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Developmental Effects of Perinatal Lead Exposure on the Cerebellum of Wistar Rat Pups

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Abstract

Lead (Pb) exposure is a persistent global health problem, with the heaviest burden in developing regions such as Africa. In Nigeria, repeated cases of Pb poisoning due to mining and environmental contamination have raised serious public health concerns. The cerebellum, which plays a critical role in motor coordination, balance, and cognitive processes, is highly susceptible to toxic insults during development. However, the specific timing of Pb exposure that produces the greatest cerebellar damage remains uncertain. This study examined the timing-dependent neurotoxic effects of Pb acetate on the histology of the developing cerebellum in Wistar rats, with emphasis on structural alterations. Twenty-four Wistar rats were assigned into four groups: control, prenatal Pb exposure, postnatal Pb exposure, and gestation-through-lactation Pb exposure groups. Lead (500 ppm) was administered through drinking water during the designated exposure periods. Following sacrifice, brain tissues were harvested, fixed, and processed for histological examination of the pons using hematoxylin and eosin staining. Photomicrographs were examined for histopathological changes. The control group showed normal cerebellar cytoarchitecture with intact Purkinje cell, molecular, and granular layers. Pb-exposed groups exhibited marked neuropathological alterations including Purkinje cell degeneration, fibrosis, pyknosis, karyolysis, karyorrhexis, astrocytosis, perineural vacuolation, vascular congestion, and areas of necrosis. These findings indicate that Pb disrupts cerebellar integrity in a timing-dependent fashion, with combined prenatal and postnatal exposure causing the most severe damage. Pb acetate exposure during vulnerable developmental windows induces profound cerebellar damage, characterized by neuronal loss, vascular changes. The perinatal stage appears to be the most sensitive period for Pb-induced neurotoxicity. These results underscore the need for strict preventive measures to minimize early-life Pb exposure and its long-term neurodevelopmental consequences.

Keywords: Lead acetate, Cerebellum, Neurotoxicity, Wistar rats, Histopathology.

INTRODUCTION

Lead (Pb) exposure contributes to almost 1% of the global burden of disease, with the highest burden occurring in developing regions such as Africa and Southeast Asia (Fewtrell et al., 2004). In Africa, Pb-related disease burden is largely associated with mental disability in children and cardiovascular disease in adults, accounting for 75% and 25% of disability-adjusted life years (DALYs), respectively (Norman et al., 2007). In West Africa, several environmental sources of Pb exposure exist, including mining, battery recycling, contaminated soil, dust, water, and air pollution, posing significant health risks to local populations (Obeng-Gyasi, 2022). Pb levels in many West African countries exceed the WHO recommended limits for drinking water and air quality [World Health Organization (WHO), 2017; WHO, 2000]. In Nigeria, recurrent outbreaks of Pb poisoning linked to artisanal and open-cast mining activities in Zamfara, Plateau, and Niger States have raised major environmental and public health concerns (Tirima et al., 2016; Orisakwe et al., 2017; Umar-Tsafe et al., 2019).

Lead is a well-established neurotoxin with a particular affinity for the developing brain. Previous studies have

demonstrated high Pb accumulation in brain regions associated with cognition and motor coordination (Mykkanen et al., 1982). Although much attention has focused on the hippocampus because of its role in learning and memory (Marchetti, 2003; Sharifi et al., 2010), evidence suggests that other brain regions, including the cerebellum, may also be vulnerable to Pb-induced neurotoxicity. Beyond its traditional role in motor control, the cerebellum contributes to cognitive processing, behavioural regulation, and learning, making it a relevant target in developmental neurotoxicity studies.

Despite growing evidence linking developmental Pb exposure to impaired neurodevelopment, the critical exposure period and underlying neuropathological changes remain unclear. Some studies report that developmental Pb exposure produces persistent neurocognitive deficits, whereas others suggest that such effects may diminish over time. Furthermore, limited information exists regarding the histopathological effects of Pb on the cerebellum during different developmental exposure periods. Therefore, this study aimed to investigate the effect of Pb exposure on the histopathology of the cerebellum.

EXPERIMENTAL DESIGN

Experimental Animals

A total number of 24 (8 males and 16 females) apparently healthy adult Wistar rats with an average weight of 180g procured from National Veterinary Research Institute, Vom, Jos, Nigeria, were used for the study. The animals were grouped in a ratio of one (1) male to two (2) females based on the method used by Abdu *et al.* (2020). The animals were kept to acclimatize for 14 days in a conducive animal house in the Department of Human Anatomy, Northwest University, Kano, Nigeria. They were fed on a commercial pellet diet and allowed access to food and water *ad libitum*.

Determination of Oestrus Cycle

The stages of the oestrous cycle (proestrus, oestrus, metestrus, and diestrus) were determined according to the method of Abdu *et al.* (2020). Vaginal smears were obtained using normal saline and examined under a light microscope at ×10 magnification. Cycle staging was based on the predominant cell types observed: nucleated epithelial cells in proestrus, cornified anucleated cells in oestrus, a mixture of leukocytes and epithelial cells in metestrus, and predominantly leukocytes with mucus in diestrus (Shreya et al., 2025).

Animal Grouping

Female rats with regular oestrous cycles were identified and mated by housing two females with one male overnight. Gestational day 1 (GD1) was confirmed by the presence of spermatozoa in vaginal smears (McClain & Becker, 1975; Azza et al., 2016). Pregnant dams were then housed separately and assigned into four experimental groups, each consisting of four pregnant females and two males used for mating.

Group I (Control): Pregnant dams received feed and water ad libitum throughout the study.

Group II (Prenatal exposure): Pregnant dams received 500 ppm lead acetate in drinking water from GD6 to GD21 to evaluate the effect of gestational Pb exposure on offspring development (Goma & Mahrous, 2013; Basha & Reddy, 2015).

Group III (Postnatal exposure): Offspring were exposed to 500 ppm lead acetate through maternal drinking water from postnatal day 1 (PND1) to PND21 to assess the effects of postnatal Pb exposure during lactation.

Group IV (Perinatal exposure): Pregnant dams received 500 ppm lead acetate from GD6 through PND21, thereby exposing offspring during both gestation and lactation (Reddy et al., 2007; Barkur & Bairy, 2014).

The exposure period from GD6 to PND21 was selected to coincide with critical stages of neurodevelopment, including implantation, fetal brain development, and early postnatal maturation, during which Pb exposure is known to produce higher blood Pb levels and greater neurotoxic effects (Bunn et al., 2001).

Animal Sacrifice

At the end of the designated Pb exposure period, rat pups were sacrificed at postnatal day 21 (PND21). The pups were anaesthetised with ether (Trust Chemical Laboratories, Cat No. 67663, Hangzhou, China), after which the skull was carefully opened along the sagittal

suture using surgical instruments, and the whole brain was harvested following the method described by Owolabi et al. (2014).

Retrieval and Fixation of Cerebellum

The whole brain was fixed in Bouin's solution for 24 hours, after which the cerebellar hemispheres were carefully isolated according to the methods of Seibenhener and Wooten (2012) and Rosińczuk et al. (2015). The cerebellar tissues were appropriately labelled, oriented in a sagittal plane, and placed in tissue cassettes for histological processing and embedding (Hosseini-Sharifabad & Nyangaard, 2007).

Light Microscopy Study of the Cerebellum

The fixed tissues were subjected to routine histological tissue processing based on the method described by Bancroft and Gamble (2008).

Tissue Processing

Graded ethanol was used to dehydrate the hippocampal tissue by removing residual fixatives and water, following Suvarna et al. (2019). The tissue was then cleared in graded xylene to remove alcohol and facilitate paraffin infiltration. This process was carried out using a Leica TP1020 automated tissue processor (Leica Biosystems, IL, USA).

The cerebellum was subsequently infiltrated with molten paraffin wax in multiple stages to remove clearing agent and provide structural support for sectioning. Embedding was performed using an embedding centre comprising a paraffin dispenser (Biobase Biodustry, Shandong, China), heated mould station, and cold plate. The tissue was oriented sagitally in a mould, fully embedded in molten wax, covered with a cassette, and allowed to solidify on a cold plate to form paraffin blocks.

Sectioning of Cerebellum

The cerebellar tissue block was mounted vertically on a Leica RM2125 RTS rotary microtome (Leica Biosystems, IL, USA) and serial sections of 5µm thick were cut and floated out on a 40°C distilled water in a water bath. Sections were then carefully lifted using clean glass slides and allowed to air dried for 30 minutes before it was placed in an oven at 40°C to deparaffinize.

Staining Procedure

The cerebellar sections were stained using haematoxylin and counterstained with eosin to demonstrate the histo-architecture based on the method described by Cardiff *et al.* (2014).

Photomicrography of Cerebellum

The H&E-stained slides were examined under different magnifications using Leica DM500 microscopes (Leica Biosystems, IL, USA), with a motorized x-y specimen stage. Photomicrographs were taken with a Leica ICC50 HD camera (Leica Biosystems, IL, USA) mounted on the microscope at the magnification of ×100 followed by ×400.

RESULTS

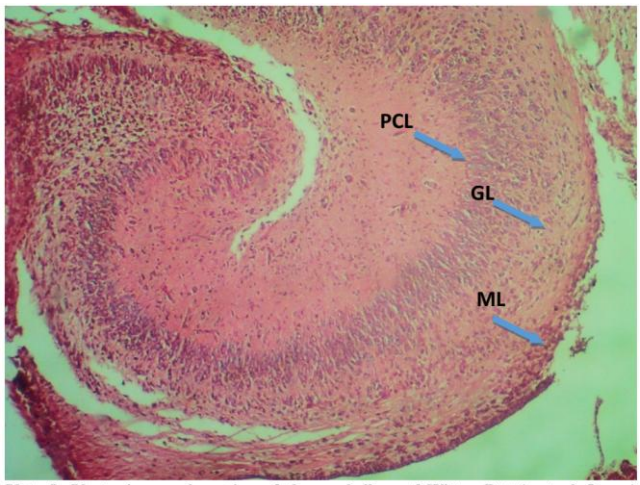

Plate I: Photomicrograph section of the cerebellum of Wistar Rat (control Group) Showing Purkinje cell layer (PCL), Molecular layer (ML) and Granular layer (GL) (H&E ×100).

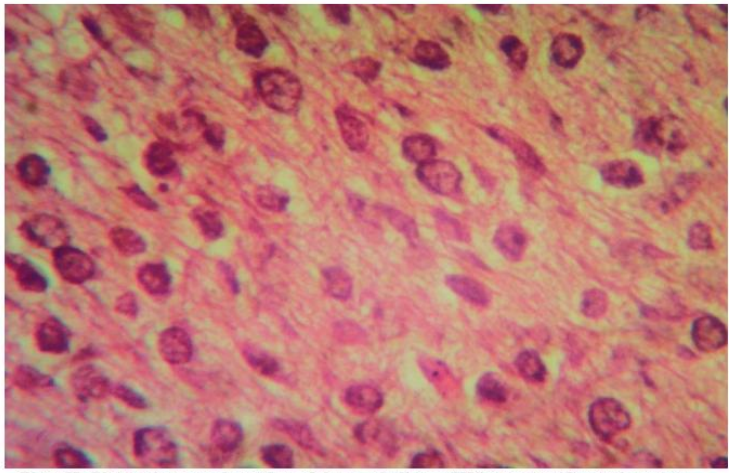

Plate II: Photomicrograph section of the cerebellum of Wistar rats (Control group) (H&E ×400).

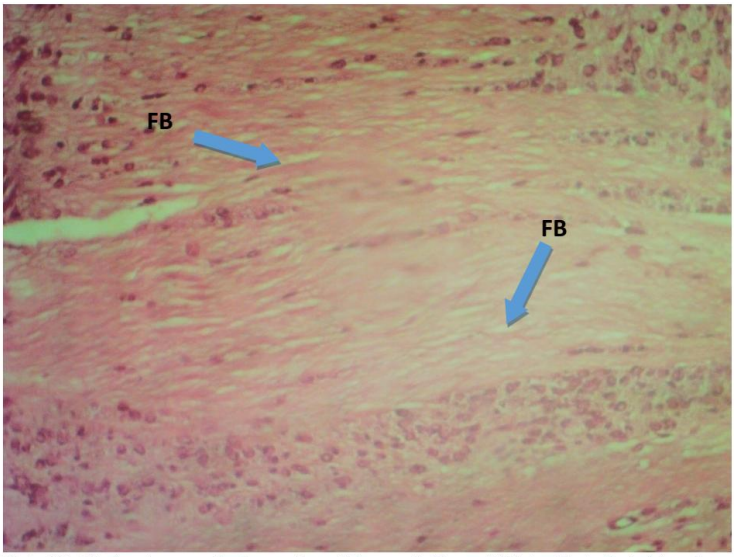

Plate III: Photomicrograph of a section of the cerebellum of Wistar Rat administered 500ppm of lead acetate from GD6 to parturition (Prenatal Group) showing area of Fibrosis (FB) (H & E ×100).

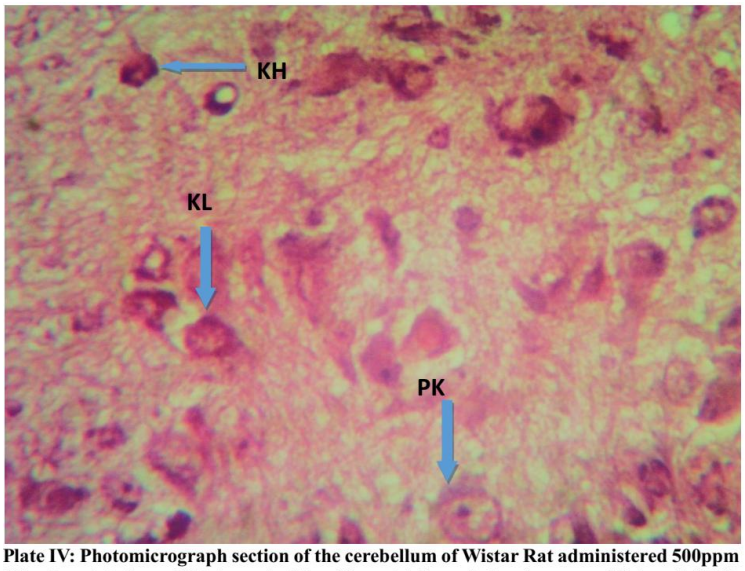

Plate IV: Photomicrograph section of the cerebellum of Wistar Rat administered 500ppm 0f lead acetate from GD6 to parturition (Prenatal Group) showing area of Pyknosis (PK), karyolysis (KL) and Karyorrhexis (KH) (H & E ×400).

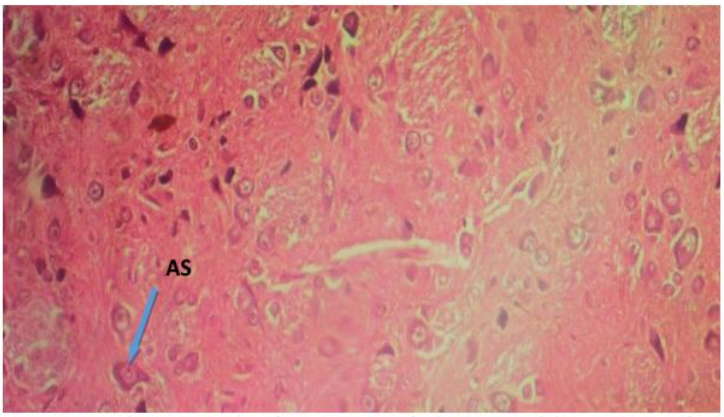

Plate V: Photomicrograph section of the cerebellum of Wistar Rat administered 500ppm of lead acetate from parturition to weaning (Postnatal Group) Showing area of Astrocyte (AS) (H & E ×100).

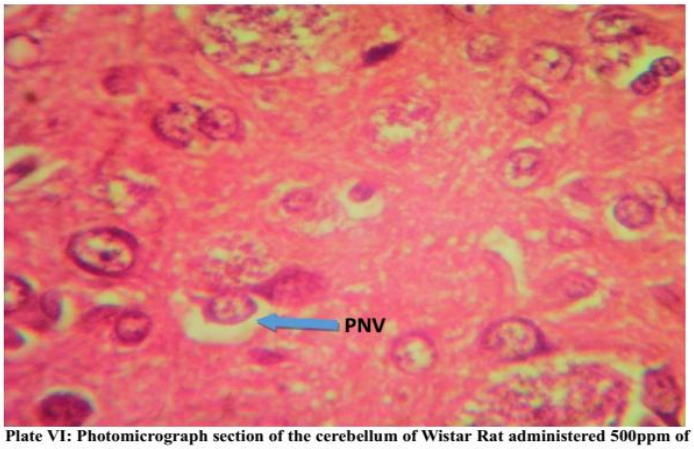

Plate VI: Photomicrograph section of the cerebellum of Wistar Rat administered 500ppm of lead acetate from parturition to weaning (postnatal group) Showing area of Perineural Vacuolation (PNV) (H & E ×400).

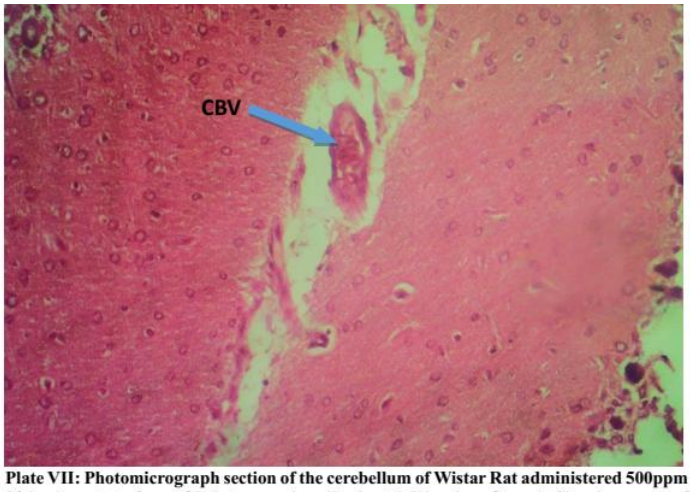

Plate VII: Photomicrograph section of the cerebellum of Wistar Rat administered 500ppm 0f lead acetate from GD6 to weaning (Perinatal Weaning Group) Showing area of Congested Blood Vessel (CBV) (H&E ×100)

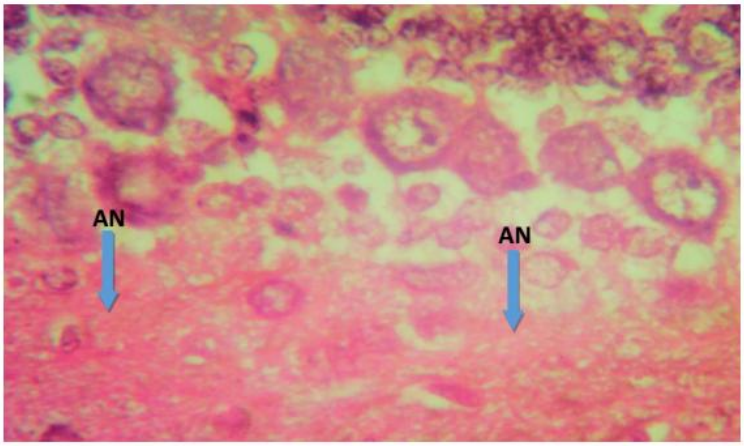

Plate VIII: Photomicrograph section of the cerebellum of Wistar Rat administered 500ppm of lead acetate from GD6 to weaning (Perinatal Weaning Group) Showing Areas of Necrosis (AN) (H&E ×400).

DISCUSSION

The present study demonstrates that developmental exposure to lead acetate induces significant histopathological alterations in the cerebellum of Wistar rats, with the severity of damage varying according to the timing and duration of exposure. The preserved cerebellar architecture observed in the control group, including intact Purkinje cells and well-organized molecular and granular layers, is consistent with the normal histological features of healthy cerebellar tissue described in rodents (Altman & Bayer, 2019; Sillitoe & Joyner, 2022).

Prenatal exposure to Pb produced fibrosis and nuclear degenerative changes, including pyknosis, karyolysis, and karyorrhexis, suggesting early neuronal injury. These findings support previous reports that Pb readily crosses the placental barrier and disrupts critical neurodevelopmental processes such as neuronal proliferation, differentiation, calcium signalling, and synaptic maturation during gestation (Basha et al., 2015; Mason et al., 2020). The observed nuclear changes likely reflect oxidative stress-mediated DNA damage and apoptotic cell death, as Pb exposure is known to increase reactive oxygen species production while suppressing endogenous antioxidant defenses (Flora et al., 2021).

The presence of fibrosis further suggests chronic tissue injury associated with reactive gliosis and extracellular matrix deposition. Since Purkinje cells undergo rapid maturation and synaptic refinement during gestation, interference at this stage may result in persistent structural abnormalities within cerebellar circuitry (Rice & Barone, 2020). Such developmental disruption may ultimately impair motor coordination, cognitive processing, and behavioural regulation, functions increasingly recognized to involve the cerebellum.

Postnatal Pb exposure was characterized by astrocytosis and perineural vacuolation, indicating reactive gliosis and neuronal stress. Astrocyte activation is a recognized response to neurotoxicity and may initially represent an attempt to maintain neurotransmitter balance and neuronal survival following toxic insult. However, prolonged astroglial activation can exacerbate oxidative stress and neuroinflammation, thereby contributing to neuronal dysfunction (Sidoryk-Wegrzynowicz & Aschner, 2018; Lee et al., 2021). Experimental evidence also indicates that Pb disrupts neurotransmitter regulation and induces inflammatory signalling pathways in the developing brain (Kurhaluk et al., 2024).

Perineural vacuolation observed in this group may indicate oedema, axonal degeneration, or myelin disruption. This finding is particularly important because the postnatal cerebellum undergoes intense synaptogenesis, neuronal migration, and myelination. Interference with these processes may impair neuronal connectivity and signal transmission. Previous studies showing that Pb alters oligodendrocyte maturation and myelin protein synthesis provide mechanistic support for these observations (Caito & Aschner, 2019).

The most severe cerebellar lesions were observed in animals exposed continuously from gestation through lactation, where vascular congestion, extensive vacuolation and necrosis were evident. These findings suggest that prolonged exposure across multiple developmental windows produces cumulative neurotoxic effects. Continuous Pb exposure likely amplifies oxidative stress, mitochondrial dysfunction, excitotoxicity, and inflammatory responses beyond the compensatory capacity of developing neural tissue. Similar studies have shown that cumulative Pb burden produces greater structural and functional brain injury than exposure confined to a single developmental period (Cecil et al., 2021; Neal & Guilarte, 2022).

Necrosis and vascular congestion observed in the perinatal exposure group may also indicate impaired cerebral perfusion and hypoxic injury secondary to

Pb-induced vascular dysfunction. Such widespread structural damage is likely to compromise cerebellar circuitry involved in motor coordination, learning, emotional regulation, and sensory integration. These findings align with reports that developmental Pb exposure contributes to long-term motor and cognitive impairments through progressive disruption of neuronal integrity and synaptic plasticity (Schmahmann, 2019; Stoodley & Schmahmann, 2018; Buckner, 2013; Parithathvi et al., 2024; Tobalu & Enogieru, 2025).

CONCLUSION

This study provides histological evidence that early-life lead exposure disrupts cerebellar development in a stage-dependent manner, with combined prenatal and postnatal exposure producing the most profound neuropathology. The findings reinforce the need for stronger environmental controls to prevent early-life Pb exposure and its long-term neurodevelopmental consequences.

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