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Chronic Administration of Lead Acetate Altered the Histology of the Cerebellum of Wistar Rats

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ABSTRACT
Lead acetate is one of many compounds of lead. This study examined the effects chronic administration of lead acetate on the histology of the cerebellum. Twenty (20) adult Wistar rats of both sexes of weight ranging between 100 g – 150 g were used for the experiment. The animals were kept in the animal house and randomized into four groups: A, B and C served as the experimental groups while D served as the control group. The animals received rat chow and distilled water *ad libitum*. Groups A, B, and C received in addition to their distilled water and feed, a solution containing 30 g of lead acetate readily dissolved in 1litre of distilled water via oral administration at different doses of 400 mg/kg, 800 mg/kg and 1200 mg/kg per body weight for 21 days respectively. On day 22 of the experiment, the animals were euthanized with ketamine hydrochloride and sacrificed by the cervical dislocation method. The cerebellum was carefully harvested and fixed in 10% formol calcium and routine tissue processing performed. Lead acetate administration significantly ($P < 0.05$) reduced the body weight of the animals and also induced histological alterations in the cell bodies of the Purkinje cells of the cerebellar cortex of the experimental groups relative to the control group.

Key words: Lead acetate, Cerebellar cortex, Purkinje cells, Body weight, Wistar rats.

INTRODUCTION
Lead, (Latin *plumbum*) is a neurotoxic heavy metal that was one of the first known metals. Lead with the atomic number of 82 is found in group 14 and period 6 of the periodic table. It is one of the potential environmental toxicants as it is highly poisonous and regardless of if inhaled or swallowed, causes severe damage to almost every organ and system in the body[1]. It is a white, poisonous, crystalline compound usually prepared commercially by dissolving litharge in acetic acid. Although it is neurotoxic and highly poisonous, it has been found useful in the manufacture of paints, pigments of chrome and pesticides[2]. Lead has also been found useful in the lining of pipes, manufacture of varnish drier, metallic lead beads, electric cable sheathing, casting of lead weights and bullets, electrodes in lead-acid batteries, mordant in dyeing, printing cottons and in making other lead compounds[3,4].

Long term exposure to lead is detrimental to the health of humans and absorption of lead into the body system over a long period of time predisposes to lead poisoning. Lead is a well-known environmental toxicant that is rapidly absorbed in the bloodstream and have adverse effects on the central nervous system, the cardiovascular system, the kidneys, and the immune system[5] . Lead toxicity causes a variety of disorders in the body which includes brain damage, increase in blood pressure, anemia, nephropathy, infertility, indigestion[6].

The cerebellum is a prominent hindbrain structure and is also called the little brain. It lies over the pons and fourth ventricle in the posterior cranium just beneath the occipital lobe of the cerebral hemispheres and also overlies the brainstem extending over much of its dorsal aspect. It is attached to the brainstem by the cerebellar peduncles and together, the brainstem and cerebellum constitute the hindbrain[7]. The cerebellum is concerned with motor coordination of movements, posture, and balance and may be involved in some cognitive tasks such as in attention and language and also helps in the regulation of fear and pleasure responses[8]. The cerebellum is composed of a three-layered cortex and deep nuclei which relay information from the cerebellar cortex to the thalamus[7]. The Purkinje cells of the cerebellum functions as the main output from the cerebellum in its coordination of body movement, balance and posture[9]. The cerebellum, detects and attenuates the difference, or "motor error," between an intended movement and the movement actually performed. The cerebellum uses this information about discrepancies to mediate both real-time and long-term reductions in these inevitable motor errors (the latter being a form of motor learning). As a result, patients with cerebellar damage exhibit persistent errors in ongoing movement.

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^{+} ions (Pb^{2+}) thus concentrating it in the brain[10,4]. Neurons are vulnerable to increases in Reactive Oxygen Species (ROS) levels due to reduced capacity to detoxify ROS[11]. which may be generated by lead intoxication thus altering the histomorphology and neurological functions of the cerebellum. As it is almost impossible to exonerate the effect of lead once it enters the body, preventive measures are the preferred option considering toxicity[12]. Hence, this study is aimed at investigating the consequence(s) of chronic lead acetate administration on possible alteration of the body weight and histology of the cerebellum of rat.

MATERIALS AND METHODS

Experimental Animals: A total of twenty (20) adult Wistar rats of both sexes were used for the experiment. The rats, weighing between 100 – 150 g were purchased from the Central Animal House, Department of Anatomy, College of Medicine, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria. They were housed in clean and well-ventilated plastic cages with, provided with rat pellets and water *ad libitum* for the duration of the experiment. Rats were randomly assigned into four groups (A-D) N=5. D served as control group while the experimental groups were A, B, and C. The animals were maintained under standard conditions of temperature and humidity with alternating 12 h light/dark cycles and allowed to acclimatize for two weeks prior to the onset of the experiment. They were weighed weekly before and during the experiment for close monitoring of their wellbeing.

Chemicals: Lead acetate **[**Formula: $Pb(C_2H_3O_2)_2{\cdot}3H_2O$**]**, purchased from the main research laboratory, University of Ilorin, Kwara state was manufactured by May & Baker Ltd, Dagenham England, Batch number L54/18/90. Ketamine hydrochloride was manufactured by Rotex Medica, Trittau, Germany, batch number 80225.

Preparation and administration of Lead acetate: A total amount of 30 g of lead acetate was weighed accurately and dissolved in 1liter of distilled water to form the stock solution. The lead acetate is readily soluble in water and with the mixture well shaken, the solution of each group of experimental animals was calculated depending on their dose and average body weight and was administered orally once a day early in the morning for 21 days using oral gavage.

Research design: Following two weeks of acclimatization, the twenty (20) Wistar rats were randomized into four groups of five (5) each (Groups A, B, C, and D) and treated as follows:
Group A: received lead acetate orally at 400 mg/kgbw once daily for 21 days.
Group B: received lead acetate orally at 800 mg/kgbw once daily for 21 days.
Group C: received lead acetate orally at 1200 mg/kgbw once daily for 21 days.
Group D: Control rats received rat chow and distilled water sham (2 mls/kg) for the period of the study.
The lowest toxic dose of lead acetate (via oral administration) is 790 mg/kg and 1100 mg/kg over 14 days[13]**.** The rats were thereafter monitored daily for any symptoms of toxicity and general wellbeing.

Sample collection and histological preparation: On the 22nd day of the experiment, all animals in all groups were weighed and then euthanized using ketamine hydrochloride (100 mg/kg) i.p. followed by cervical dislocation method after which the brains were quickly dissected out and the cerebellum separated and preserved for histology by fixing in 10% formol calcium for three days before being processed for routine paraffin wax embedding technique.

Histology: The cerebellum from each group was obtained and homologous sampling was assured by obtaining transverse sections of the right cerebellum from each specimen from the paravermal zone portions of the cerebella for uniformity. The tissues were embedded in paraffin wax using molten wax dispenser Leica EG 1150H and then cooled over Leica EG 1150C. Solidified tissues were sectioned using Leica RM 2135 at 5 µm thickness using rotary microtome and then stained with Haematoxylin and Eosin. After staining, the slides were viewed with an Olympus CH (Japan) light microscope with 16x objective. The image capturing was performed with a Sony DSC-W610 digital camera (Japan).

Statistical data analysis: Data obtained from various analyses were expressed as mean ± SD and analyzed using t-test and GraphPad prism version 6.0 windows Graph pad software, SanDiego, California USA. Comparisons between groups and control were made using one-way ANOVA (Analysis of Variance) test with confidence interval calculated at 95% and the level of the statistical significance set at $p \leq 0.05$.

RESULTS

Physical/ General observation: On the first day of administration, animals stretch their heads for few seconds after administration which was followed by reluctance to administration, feeding and drinking of water in subsequent days. On the fourth day of the administration, a death was recorded in group C (1200 mg/kgbw) while administering, frequent defecation and blood discharge from vulva was also noticed. At about a week into administration, animals became less alert, nose became red while administering, beddings got dirty more frequently and another death was recorded in group C (1200 mg/kgbw). Animals in group C were hyperactive when touched while groups A and B showed little or no reluctance to administration.

Two weeks into administration, body weight showed an increase ($P \leq 0.05$) in Groups A, C and Control (D) but was maintained in Group B. More bloody discharge was

observed from vulva in group B animals and in group C, animals showed little or no reluctance to administration. On the 16th day of administration, a death was recorded in group A (400 mg/kgbw) and reduced motor activities was observed as experimental animals in Groups B and C showed reduced movement in their cages following lead administration. On days 18 and 19, animals showed further submission to administration and also reluctance to feeding and water intake.

Weight measurement on the 21st day showed increase in body weight in Groups A, B and D but significant reduction in C.

Table : Effect of Lead acetate administration on the body weight of Wistar rats

GROUPS	Initial Mean Weight (g)	Final Mean Weight (g)	Weight gain	% Weight gain
GROUP A	137.20±1.79	145.60±1.67*	8.40±0.89*	6.12*
GROUP B	128.00±2.45	146.80±0.84*	18.80±1.92*	14.69*
GROUP C	141.60±2.61	125.20±3.90*	-16.40±2.97*	-11.58*
CONTROL(D)	146.80±1.09	190.20±3.49	43.40±2.61	29.56

The table above shows the mean and percentage body weight gain of animals across all the groups during the course of the administration. Data are expressed as mean ± standard deviation of 5 rats in each of the groups. * Significance ($P \leq 0.05$) versus control (D).

The percentage change in weight was calculated using the formula below:

$$\% \text{ change in weight} = \frac{\text{Change in weight}}{\text{Pre-treatment weight}} \times 100$$

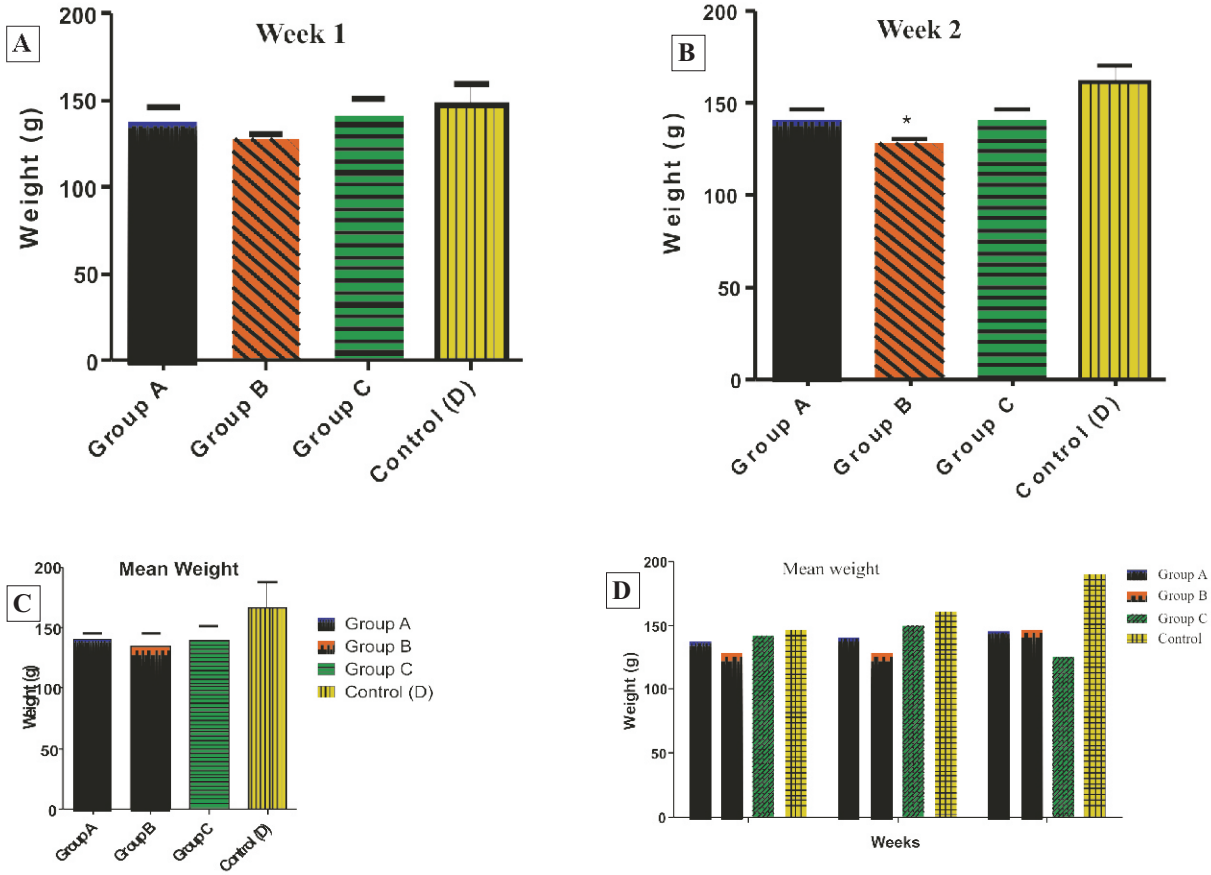

Figure 1: Bar chart showing body weights across groups. The above graphs show the body weight of animals for the administration period of three weeks. A: Week 1; B: Week 2; C: Week 3; D: Mean weight; E: A combined bar chart of the mean weights in the control and treated groups during the period of administration; In B (Week 2), administration of PbAct showed a significant reduction (*) in the body weight of animals in group B at $P \leq 0.05$ compared to the control. Also in C (Week 3), there were significant reductions (*) in body weight of animals in Group A and C at $P \leq 0.05$ compared to the control as a result of PbAct administration. Values are expressed as mean ± standard

deviation of 5 rats in each of the groups. PbAct, Lead acetate. * Significance ($P \leq 0.05$) versus control (D)

Histological alteration of the rat Cerebellum: Figure 2 shows that while the Purkinje cells histology was normal in group D and aligned in a straight line, various dimensions of alterations are observed as depicted in the cerebellum of rats treated with different dosages of lead acetate.

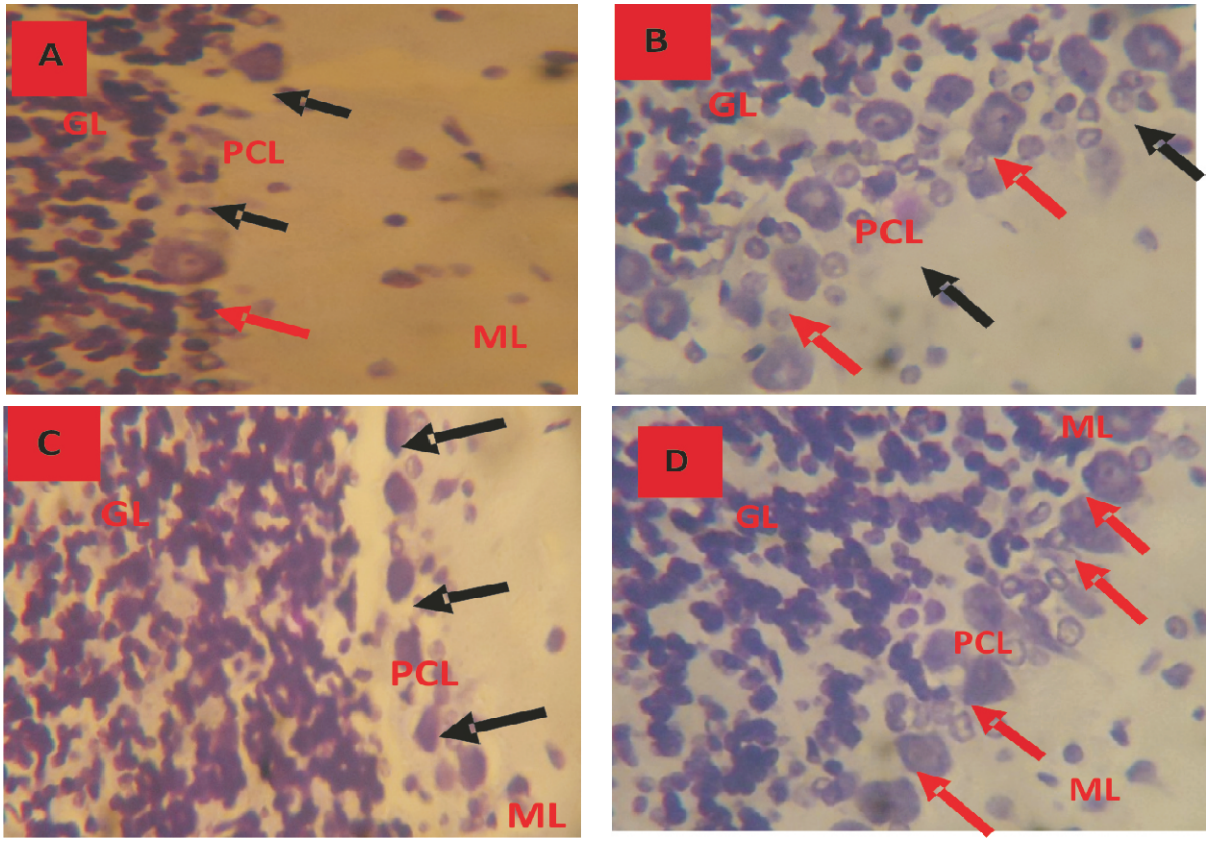

Figure 2: Representative Light Photomicrographs of stained sections of cerebellum of rats: (A) PbAct-treated 400mg/kgbw; Purkinje cells appear deformed and shrunken but few are normal in shape and size (B) PbAct-treated 800mg/kgbw; Few Purkinje cells are normal in shape and size; Purkinje cells appear in multilayers (C) PbAct-treated 1200mg/kgbw; Complete eosinophilia of the Purkinje cell bodies; Purkinje cells are shrunken in size and severely deformed; Nucleli are totally absent (D) Control; Purkinje cells appear normal and are aligned in a straight line.
Red arrow: Normal cells; Black arrow: shrunken cells; ML- Molecular layer, PCL- Purkinje cell layer, GL- Granular layer, PbAct- Lead acetate. H&E. X400

DISCUSSION

This study demonstrated that lead acetate induced weight reduction in treated rats as shown in the group which received the highest dose of lead acetate orally at 1200 mg/kgbw when compared with the control. This is supported by reports of Madkour (2020) who reported that high levels of lead in the body or blood can result in weight loss. This may be due to oxidative stress as a result of formation of free radicals which in turn interfere with body functions such as damage to lipids, proteins, enzymes and DNA. Lead depletes the gluthathione and protein–bound sulfhydryl groups, resulting in the production of Reactive Oxygen Species (ROS)[14].

Our findings also indicated that chronic lead treatment altered the microanatomy of the cerebellum of the Wistar rats. Al-Naimi *et al.*[15] had reported that the alteration of the microanatomy of the cerebellum of rats by lead acetate was shown by the loss of the basophilic staining of the Purkinje cell nuclei[15], which we also demonstrated in this work. Purkinje cells are the predominant output source of the cerebellar cortex, neuronal cell death may lead to poor control and processing of new neuronal protein synthesis necessary for axonal flow and the maintenance of the integrity of the Purkinje neuron[16]. Afferent fibres to the paravermal zone of the cerebellum from which the specimen was obtained come from the spinal cord, brain stem and

cerebral cortex. Cellular degeneration of the Purkinje neurons from this zone might therefore lead to disorder of movements in the affected rats as evident in the reduction of movement which is related to locomotor activity[17]. Lead's ability to damage the brain have been attributed to its ability to penetrate the blood-brain barrier (BBB) by substituting for calcium ions[4].

Compared to other organ systems, the nervous system is regarded to be the most sensitive for lead induced toxicity[18,5]. At higher levels, lead has been reported to cause permanent brain damage and even death[19]. As the nervous system is the primary target for the low levels of lead exposure, more attention has been directed towards lead poisoning and various ameliorative measures have been applied in researches[20]. Unfortunately, lead poisoning cases continue to occur very rampantly and is now of major concern in many countries. The lead-induced Purkinje cell damage may cause cerebellar injury leading to gait, movement and posture impairments[21].

CONCLUSION

The present study demonstrated histological evidence that chronic administration of lead acetate induced weight loss and degenerative changes in the cerebellum of Wistar rats which received different doses of 400, 800 and 1200 mg/kgbw for 21 days. This suggests that more research should be done into the management of lead toxicity and also advise the avoidance of both occupational or environmental exposure.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study. The authors alone are responsible for funding of this research and the content and writing of this paper.

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