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## Lead Poisoning causes Histoarchitectural Disruptions in Blood Marrow, Brain Regions and Muscles

### *Histological Observations of Lead Poisoning Effects on Vital Body Tissues of Murine Models: Part I*

Joshua O Owolabi<sup>1\*</sup>, Philip O Ogunnaike<sup>1</sup>, Joshua A. Adeyeye<sup>2</sup>

<sup>1</sup>Department of Anatomy, Ben Carson Sr. School of Medicine, Babcock University, Nigeria

<sup>2</sup>Department of Nutrition and Dietetics, Ben Carson Sr. School of Medicine, Babcock University, Nigeria

**Abstract** The damaging effects of lead poisoning effects to various organs of the body have been severally reported; as well as the health complications they produce. There are however relatively few reports on the nature of such effects on body tissues- histology, most publications have rather addressed the resulting complications. The proper understanding of the nature of the effects of lead poisoning on body tissues could help in understating the cause of the complications and may provide insights into better ways of managing lead poisoning effects. This research investigated the effects of lead poisoning on vital body tissues histology. Twelve Wistar rats were distributed into two groups: Group A being the control and Group B was administered 50mg/kg body weight of lead-in-water for a period of 28 days that the experiment lasted. The tissues were excised, fixed in formal saline and processed using the routine Haematoxylin and Eosin staining technique. Photomicrographs were obtained and analysed critically using qualitative histological principles. Lead produced observable deleterious effects on all tissues tested, the extent however varies greatly: from its extensively disruptive effects on the brain tissues. Brain cerebral and cerebellar cortices show neuronal damage and morphological disruption. Cardiac muscle also showed signs of damage. Skeletal muscle simply had slight myocytes and fibre distortions. Lead poisoning effects was observed in all the tissues.

**Keywords** Lead Poisoning, Tissues Histology, Wistar rat

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#### Introduction

Lead poisoning is no doubt an age long global health challenge [1]; a notorious environmental metallic poisoning [heavy metal] that is capable of producing greatly deleterious effects on most body tissues. Its health effects are greatly influenced by its inadvertent introduction into the environment in several ways and forms as well as its peculiar manners of occupational exposures. Many affected people are usually ignorant of lead poisoning and its unpleasant effects on health [2-3] and this does not make them seek remedial solutions promptly. Lead poisoning whether acute or chronic could produce general health compromising effects [4-5] and its effects on individual tissues such as the brain, heart, testis, eye, skeletal muscle, bones, blood, kidney, have been severally reported [3, 6]. A major factor that is responsible for the notoriety of lead effects on body tissues is the mechanisms by which it interacts with body cells- which are the building blocks of the body; and these mechanisms are almost generally feasible for all body cells. Consequently, lead could influence most cells' physiological conditions; alter their intra-cellular bio-dynamics as well as their signals and interactions with one another. The overall effect is that lead produces extensive deleterious effects on body tissues [7-8]. Consequently, lead could induce cell deaths and cause



tissues' disruption, induce anomalies such as carcinogenesis, inhibit cells normal growth and development or constitute pathological biochemicals in forms of ions and compounds that negatively interact and influence body's biochemistry.

It should be noted that despite numerous reports on lead poisoning; there is the need for many more critical investigations especially as long as it remains a foremost environmental health hazard. In addition, structured and strategic research- other than individual case study reports and results from poorly monitored procedures- such as induced experimental conditions would produce measurable, accurate and precise outcomes that could greatly help in developing strategies to combat this global and almost eternal health challenge. More specifically, this research is a strategic attempt to investigate the effects of lead poisoning on a dozen vital tissues in vivo. Most reported investigations basically examined bio-by-products to measure lead poisoning effects. However, considering the dynamic nature of physiological chemical activities within body systems; such investigations might not have been direct observations of the original effects. More so, this is one of the few attempts to observe tissues' ultra structures as being affected by lead poisoning under the same conditions of treatment. It is a huge opportunity to contribute greatly to knowledge towards solving the problems of lead poisoning. A dozen vital tissues including the brain [cerebrum, cerebellum and hippocampus], bone marrow, liver, kidney, heart muscle, skeletal muscle, lung, spleen, testis and epididymis were critically investigated histologically.

### Materials and Methods

Twelve [n = 12] Wistar rats were distributed into two groups of six animals: Group A served as control and were fed *ad libitum*. Group B was administered 50mg/kg body weight of lead-in-water via the gastric route with the aid of suitable orogastric canula for a period of 28 days that the experiment lasted. The tissues were excised, fixed in formal saline and processed using the routine Haematoxylin and Eosin [H&E] staining technique [9]. Photomicrographs were obtained using the Accuscope Photomicrographic Set and analysed critically using qualitative histological principles. The effects of lead poisoning for the cellular and extra-cellular elements and their relationships were analysed as well as the general histo-architecture for each tissue.

### Results

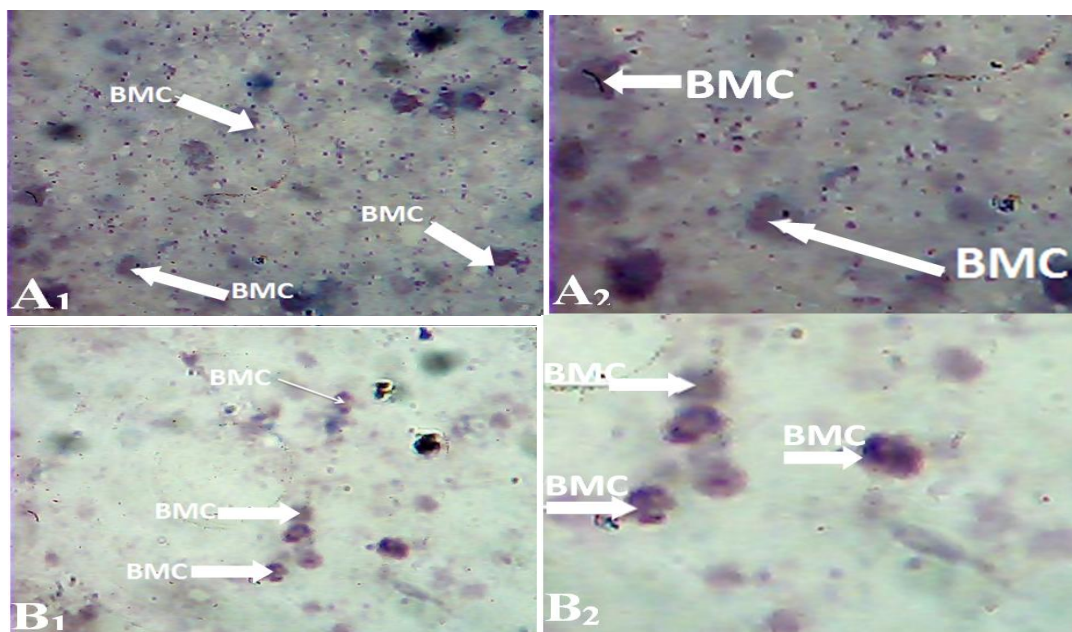


Figure 1: Photomicrographs of the bone marrow of experimental animals; A1 and A2 are illustrations of the Control Group A bone marrow cells at X160 and X640. Photomicrographs portray features of normal bone marrow elements- cells are identifiable; B1 and B2 are photomicrographs of the Group B exposed to lead poisoning at X160



and X640; cells are relatively less in number, stain pale and morphology is heterogeneous. [BMC = Bone Marrow Cells]

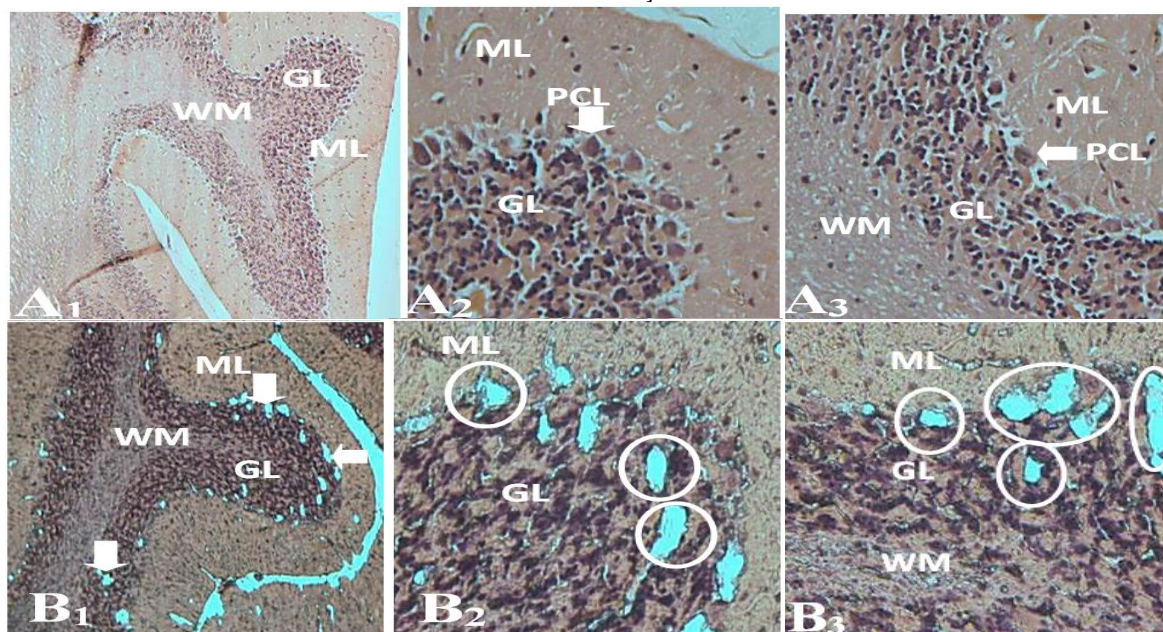


Figure 2: Photomicrographs of the cerebellar cortex of experimental animals; A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub> are illustrations of the Control Group A cerebellar cortex at X160 [cross section] X640 [molecular and granular layers and X640 [molecular and granular layers and white matter]. All photomicrographs portray features of normal cortex; cerebellar cortex layers are well defined. B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub> are photomicrographs of the Group B exposed to lead poisoning at X160 [cross section] X640 [molecular and granular layers and X640 [molecular and granular layers and white matter]; cerebellar cortex layers are poorly defined, cells' morphology is greatly altered and the neuropil shows extensive tissue damage. [WM= White Matter; GL= Granular Layer; ML= Molecular Layer; PCL= Purkinje Cell Layer].

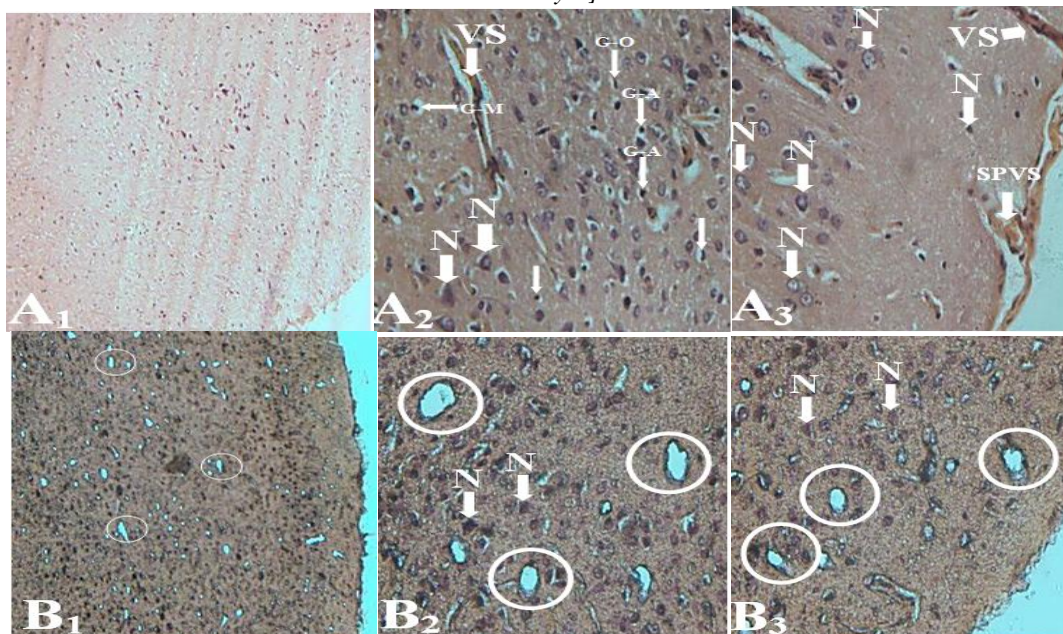


Figure 3: Photomicrographs of the cerebrum of experimental animals; A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub> are illustrations of the Control Group A cerebral cortex at X160 [cross section] X640 deeper layers and X640 [superficial layers]. All photomicrographs portray features of normal cortex; neurons and glia are identifiable and the neuropil is well defined. B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> are photomicrographs of the Group B exposed to lead poisoning at X160 [cross section]; X640



[deep layers] and X640 [superficial layers]; features of extensive histo-architectural disruptions are numerous, cells' morphology is greatly altered and the neuropil shows extensive damage. [N = Neuron; VS= Vascular Structure; G-A= Glia- Astrocyte; G-O= Glia- Oligodendrocyte; G-M= Glia-Microglia; SPVS= Sub-Pial Vascular Structure]

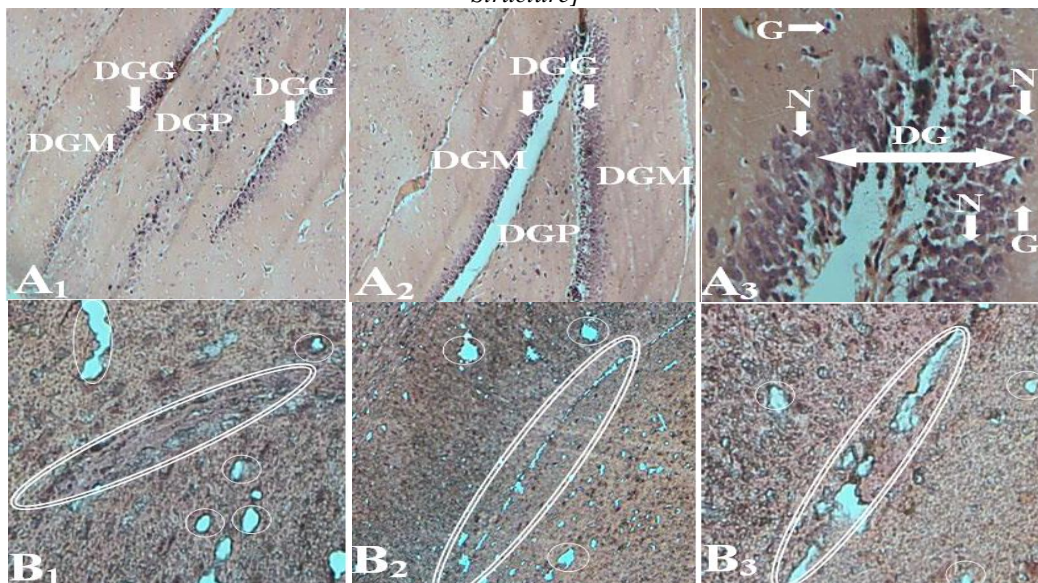


Figure 4: Photomicrographs of the hippocampus of experimental animals; A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub> are illustrations of the Control Group A hippocampus at X160, X160 [cross sections] and X640 [dentate gyrus]. All photomicrographs portray features of normal cortex; layers and parts of the hippocampus are well defined. B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> are photomicrographs of the Group B exposed to lead poisoning at X160, X160 [cross sections] and X640 [dentate gyrus]; there is extensive histo-architectural disruption of the hippocampus, there is extensive damage to cells which morphology is greatly altered and the neuropil shows end glia are relatively numerous. [DGG= Dentate Gyrus Granular Layer; DGM= Dentate Gyrus Molecular Layer; DGP= Dentate Gyrus Polymorphic Layer; N= Neuron; G= Glia; DG = Dentate Gyrus]

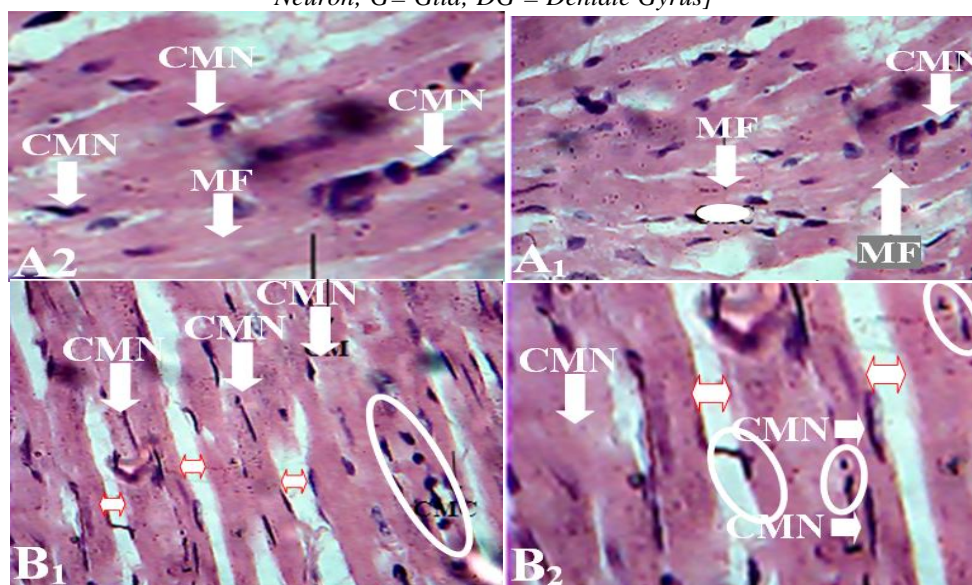


Figure 5: Photomicrographs of the cardiac muscle of experimental animals; A<sub>1</sub> and A<sub>2</sub> are illustrations of the Control Group A cardiac muscle at X160 and X640. Photomicrographs portray features of normal cardiac muscle- cells are well defined; B<sub>1</sub> And B<sub>2</sub> are photomicrographs of the Group B exposed to lead poisoning at X160 and X640; cells are relatively heterogeneous. [CMN- Cardiac Myocyte Nucleus; MF- Muscle Fibrils].

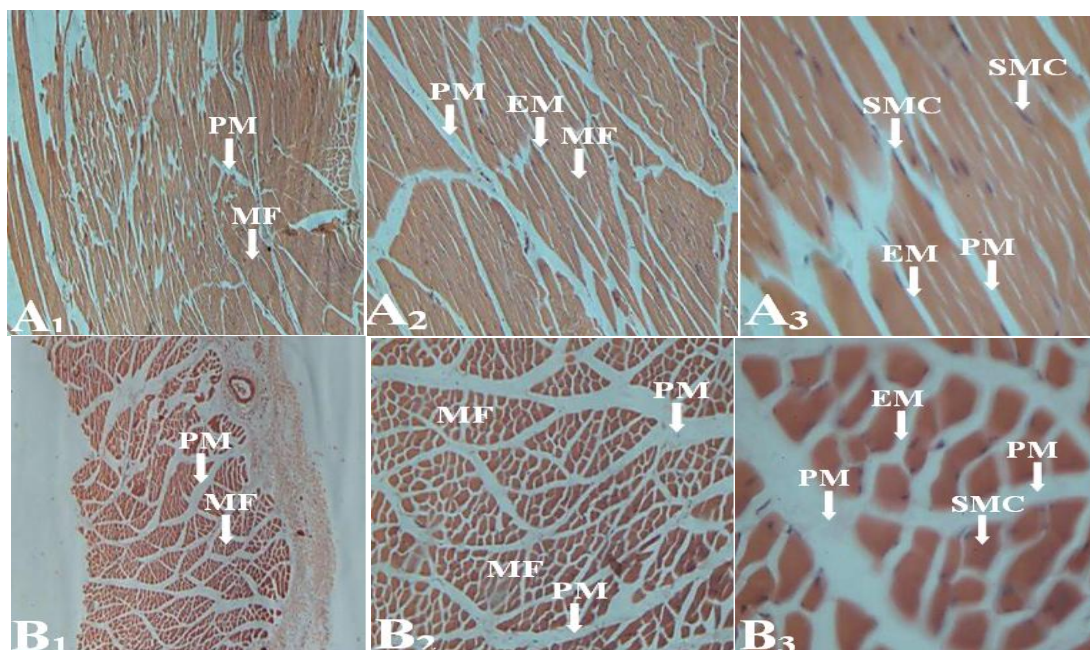


Figure 6: Photomicrographs of the skeletal muscle of experimental animals; A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub> are illustrations of the Control Group A skeletal muscle X160[general histo-architecture] X160 [fibre organisation] and X640 [myocytes and fibres]. All photomicrographs portray features of normal lung; histo-architecture is well defined. B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> are photomicrographs of the Group B exposed to lead poisoning at X160[general histo-architecture] X160 [fibre organisation] and X640 [myocytes and fibres]; there are few and mild features suggesting tissue damage- cells are relatively less prominent and fibre and myocytes present less compact organisation. [MF = Muscle Fascicle; PM= Perimysium; EM= Endomysium; SMC= Skeletal Muscle Cell]

## Discussion

### Bone Marrow

Bone marrow cells are demonstrated in the photomicrographs A<sub>1</sub> and A<sub>2</sub>; though the specific type or stage of development cannot be ascertained; their relative morphology show healthy cells, obviously of various sizes and most likely at various stages of development; some are supposed to be primitive and pluripotent while a few others could have differentiated but at various stages of development. On the other hand, the bone marrow tissue of the Group B animals administered lead in the course of the experiment portrays features that could differentiate it from the control: the cells are quite less prominent and relatively less abundant. The photomicrographs [B<sub>1</sub> and B<sub>2</sub>] present less abundant *background* materials or elements, indicating either a less haemopoietically active marrow or a marrow being relatively richer in adipose tissue. More so, much of extracellular elements and cells could have been destroyed. Every possible explanation for the relatively less active or functional marrow will still be a pointer to a compromise in the series of physiological activities that take place in the bone marrow, especially the formation of blood cells of various types- haemopoiesis. The implications could be numerous; primary ones would however include compromises in erythrocyte percentage [packed cell volume] and the body's immunity through white blood cells functions.

There are several reports implicating lead toxicity for producing deleterious effects on the hematopoietic system as well as other tissues such as liver kidney and the brain [10-12]. Nagaraja *et al.*, [13] reported lead poisoning to have produced toxic effect in the bone marrow causing increased lipid peroxidation and depletion of antioxidant enzymes. Lead poisoning in experimental dogs caused increases in marrow segmented neutrophils and myeloid series cells, and increased myeloid: erythroid ratios [14]. The overall effects of lead poisoning can result in anaemia of lead poisoning, as reported by Waldron [15]; in addition to morphological deformation and functional anomalies of tissue. Results from this investigation confirm these earlier results and provide additional structural evidences.



### Brain Cerebellum

The cerebellum of the Control Group A has well defined histo-architecture and all other features have normal morphology. The treated Group B cerebellum at various magnifications has features of tissue damage. The circled features in Figures B<sub>2</sub> and B<sub>3</sub> are signs of tissue damage- both to the cells and the neuropil. The extensive damage to the tissue is more obvious within the Purkinje cells layer. The Purkinje cells are not observable on photomicrographs [B<sub>2</sub> and B<sub>3</sub>], suggesting that the toxic effects of lead poisoning could have destroyed the cells or grossly deformed them morphologically. Either of these would no doubt compromise the normal functions of these cells. Granular cells also portray morphological distortion; they are quite poorly defined with respect to the control. Regions of tissue damage are also observable within the layer of granular cells; though not are much as it is in the bordering region of Purkinje cells. The molecular layer and the white matter are also affected; structures within these regions are poorly defined relative to the control. At the lowest magnification used, [B<sub>1</sub>] relative to the control [A<sub>1</sub>] shows that there is tissue damage across the entire region of the cerebellar cortex; these effects are however most disruptive in the layer of the Purkinje cells. These are obvious implications that lead poisoning could cause overall damage to the tissue of the cerebellum, destroying or deforming the cells as well as their processes. This would, by implication result in complications associated with cerebellar malfunctions. Chronic perinatal exposure to low dose lead caused marked degeneration and cell loss of the adult cerebellum particularly the Purkinje neurons [16]. In the human brain, lead-induced damage has been reported to occur preferentially in the cerebellum as well as other brain areas including cerebral cortex and hippocampus [17]. Lead has also been found to alter the molecular mechanisms of normal brain development in such manners that may result in motor skill development [18] and anomalies such as postural disequilibrium [19].

### Brian Cerebrum

Photomicrographs of the cerebral cortex of the control Group A animals [labelled A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub>] show a normal histo-architecture. The neurons are observable across the various layers of the cerebellar cortex in their various morphological forms and sizes [A<sub>1</sub>]; various layers of the cerebellar cortex also have the cells within them. The morphology of the cells conform to their relative position in a normal cortical arrangement beginning from the peripheral molecular layer cells [A<sub>3</sub>] to the deeper-layer neurons [A<sub>2</sub>]. Conversely, the cerebellar cortex of the Group B animals exposed to lead poisoning effects [illustrated as A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub>] show several signs of tissue histological anomalies. The lowest used magnifications [B<sub>1</sub>] show several signs of tissue damage that could be observed across all the layers of the cortex; suggesting that the deleterious effects of lead targeted all the layers, damaged cells, supposedly their processes, thus creating localised areas of extensive tissue damage within the cortex. The neuropil, relative to the control also show signs of disruption. Observable neurons show signs of morphological disruption and they are polymorphic even within similar brain regions; there are also signs of karyorrhexis (nucleus fragmentation prior to cell death) [20].

Lead has been previously reported to cause apoptosis of cells and disorders of neurons and glia in the brain; it also disrupted the ultra-structures [21-24]. These reports all share similarities with the current results.

### Brain Hippocampus

The hippocampus histological representation for the control Group A with emphasis on the dentate gyrus is illustrated at different magnifications in Figure 4- A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub>. The various regions are observable and well defined- molecular layer [DGM], granular layer [DGG] and the polymorphic layer [DGP]. The constituent cells are also normal in morphology and organisation; these include the neurons [N] and the glia [G]. Figures B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>, however, are photomicrographs illustrating the hippocampus of the animals exposed to lead poisoning and they portray numerous features of histo-morphological anomalies. The most striking is the total disruption of the entire hippocampus structural architecture and cellular organisation in such a serve manner that all the basic typical features of the hippocampus organisation are lost. The tissue *zones* cannot be defined as being separate in form from one another. Cells have lost their morphological identity and the tissue has assumed an *amorphous* form. Neurons appear to have been largely destroyed and numerous glia; typically astrocytes form the background of the abnormal



neuropil- this observation suggests excessive gliosis due to neuronal death. There are also several intra-tissue *spaces* created by damaged tissues [circled features in B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>].

These observations altogether showed that lead produced extensive structural disruption on the histo-architecture of the hippocampus; these tissue-deforming effects would no doubt compromise the functions of the hippocampus to a very large extent. These effects could produce severe anomalies of the hippocampus that might not be reversible due to the nature of the response of brain tissue to disruptive effects that may require repair or reparation. It is logical to note that by virtue of brain tissue nature, such effects are likely irreversible, thus producing permanent damage to this portion of the brain tissue.

Previous reports on lead effects on hippocampal structure, function and associated functions included astrocytic reactions [25], alteration of hippocampal ultra structures and compromise in short-term and long term memory in models [26] as well as reduction in prenatal neurogenesis [27]. It is important to note that extensive cell death, tissue histoarchitecture disruption and subsequent loss of cell processes' integrity would compromise commissural interconnections and eventually compromise the functions of the hippocampus which is greatly associated with memory [28]. Notably, the hippocampus is vital to mental functions including memory, cognitive function, and mood regulation and it is particularly vulnerable [29-30]; this may account for the largely extensive histological disruption observed in this investigation. However, this part of the brain is unique due to the possibility of adult neurogenesis within it [31-36]. This could be a subject for further investigation as it may lead to remedial solutions for victims of lead poisoning with damaged hippocampal formations, whose associated mental functions are subsequently compromised.

### **Cardiac Muscle**

The Control Group A cardiac muscle [Photomicrographs A<sub>1</sub> and A<sub>2</sub>] have normal morphologically defined cells, with their nuclei [CMN] and the muscle fibrils [MF] observable. Bundles of fibres also have the characteristic branching pattern of the cardiac muscle. Generally, the histo-architecture portrays characteristics of normal muscle tissue. A critical look at the Group B cardiac muscle [Photomicrographs B<sub>1</sub> and B<sub>2</sub>] reveals some variations relative to the Control Group A. Nuclei of several cardiac muscle cells are deformed and heterogeneous; a few cells appear unusually thin and elongated. There are unusual clusters of cells that appear to have unusual morphology as well [see circled cell clusters in Photomicrographs B<sub>1</sub> and B<sub>2</sub>]. Furthermore, rather than the normal branching pattern of muscle fibre bundles as seen in the Control Group A, there are extensive gaps, and usually not branching unto one another- showing sign of histo-architectural disruption [see double headed red-outline arrows on B<sub>1</sub> and B<sub>2</sub>].

The observed anomalies in the morphology, distribution and organisation of the nuclei in the cardiac muscle photomicrographs of the Group B cardiac muscle exposed to lead poisoning as well as the observed signs of general tissue organisation disruptions are pointers to the deleterious effects of lead on the tissue. It also shows that lead poisoning affected the basic elements constituting the tissues and produced observable effects. Poor muscle cells and accompanying fibre disorganisation are possible indicators of muscle wasting, especially if there is continuous assault with no opportunity for recovery. Another possible consequence is the weakening of the muscle and consequent compromises in their contractility- which is basically the determinant of the heart muscle effectiveness. Altogether, there are strong indications that the lead poisoned heart muscle would be weak, histologically distorted and would consequently be physiologically abnormal. Lead poisoning has been linked with cardiovascular disorders [37]; it also reportedly reduced tension development and the myosin ATPase activity of the rat right ventricular myocardium [38] and produces cardiotoxic effects [39]. Lead poisoning has also been implicated in left ventricular hypertrophy cases in United States adults.

### **Skeletal Muscle**

Muscle fascicles are well defined and bundled by the perimysium in the control Group A skeletal muscle while the myocytes and the endomysium around the muscles fibres are also observable. All the basic features that define the skeletal muscle as observed in the Control Group A are also observable in the Group B exposed to lead poisoning. There are no signs of extensive histological disruption; the relative prominence of inter-fibre and inter-fascicular



spaces as observable on all the three photomicrographs could be pointers to possibility of individual fibre shrinkage and consequently, fascicles and whole muscle shrinkage- a sort of relative atrophy [as opposed to hypertrophy]. On the other hand, it could be that muscle cell physiological volume increase [physiological hypertrophy] is limited due to lead poisoning and relative to the unaffected normal tissues [such possibility cannot be overruled since the mechanism cannot be established but only the resultant effects].

Altogether, these features do not point to destruction of muscle cells cum tissues; but could produce mild retardation or limitation to physiological hypertrophy, causing mild muscle wasting, relatively. Physiological consequences may include muscle weakness as frequently reported to be a major effect of lead poisoning on the skeletal muscle. Not many literatures are available on the effects of lead poisoning on skeletal muscle histo-architecture; muscle weakness has however been reported as a major effects of lead poisoning [40-41].

### Conclusion

This investigation attempted to observe and analyse the nature of lead effects on the structures of most vital tissues of the body. Lead produced observable deleterious effects on all tissues tested, the extent however vary greatly: from its extensively disruptive effects on the brain and kidney tissues to the relatively mild effects on the skeletal muscle- which showed least signs of lead toxic effects. Brain cerebral and cerebellar cortices show neuronal damage and morphological disruption. Cardiac muscles also showed evidences of damage. This investigation showed the disruptive and deleterious effects lead toxicity to body health and tissues generally. It also shows that the nature and extents of tissues structural disruptions could vary.

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