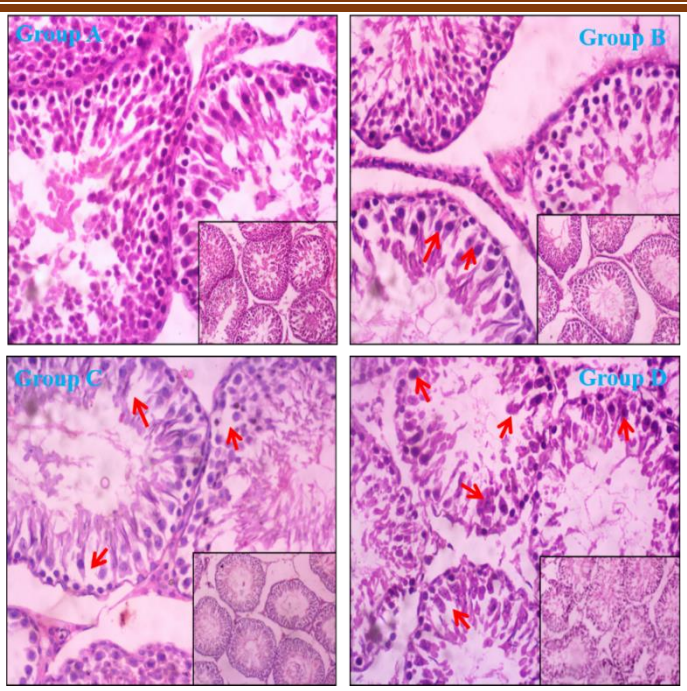


Figure 2. Representative photomicrographs of testicular section of experimental rats viewed under light microscope: Group A: 1 ml/kg b.wt distilled water (control), showing no visible lesion; Group B: 60 mg/kg b.wt lead acetate, showing mild scanty sperm precursor (spematogonia) cells (arrow); Group C: 2.5 mg/kg b.wt sodium arsenite, showing erosion of the seminiferous tubules; Group D: 60 mg/kg b.wt lead acetate + 2.5 mg/kg b.wt sodium arsenite, showing arrested development of the primary and secondary spermatogonia evidenced by increased number of immature cells (large cytoplasm to nuclear ration) to maturing sperm cells (red arrows). H&E stain at x100 (embedded) and x400 Magnification.

Similarly, the cerebral cortex of the brain was observed and there was no visible lesion seen in the control Group A (Figure 3). However, animals in Groups B and D shows histopathological lesions in form of neuronal degeneration evidenced by nuclear pyknosis, karyorrhexis and necrosis evidenced by karyolysis; and focal areas of neuronal degeneration evidenced by karyolysis and increased eosinophilia respectively (Figure 3). Moreover, the lesion in Group C was mild multifocal areas of neuronal degeneration and necrosis with pyknotic nucleus (Figure 3).

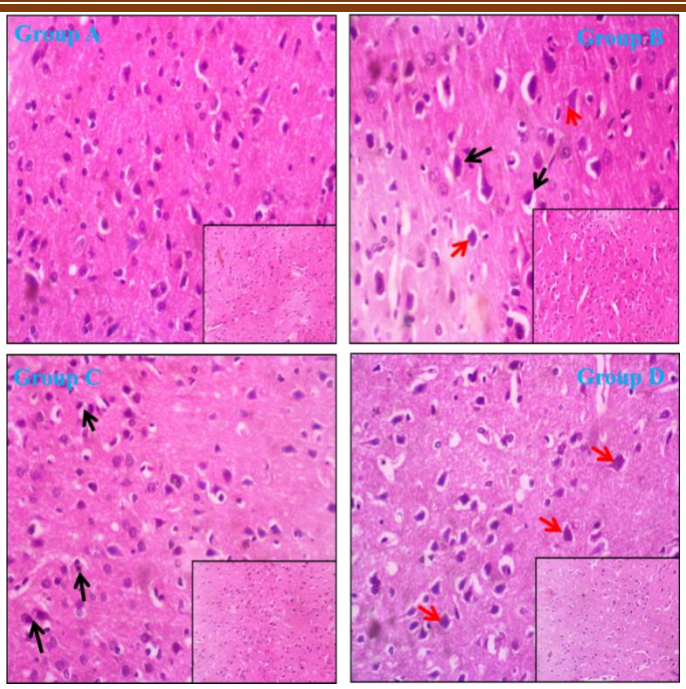


Figure 3. Representative photomicrographs of cerebral cortex of experimental rats viewed under light microscope: Group A: 1 ml/kg b.wt distilled water (control), showing no visible lesion; Group B: 60 mg/kg b.wt lead acetate, showing neuronal degeneration evidenced by nuclear pyknosis, karyorrhexis (black arrows) and necrosis evidenced by karyolysis (red arrows); Group C: 2.5 mg/kg b.wt sodium arsenite, showing multifocal areas of neuronal degeneration and necrosis (black arrows) with pyknotic nucleus; Group D: 60 mg/kg b.wt lead acetate + 2.5 mg/kg b.wt sodium arsenite, showing focal areas of neuronal degeneration evidenced by karyolysis and increased eosinophilia.(Red arrows). H&E stain at x100 (embedded) and x400 Magnification.

Histological analysis of the lungs showed no noticeable lesion in the tissue sections of animals treated with distilled water in Group A. Those treated with lead acetate and sodium arsenite shows derangement in their histology (Figure 4) in Group B, sodium arsenite caused severe interstitial congestion with infiltration of inflammatory cell; neutrophils. The histology of animals in Groups C and D show severe interstitial congestion and haemorrhage with fibrin exudation and severe lymphocytic infiltration with a few plasma cells and macrophages; and moderate interstitial congestion with infiltration of polymorphonuclear (neutrophils) and mononuclear (macrophages, lymphocytes) cells respectively (Figure 4).

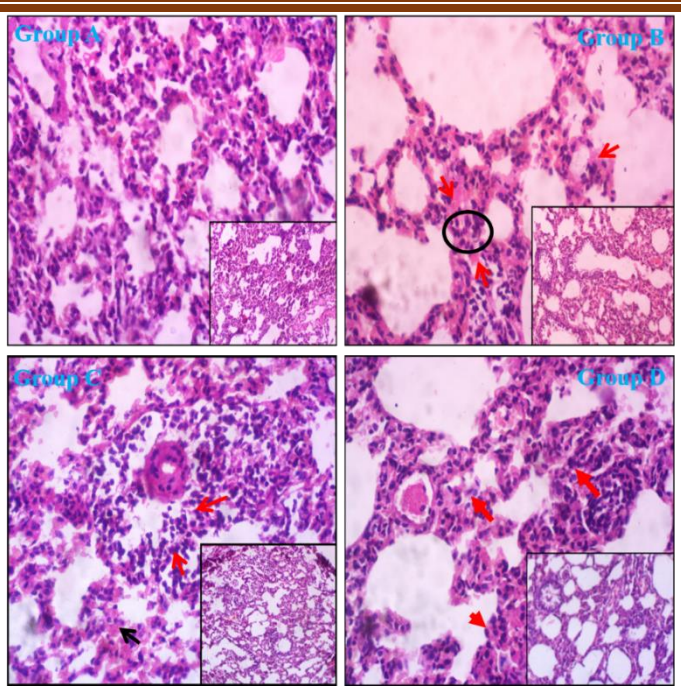


Figure 4. Representative photomicrographs of lung section of experimental rats viewed under light microscope. Group A: 1 ml/kg b.wt distilled water (control), showing mild interstitial congestion; Group B: 60 mg/kg b.wt lead acetate, showing severe interstitial congestion (red arrow) with infiltration of inflammatory cell; neutrophils (circle); Group C: 2.5 mg/kg b.wt sodium arsenite, showing severe interstitial congestion and haemorrhage with fibrin exudation (black arrows) alongside severe lymphocytic infiltration with a few plasma cells and macrophages (red arrows); Group D: 60 mg/kg b.wt lead acetate + 2.5 mg/kg b.wt sodium arsenite, showing moderate interstitial congestion with infiltration of polymorphonuclear (neutrophils-red arrows) and mononuclear (macrophages, lymphocytes) cells. H&E stain at x100 (embedded) and x400 Magnification.

The histology of the heart showed locally extensive area of myocardial degeneration and necrosis evidenced by loss of striations and macrophages in Group B (Figure 5) and Group C showing a focal area of myocardial degeneration with few macrophages. Group D histology showed multifocal areas of degeneration and necrosis as observed with evidence of loss of striations. i.e. Zenker's necrosis. The control group shows no noticeable lesion (Figure 5).

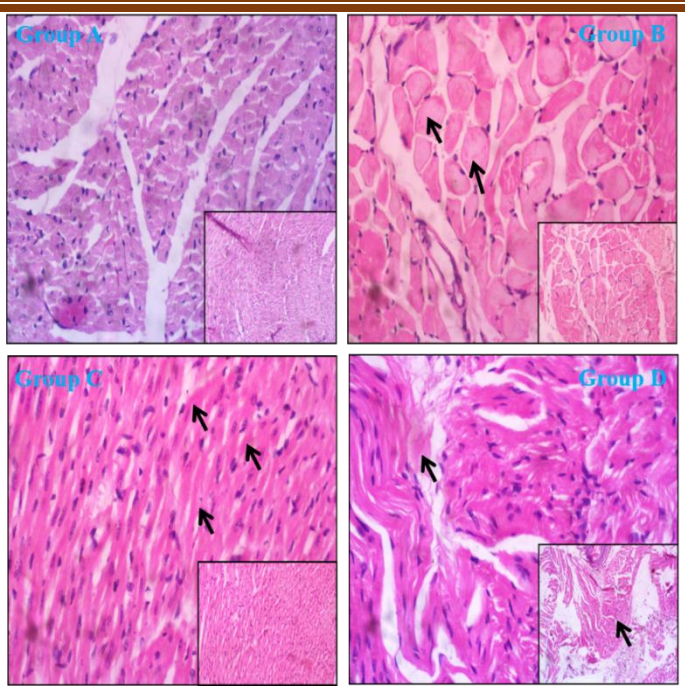


Figure 5. Representative photomicrographs of heart section of experimental rats viewed under light microscope: Group A: 1 ml/kg b.wt distilled water (control), showing no visible lesion; Group B: 60 mg/kg b.wt lead acetate, showing locally extensive area of myocardial degeneration and necrosis evidenced by loss of striations and macrophages (arrows); Group C: 2.5 mg/kg b.wt sodium arsenite, showing a focal area of myocardial degeneration with few macrophages (arrows); Group D: 60 mg/kg b.wt lead acetate + 2.5 mg/kg b.wt sodium arsenite, showing multifocal areas of degeneration and necrosis that are evidenced by loss of striations (black arrows). H&E stain at x100 (embedded) and x400 Magnification.

In our study, we observed that the relative weights of the treated animals' organs were not significantly different relative to the control in all the groups except for the animal co-exposed to lead and arsenite that has a significantly reduced relative weight for spleen. There are established reports of the loss in body weight gain or weight reducing properties of arsenic in some animals' model [25, 26, 27]. Jain *et al.* [28] reported similar loss of body weight gain in their animal model when co-exposed to arsenic and nicotine. Since there was no significant loss in relative weight gain of the organs in the present study, it could be suggested that those reports of loss in body weight gain caused by arsenite and lead was targeted at the cutis and not the viscera. Dermal toxicity of arsenite has been widely reported [29, 30, 31], and this might not be unrelated to the loss in weight gain obtained in this study. However, this claim needs further investigation.

The report of Souza *et al* [32] corroborates our present study,-where arsenite could not cause any changes to the relative weight of organs assessed.

Leucocytosis or increased white blood cell is an indicator of poisoning and stress [33]. It can also be seen during the response of the body system to foreign antigens [34]. This leucocyte mobilization into the circulation could be a resultant effect of immune system stimulation against infectious agents or chemicals [9]. In our study, the non-significant increase in the total white blood cell and significant increase in the differential count especially neutrophil and monocytes is indicative of the immune system trying to wade off foreign substance or chemicals, in this case, lead and arsenite. However, it was reported that in chronic exposure to arsenic, leukopenia is almost universally seen [29]. Furthermore, thrombocytopenia (decrease platelets count in blood) has been reported to frequently occur with arsenic exposure [29]. Meanwhile, in their study, Ola-Davies and Akinrinde [9] reported thrombocytosis (increase platelets count) in arsenite-treated animal for 7 days. This was reported to be an indication of immediate ⁻type of hypersensitivity to arsenic exposure. However, the thrombocytopenia observed in our study in the treated groups might be due to the extended day of exposure, 14 days. The reduction was significant in animals co-exposed to lead and arsenic which could be synergistic in nature (Figure 1). This finding is also similar to what was reported by Ikewuchi [35].

Histological examination in our study suggests that both arsenic and lead are toxic to the testes, brain, lungs and heart when the animals were individually and simultaneously exposed to the toxicants. This was reflected in the histological changes in the testes (Figure 2), cerebral cortex (Figure 3), lungs (Figure 4) and heart (Figure 5). Odunola *et al* [18] in their study reported mild infiltrative haemorrhage in the lungs of animals exposed to lead and arsenic while other organs were having normal architecture. Ola-Davies and Akinrinde [9] and Samuel *et al.*, [13] also reported alterations in the histology of liver and testes of rats exposed to arsenite. The long term exposure of animals to lead and arsenic could have caused the severity of lesions observed in our study. The infiltration by inflammatory cells together with degenerative and necrotic changes in the assessed tissues is in tandem with the elevation in both total and differential white blood cell observed in the study.

CONCLUSION

In the present study, we have assessed the relevant blood indices and histology of some vital organs of male Wistar rats exposed individually or simultaneously to lead acetate and sodium arsenite. This is important for proper understanding of the degree of damage and the organs that are susceptible to the toxicities induced by these environmental toxicants. It was demonstrated that there was a possibility of stress induction and inflammatory responses in the treated rats due to the increase in the values of the white blood cells and its differentials together with histological alterations in tissue architecture. Combination of the lead and arsenite could not cause a greater degree of toxicities in the assessed tissues.

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