



(RESEARCH ARTICLE)



Neurohistological changes in the cerebellar cortex of adult Wistar rats following lead acetate induced cortical damage

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GSC Biological and Pharmaceutical Sciences, 2021, 15(03), 182–188

Publication history: Received on 24 April 2021; revised on 10 June 2021; accepted on 12 June 2021

Article DOI: <https://doi.org/10.30574/gscbps.2021.15.3.0141>

Abstract

This study investigated the neurohistological effect of lead acetate on cerebellar cortex of adult wistar rats. Lead is a common industrial poisonous substance that its prevalence in the environment exhibits toxic effect which makes different organs & tissues especially the central nervous system vulnerable to lead exposure. Lead is however, found useful applications in diverse items of daily needs like paints, water pipes, car batteries, leaded gasoline, ammunition, cosmetics, hair dye, airplanes, shielding for x-ray machines.

Thirty-six (36) adult wistar rats of both sexes weighing between 120-250 grams were randomly grouped into four groups. Group A, B, C and D each group containing seven (9) rats. Group A rats served as the control, and was maintained on standard feed and water for 28 days, group B, C and D rats were treated orally once daily with 0.09g/kg, 0.18g/kg and 0.2g/kg of lead acetate respectively for 28 days. The weights of the wistar rats were recorded on weekly basis during the treatment. All the wistar rats in group A, B, C and D were sacrificed by cervical dislocation on the 29th day of the treatment. The brain was removed and weighed with a sensitive balance and the cerebellum of each rats was then fixed in 10% formol saline, the tissue was processed and stained with Hematoxylin and Eosin for histological study.

Results showed that the mean body weights of the wistar rats significantly decreased in the treated groups when compared with the control group. The mean brain weights of the lead treated groups showed a significant decrease when compared to the control group. Histological study of the brain (cerebellar cortex) of the treated groups demonstrated degenerative changes revealed shrinkage, reduced sized and cellular loss of the Purkinje cells with vacuolations in the Purkinje cell layer compared with normal cerebellar histoarchitecture in the control.

The study concluded that lead acetate has a neurotoxic effect on the cerebellar cortex of adult wistar rats which may ultimately impair some cerebellar functions.

Keywords: Lead acetate; Cerebellar cortex; Neurotoxicity; Purkinje cell; neurodegeneration

1. Introduction

Lead is a heavy and non-biodegradable, low melting metal that occurs naturally in the earth's crust, noted for its toxicity and regarded as a potent occupational toxicant [1]; Children may be exposed to peeling or flaking lead-based paint or weathered powdered paint when engaging in activities that increase exposure [1]. Lead toxicity is a hazard with the potential of causing health effects which may affect the nervous, hematopoietic, hepatic and renal systems, eliciting various disorders [2]. The mechanism of toxicity common to all toxic metals, including lead, is via oxidative stress leading to production of lipid peroxidation (LPO) secondary to generation of free radicals in the tissues. Reactive oxygen

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species (ROS) results in damage to various biomolecules like DNA, enzymes, proteins and membrane-based lipids in addition to simultaneously impairing the antioxidant defence system [3].

The Purkinje cells of the cerebellum function as the main output from the cerebellum in its coordination of body movement, balance and posture [4]. Lead as a toxicant reportedly affects the central nervous system due in part to its ability to cross the Blood-Brain Barrier (BBB) by substituting other bivalent cations like Ca^{2+} , Mg^{2+} , Fe^{2+} with Pb^{2+} ions (Pb^{2+}) thus concentrating it in the brain [5]. Neurons have a reduced capacity to detoxify ROS making them vulnerable to increases in ROS levels which may be generated by lead intoxication thus altering the histology and neurological functions of the hippocampus and cerebellum [6].

The cerebellum is a part of the hindbrain situated in the posterior cranial fossa. It is a part of the pons and medulla oblongata, separated from two structures by the cavity of fourth ventricle; it is covered by tentorium cerebelli and is connected to brain stem by three cerebellar peduncles. It consists of two laterally, large hemisphere which are united by midline vermis. The cerebellar deep nuclei are the sole output structures of the cerebellum. These nuclei are encased by a highly convoluted sheet of tissue called cerebellar cortex, which contains almost all the neurons in the cerebellum. A cross section through the cerebellum reveals the intricate pattern of folds and fissures that characterize the cerebellar cortex. The cerebellar cortex consists of three layers: molecular layer, Purkinje cell layer, and granule cell layer. The molecular layer is the outer- most layer and is largely a synaptic layer, containing the connections of a number of neurons (e.g., basket and stellate cells) with the dense dendritic arborizations of the Purkinje cells, whose cell bodies are the predominant component of the Purkinje cell layer. The innermost layer of the cortex is the granule cell layer containing Golgi cells, Lugaro cells, unipolar brush cells, and the highly abundant granule cells. Almost all of the neurons of the cerebellar cortex use either the excitatory neurotransmitter glutamate or the inhibitory neurotransmitter gamma-aminobutyric acid [7].

The cerebellum receives information from the spinal cord, and other part of the brain and then regulates motor movement. The cerebellum coordinates voluntary movement such as posture, balance, coordination and speech, resulting in smooth and balanced muscular activity. Damage to the cerebellum can lead to loss of coordination of motor movement, inability to judge distance and when to stop (dysmetria), the inability to perform rapid alternating movements (adiadochokinesia), movement tremors, staggering, wide based walking known as ataxic gait [7].

2. Material and methods

2.1. Care for Experimental Animals

Thirty-six (36) healthy adult wistar rats of both sexes weighing between 120-250 grams were used. All the rats were acclimatized for a period of two weeks before exposure to lead, during the period of acclimatization all the rats were carefully and routinely screened, inspected and confirmed to be free of any pathological conditions. They were provided with food and distilled water ad libitum. The rats were fed with standard rat pellet purchased from bovajay food mill, Apake, Ogbomoso.

The experimental animals were housed in standard plastic cages in a serene and conducive cross-ventilated room in the Animal Holding of Anatomy department at Ladoké Akintola University of Technology, Ogbomoso, Nigeria and maintained in accordance with the 'Guide for the care and use of Laboratory Animals' prepared and compiled by the National Research Council [8].

2.2. Experimental design and grouping

The rats were separated randomly into 4 groups of nine each. Group A was the control, while Group B, C and D were the lead-treated groups as follows

Group A: rats received stock diet and distilled water, they served as control.

Group B: Experimental animals received stock diet and 0.09mg/kg of lead acetate orally once daily for 21 days

Group C: Experimental animals received stock diet and 0.18mg/kg of lead acetate solution orally once daily for 21 days

Group D: Experimental animals received stock diet and 0.2mg/kg of lead acetate solution orally once daily for 21 days

Lead was dissolved in distilled water for oral administration. It was administered with the use of oral cannula for the rats in groups B, C, and D once per day for 21 days.

All the rats in each group were weighed with a weighing scale weekly during the treatment period to monitor changes in their body weights. At the end of treatment, the body weights of all the rats in each group were taken and compared with their initial body weights before the onset of lead administration.

2.3. Collection of Brain Sample

The animals were sacrificed after the last dose of lead acetate administration using the cervical dislocation method. The head and cranium were carefully removed with a surgical blade and the brain was removed, weighed before half of the cerebral cortex was cut and homogenized for assessment of oxidative stress parameters while the whole of cerebellum was quickly fixed in formal calcium for histological evaluation using Hand E staining technique.

2.4. Statistical analysis

Data was express as mean \pm SEM. The difference was compared for statistical analysis by student's test. Descriptive and inferential statistics was applied to the results. All statistical analysis was performed using SPSS version 6.0.

Table 1 Mean \pm S.E.M of the body weights of Wistar rats before and during administration

| Period/ Week | Group A | Group B (0.09g/Kg) | Group C (0.18g/Kg) | Group D (0.2g/Kg) |
|-----------------|------------------|-----------------------|-----------------------|----------------------|
| Week 0 | 223.3 \pm 8.94 | 225.3 \pm 7.42 | 187.6 \pm 6.27** | 152.2 \pm 12.38*** |
| Week 1 | 229.3 \pm 8.77 | 220.5 \pm 7.49 | 186.4 \pm 8.79** | 142.3 \pm 5.75** |
| Week 2 | 226.0 \pm 8.45 | 203.7 \pm 17.15 | 194.5 \pm 7.38* | 155.3 \pm 5.06*** |
| Week 3 | 216.4 \pm 8.69 | 200.0 \pm 9.09 | 178.0 \pm 8.75** | 141.5 \pm 5.44** |
| Week 4 | 199.7 \pm 7.67 | 187.7 \pm 11.59 | 186.3 \pm 5.18 | 147.1 \pm 5.29*** |

Significance: P < 0.05, value greater than 0.05 are considered insignificant while values less than 0.05 are considered significant (*). Values were expressed as mean \pm Standard error of mean.

Table 1 above revealed the body weights of Wistar rats which started in significant decreasing from the control group (A) which decreased from mean value of 223.3 \pm 8.94 to 199 \pm 7.67.

Wistar rats in group B received a low dose of lead acetate (0.09g/kg) which caused an insignificant decrease in body weights of the wistar rats from mean value of 225.3 \pm 7.42 to 7.42 to 187.7 \pm 11.59 and in comparison, with the control group A, it is not statistically significant (P>0.05).

Wistar rats in group C received a medium dose of lead acetate (0.18g/kg) which caused a slight decrease in body weights of the rats from mean value 187.60 \pm 6.27 to 186.30 \pm 5.18 and this weight loss shows a statistical difference (p<0.05) when compared with the control group (A). Wistar rats in group D received a high dose of lead acetate (0.2g/kg) which caused significant decrease in body weights of wistar rats from mean value 152.20 \pm 12.38 to 147.10 \pm 5.29 and this weight loss is statistically significance (p<0.05) when compared with the control group (A).

Table 2 Showing weights of the brains of the wistar rats after lead acetate administration

| Groups | Brain Weights(g) | Relative Weight (%) |
|--------|-------------------|---------------------|
| A | 2.05 \pm 0.13 | 1.02 |
| B | 1.72 \pm 0.06 | 0.92 |
| C | 1.57 \pm 0.03* | 0.84 |
| D | 1.53 \pm 0.05** | 1.04 |

Significance: P < 0.05, value greater than 0.05 are considered insignificant while values less than 0.05 are considered significant (*). Values were expressed as mean \pm Standard error of mean.

Table 2 Above shows the brain weights of the wistar rats after lead exposure. The brain weights was 2.05 ± 0.13 in group A which reduced to 1.72 ± 0.06 in group B, which reduce to 1.57 ± 0.03 in group C and 1.53 ± 0.05 in group D. The brain weights were significantly reduced in group C and D ($p < 0.05$).

3. Histological observation

3.1. Group A: Control group

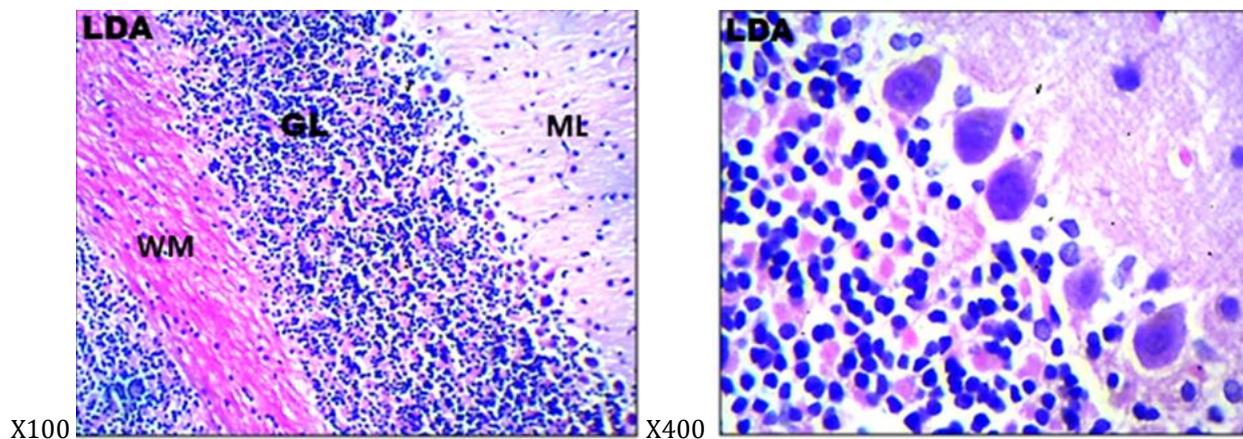


Figure A: Photomicrographs showing normal morphological presentation of the cerebellar cortex with H&E staining are observed in group A. Cellular morphology of this group is characterised by Purkinje cells with conspicuous cell bodies and dendrite that are projecting deep into the molecular layer (ML) (which has sparse nuclei), assuming the shape of a fan. Also, the granule layer (GL) in this group consists of small granule neurons which are compactly disposed.

Control Group A: H&E stained sections showed normal histological structure in the form of outer Molecular Layer (ML) which is mainly formed from fibres with few small stellate cells and basket cells. Middle Purkinje cells in the Purkinje layer (PL) are arranged in one row of large pyriform or flask shape cells, with clear nuclei, prominent nucleoli, and cytoplasm. The inner most layer contain the granular cells (GL) which are closely packed rounded small cells.

3.2. Group B

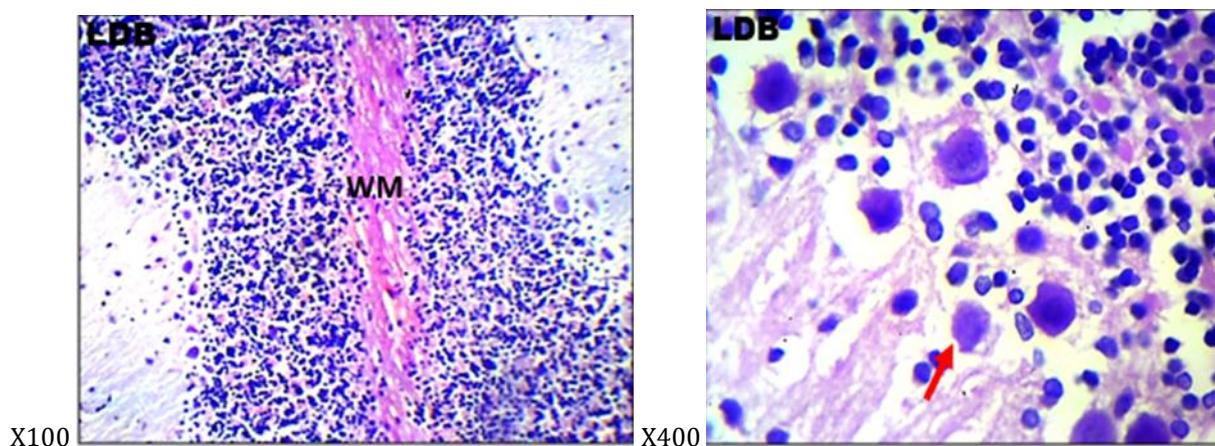


Figure B: photomicrograph of the cerebral cortex of group B Wistar rat that receive lead acetate with low dose of 0.09g/kg for 28 days and stained with H&E. It shows slight degeneration of the Purkinje cells

Treated Group B: by H&E stain the Purkinje layer showed shrinkage of the Purkinje cells (empty spaces around them) with increased acidophilia.

3.3. Group C

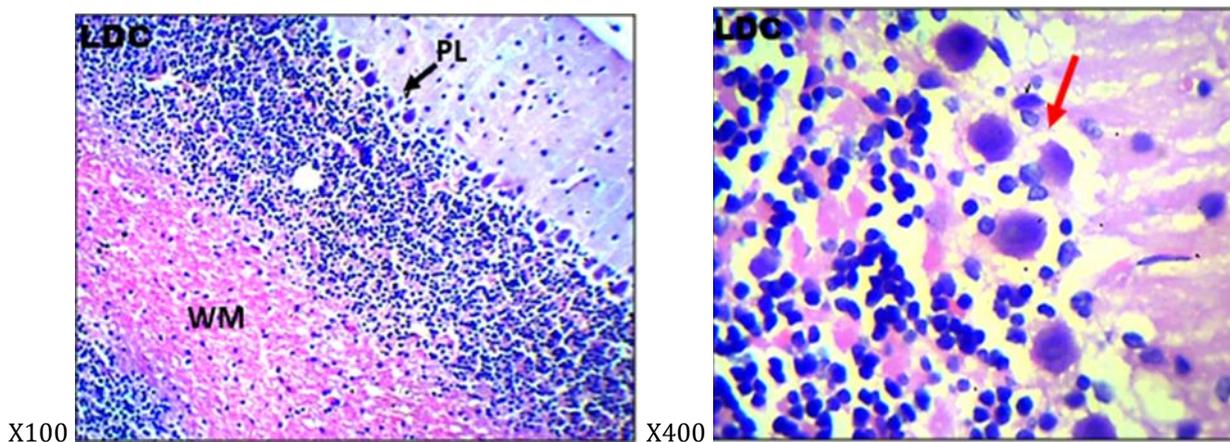


Figure C: Photomicrograph of the cerebellar cortex of group C Wistar rats that received lead acetate with a medium dose of 0.18g/kg for 28 days stained with H&E shows degenerating Purkinje cells (red arrows) with pyknotic cell bodies and short dendritic processes can be seen around the indistinctly demarcated cerebellar layers of group C. Furthermore, the neutrophils in this group appear fragmented with irregular shaped and sized neuronal cells. Notable degenerating features including perineural spaces appear to surround neurons in this group.

Treated Group C: H&E stained sections shows degenerating Purkinje cells with pyknotic cell bodies and short dendritic processes can be seen around the indistinctly demarcated cerebellar layers. Furthermore, the neutrophils in these groups appear fragmented with irregular shaped and sized neuronal cells.

3.4. Group D

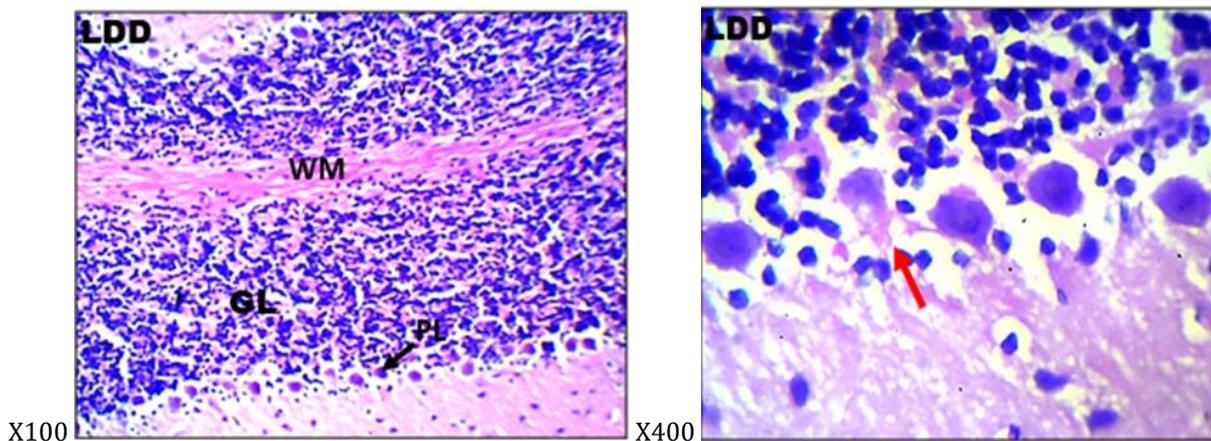


Figure D: Photomicrograph of the cerebellar cortex of group D Wistar rats that received lead acetate with a medium dose of 0.2g/kg for 28 days stained with H&E for 28 days revealed histological alterations of the cerebellar cortex layers with shrinkage and degeneration of the Purkinje and molecular layer cells with scattered glial cells. The Purkinje cells shows empty spaces between the indicating degenerating of cells compared with the control group.

Treated Group D: H&E stained sections shows degenerating Purkinje cells with pyknotic cell bodies and short dendritic processes can be seen around the indistinctly demarcated cerebellar layers. Furthermore, the neutrophils in these groups appear fragmented with irregular shaped and sized neuronal cells. There are notable degenerative features, including perineural spaces appear to surround neurons in these groups.

4. Discussion

This study investigated some effects of lead acetate on the cerebellar cortex of an adult Wistar rats in relation to histological organization of cerebellar cortical layers. Lead intoxication has been shown to affect many systems of the body. However, lead has its most detrimental and toxic effects on the central nervous system. Report has revealed that

in the nervous system, lead inhibits or blocks the receptor known as N-methyl-D-aspartate, an effective receptor involved in the maturation of brain plasticity, which are important changes in association with brain organization. The blockage of this receptor in the brain results invariably in interruption of long-term potentiation, which has the potential to limit the permanent intake and storage of newly learned knowledge. Additionally, previous investigator has indicated that elevated Blood Lead Levels (BLLs) results in impairment of the blood-brain barrier function [9]

Result of the body weights analysis from this study showed an insignificant decrease in the weights of the Wistar rats in group B (received low doses of lead acetate) when compared to the control group A. There was a significant decrease ($P < 0.5$) in the body weights of group C and D (they received high doses of lead acetate) in comparison to control group A. This significant decrease ($P < 0.5$) in the body weights and relative brain weights could be attributed to the toxic effects of lead acetate. The significant decrease in brain and body weights in lead-treated group in this study consistent with similarly reported the findings of other investigators [10]. Previous report has shown that many factors determine the neurotoxic effects of lead, which include integrity of blood-brain barrier, lead-binding proteins, cellular scavenger's e.g. glutathione and interactions with other micro-nutrients [11].

The result of the histological examination of the cerebellar tissue of lead administered rats using H and E stain revealed histological alterations of the cerebellar cortical layers with shrinkage and degeneration of the Purkinje and molecular layer cells with distorted glial cells. The Purkinje cells exhibited darkly stained nucleus with eosinophilic cytoplasm and empty spaces or vacuolations between them indicating degeneration and loss of the Purkinje cells. The alteration of the microanatomy of the cerebellar cortical layers of the lead acetate treated rats as shown by the loss of the basophilic staining of the Purkinje cell nuclei is in agreement with previous report which indicated a similar finding in their study [12]. Similarly, the neurodegenerative changes and distortions of the cortical layers observed in this study have also been revealed in other previous studies [13].

Granular cells also portray morphological distortion; they were quite poorly defined with respect to the control. Regions of tissue and neuronal damage are also observable within the layer of granular cells; though not as much as it is in the bordering region of Purkinje cells. Additionally, it has been documented that Lead produced observable deleterious effects on all tissues tested, the extent however varies greatly: from its extensively disruptive effects on the brain tissues [14]. Brain cerebral and cerebellar cortices show neuronal damage and morphological disruption which were similarly observed in this study, when wistar rats received lead acetate in their drinking water for 60 days degeneration in the neuron cells was evident. The degeneration of cerebellar cortical layers coupled with cellular loss may be due to oxidative damage induced by lipid peroxidation following lead exposure in wistar rats investigated.

5. Conclusion

This study concluded that exposure of adult wistar rats to lead acetate for 28 days produced observable distorted and neurodegenerative changes in the cerebellar cortical layers which were adversely affected.

Compliance with ethical standards

Acknowledgments

The authors appreciate the support received for the success of this study particularly from the technical staff of the department of Anatomy, Faculty of Basic Medical Sciences Ladoke Akintola University, Ogbomosho, Oyo State, Nigeria

Disclosure of conflict of interest

There is no conflict of interest

Statement of ethical approval

Animal Ethic committee approval was obtained for this research work

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