Research Article
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Lead-induced oxidative stress in postnatal developing cerebellum of Wistar rats: role of aqueous extract of *Cucumis sativus Linn* and vitamin C

Abstract

Lead (Pb) is one of the most abundant heavy metal whose toxicity causes environmental and health problems, with the brain being highly sensitive to lead toxicity. There is limited literature regarding the protection of the developing brain against lead-induced toxicity. However, plant-derived products with antioxidant activity have been useful in reducing lead-induced neurotoxicity. This study investigated the role of *Cucumissativus* (*C. sativus*) (Cucumber) and vitamin C in lead-induced oxidative stress in postnatal developing cerebellum of Wistar rats.

Twenty-five pregnant Wistar rats weighing between 160 and 180g were divided into five groups (n=5). Group I served as control and received water, group II received 50mg/kg of lead acetate, group III received 200mg/kg aqueous extract of *C. sativus*, group IV received 200mg/kg of *C. sativus* and 50mg/kg of lead acetate and group V received 200mg/kg of vitamin C and 50mg/kg of lead acetate. The interventions were administered orally using an oral gavage from the first day of gestation to postnatal day 21. Neurobehavioural assessment (forelimb grip strength and negative geotaxis) was carried out on pups of day 21 and then sacrificed. Some cerebella of pups of days 1, 7, 14, 21 and 28 were fixed in 10% formol-saline for histological and immunohistochemical evaluations, while others (day 21) were preserved in in phosphate buffered saline at 4°C and pH 7.2 for oxidative stress assays. Data were analysed using ANOVA at $p<0.05$.

Decreased body weight of Pb-treated pups on days 14 and 21, decreased forelimb grip and increased negative geotaxis, increased lipid peroxidation (LPO), decreased glutathione (GSH) levels, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities was seen in the lead-treated day 21 pups compared with the control and other treated groups. Histologically, in the cerebellar cortex, there was persistent external granular layer (EGL) on day 21 in the treated groups compared with the control, decreased molecular layer (ML) thickness and depleted Pc in the Pb-treated day 28 pups, and increased astrocyte population on day 21 pups compared with the control and *C. sativus* groups.Lead induced oxidative stress which caused behavioural deficit and morphological changes in the postnatal developing cerebellum of rats. Aqueous extracts of *Cucumis sativus* and vitamin C decreased the rate at which lead induced neurotoxicity.

Keywords: leadpoisoning, oxidativestress, *Cucumis sativus*, neurobehavioral, cerebellar development

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Introduction

Lead (Pb) is one of the most abundant heavy metals and its toxic effect causes environmental and health problems because of its stability in contaminated site and complexity of mechanism in biological toxicity.[1] Lead is widely used in industries, batteries, paints, plastic, ceramic, secondary foundries and welding. Humans and animals may be exposed to lead through food, contaminated water and air pollution caused by industrial emission and gasoline containing lead compounds.[2] Exposure to lead mainly occurs through the respiratory and gastrointestinal systems. Absorbed lead is stored in soft tissues before its conjugation in the liver, then passed to the kidney where a small quantity is excreted in urine while the remaining accumulates in various body organs, affecting many biological activities at the molecular, cellular and intercellular levels, which may result in morphological alterations,[3,4] affecting the central nervous system (CNS), haematopoietic, renal and reproductive systems.[5,6] The nervous system appears to be the most sensitive and chief target for lead-induced toxicity,[7,8] causing permanent brain damage and even death.[9] Lead has been reported to cross the blood-placental barrier and blood-brain barrier (BBB) in developing animals, accumulating in foetal tissues and causing damage to the brain, including the glial cells.[10–12]Accumulation of lead in the CNS of fetuses and young may result in encephalopathy, presenting major symptoms such as, headache, irritability, poor attention span, dullness, loss of memory, muscular tremor, hallucinations, convulsions, delirium, lack of coordination, ataxia, paralysis and coma.[13]Needleman et al.[14]reported that the developing nervous system of foetuses and young children absorb higher fraction of lead as such, are especially vulnerable to the neurological effects of lead, impairing the transduction of nerve impulses, causing muscular weakness, especially of the exterior muscles, fatigue and lack of muscular co-ordination.[15]

Lead has been reported to induces toxicity via generation of free radicals resulting in oxidative stress,[16,17] disrupting the prooxidant/antioxidant balance, contributing to tissue injury via oxidative damage by modifying biomolecules such as lipids, proteins and DNA.[18] Oxidativestress-related disorders in humans can be prevented

or ameliorated by supplementation with compounds containing high antioxidant activity[19] and the use of medicinal and phenolic plants possessing high antioxidant property for protection against heavy metal toxicity has become of great interest to researchers.[20]*Cucumis sativus* (Cucumber) a seasonal vegetable crop cultivated worldwide consumed fresh in salads or fermented (pickles) or as a cooked vegetable,[21]belongs to the Cucurbitaceae family. *Cucumis sativus* is a very good source of vitamins A, B6, beta-carotene and C, calcium, potassium, magnesium, phosphorus, thiamin, folate, pantothenic acid, manganese copper and dietary fiber, which support its role as a nueroprotective agent.[22] The medicinal benefits of *C. sativus* in promoting healthy hair growth, treatment of skin infections like eczema, expulsion of intestinal and tapeworms and lowering of blood pressure are well documented.[23,24] Pharmacologically, the hepatoprotective,[25] antidiabetic,[26] anti-ulcer and wound healing[27] and antioxidant[28] activities of *C. sativus* have been reported but literature on the use of medicinal plants, which are affordable and easily accessible in ameliorating or protecting Pb neurotoxicity is limited. *Cucumis sativus* contains an anti-inflammatory flavonol called fisetin and antioxidant flavonoids called quercetin which help improves memory and protecting nerve cells in neurodegenerative illnesses such as aging, cancer, Alzheimer's disease and chronic heart disease.[29] Considering the regulatory functions of the cerebellum in motor co-ordination, equilibrium, smooth eye movements and maintenance of muscle tone,[30] the documented toxicity of lead exposure and the antioxidant property of *C. sativus*, this study was designed to investigate the protective effects of aqueous extracts *C. sativus* on lead-induced oxidative stress in the postnatal developing cerebellum of Wistar rat.

Materials and methods

Preparation of aqueous extract of *Cucumis sativus*

Cucumis sativus (Cucumber) was purchased from Bodija market in Ibadan, Oyo state, Nigeria, identified and authenticated at Forestry Research Institute of Nigeria, FRIN (FHI: 112307). The vegetables were washed thoroughly in clean water, cut into smaller pieces and then blended until puree is formed while gradually adding water. The Puree was allowed to steep with hot water for about 15-20 minutes and then strained and the filtrate was preserved in a refrigerator at adequate temperature (2-8°C) until when needed. The extract at a dose of 150 mg/kg body weight was administered orally.

Experimental animals

Twenty five (25) female Wistar rats weighing between 160 and180g were housed in the central animal house, College of Medicine, University of Ibadan, Nigeria. They were acclimatized to laboratory room conditions (12 hour dark/light periods) for two week before the onset of the experiment. The rats were fed with rat chow, and water ad libitum. The rats were mated and pregnancy confirmed by the presence of vaginal plug or smear and taken as the first day of conception. All the animals received humane care according to criteria outlined in the Guide for the Care and Use of Laboratory Animals (prepared by the National Academy of Science and published by the National Institutes of Health, 2011).

Experimental design

The pregnant rats were divided into five groups (n=5) as follows;
Group I: Received distilled water daily and served as control group

Group II: Received 50 mg/kg per lead acetate, daily[31]

Group III: Received 150 mg/kg per *C. sativus*, daily

Group IV: Received 150 mg/kg *C. sativus* + 50mg/kg lead acetate, daily

Group V: Received 200 mg/kg vitamin C[32] + 50mg/kg lead acetate, daily

The interventions were administered orally, between the 8 and 10 am using oral gavage from day 1 of pregnancy through postnatal day 21. The doses administered were calculated using the average weight of the animals in each group. Aqueous extracts of *C. sativus* and vitamin C was administered one hour before administration of lead acetate.

Sacrifice of the experimental animals and brain tissue harvest

After birth, the pups of days 1, 7, 14, 21, and 28 were weighed, neurobehavioural studies was done (on pups of day 21) and sacrificed by quick cervical dislocation. The brains and cerebella of the pups dissected out, weighed and some cerebella fixed in 10% formol-saline for histological and immunohistochemical studies, while others were preserved in phosphate buffered saline at 4°C and pH 7.4 for oxidative stress evaluation.

Neurobehavioural test

Forelimb grip strength test: This test (for muscular strength and balance) involved placing the fore paws of the rat on a horizontally suspended metal wire (measuring about 2mm in diameter and 1m in length), placed one meter above a landing surface filled with soft bedding. The length of time that each rat was able to stay suspended by its forelimb alone, before falling off the wire was recorded. A maximum time of 5 minutes was allotted to each rat for this test.[33]

Negative geotaxis: The unlearned response to gravitational cues is referred to as negative geotaxis.[34]In this test (measure equilibrium), the rats were placed facing downwards and against gravity on a wooden plane, inclined at an angle of 45 degrees to the horizontal. The rats were left to re-orient themselves properly to a position where their tail pointed in the direction of gravity, and the time taken for each rat to successfully re-orient itself properly was recorded. For every rat that failed to re-orient itself, the time was recorded as 60s.[35]

Oxidative stress marker and antioxidants: The cerebellar tissues were homogenized in ice cold phosphate buffer at pH 7.4. The resulting homogenates were centrifuged at 4°C at 1500 rmp for 10 minutes and the supernatant used for oxidative stress evaluation. Protein concentrations of the cerebellar tissue were determined using Bradford method[36] and the following oxidative stress marker and antioxidants were assayed for using spectrophotometer; lipid peroxidation (LPO) in line with the method described by Varshney& Kale[37]reduced glutathione (GSH) in line with the method used by Beutler et al.[38]catalase (CAT) in accordance with Claiborne,[39] superoxide dismutase (SOD) activity following Misra and Fridovich's method[40] and glutathione peroxidase (GPx) activity by the method of Rotruck et al.[41]

Tissue processing for histological and immunohistological studies

Histological preparations: Cerebellar tissues from the pups of all

groups were fixed in 10% formo-saline, processed employing routine paraffin embedding and stained with Haematoxylin and Eosin for histomorphological evaluation. The slides were examined and evaluated under a 500-pixel Leica digital binocular microscope and the following were evaluated in the cerebellar cortex; thickness of the external granular layer (EGL) and molecular layer (ML), and Purkinje cell density and astrocyte population using the computer software, image-j.

Immunohistochemistry: Cerebellar tissues were immunostained with Glial fibrillary acidic protein (GFAP) for astrocyte population (neuroglia) using the Avidin biotin immunoperoxidase method. Briefly, cut formalin-fixed paraffin sections were treated with 3% hydrogen peroxide (H_2O_2) for 15min, to block endogenous peroxidase. Then, washed in phosphate buffered saline(PBS) and treated with GFAP primary antibody (GFAP, mouse monoclonal antibody 1:100 dilution, Leica Biosystems Inc. Ilinois, USA) at room temperature for 60 min. The sections were washed in 3 changes of PBS for 5 min each, incubated with horseradish peroxidase (HRP) secondary biotinylated anti-mouse antibodies and washed in 3 changes of PBS for 5mins. The sections were then incubated with diaminobenzidine (DAB) for 3 to 5min and counterstained with Haematoxylin solution for 2mins and blued briefly. Sections were dehydrated in alcohol, cleared in xylene and mounted in DPX. Images were captured from the cerebellar cortex with a 500-pixel Leica binocular microscope. Astrocyte population was counted using the software, imagej.

Statistical analysis

Data collected was further analysed as mean±SD employing one-way analysis of variance (ANOVA) followed by TukeyPosthoc for multiple comparison using the GraphPad prism 5.0 at $p<0.05$.

Results

We hypothesized that *C. sativus* extracts and vitamin C have no significant protective effect on lead-induced oxidative damage in postnatal developing cerebellum of rats. However, in the course of the experiment, the control and *C. sativus* group remained active throughout and there was no gross deformity in all the groups.

Effect on the body weight

There was progressive increase in the body weight of pups from day 1 through day 28 in both the control and treated groups however, a decreased body weight was observed in the lead acetate-treated group on day 14 and 21 compared with the control and on day 28 compared with *C. sativus* + Pb group at $p<0.05$ (Table 1).

Table 1 Mean body weight in grams of control and treated pups of days 1, 7, 14, 21 and 28

Group	Day 1	Day 7	Day 14	Day 21	Day 28
Control	4.98±0.04	10.6±0.90	19.8±1.09	30.0±0.40	39.8±1.50
Pb	4.82±0.04	9.40±0.55	17.6±1.14[a]	26.2±0.80[a]	36.8±1.64
C.sativus	4.98±0.11	10.2±0.44	19.4±0.89	29.6±0.84	39.8±0.44
C.sativus+Pb	5.04±0.09	10.2±0.44	19.6±1.14	28.8±2.16	41.6±4.78[b]
Vit.C+Pb	4.96±0.05	9.40±0.89	19.4±0.89	29.0±1.00	38.8±1.09

Values (n=5) are expressed as mean±SD in grams. Pb, lead acetate; *C. sativus*, *Cucumis sativus*; Vit.C, Vitamin C; ap<0.05 compared with control, bp<0.05 compared with Pb group.

Effects on Forelimb grip strength and Negative geotaxis of day 21 Pups

There was significant reduction in the forelimb grip strength and increased time spent on negative geotaxis in the Pb-treated group compared with the control at $p<0.05$ but no significant difference between the control and other treated groups at $p>0.05$ (Figure 1).

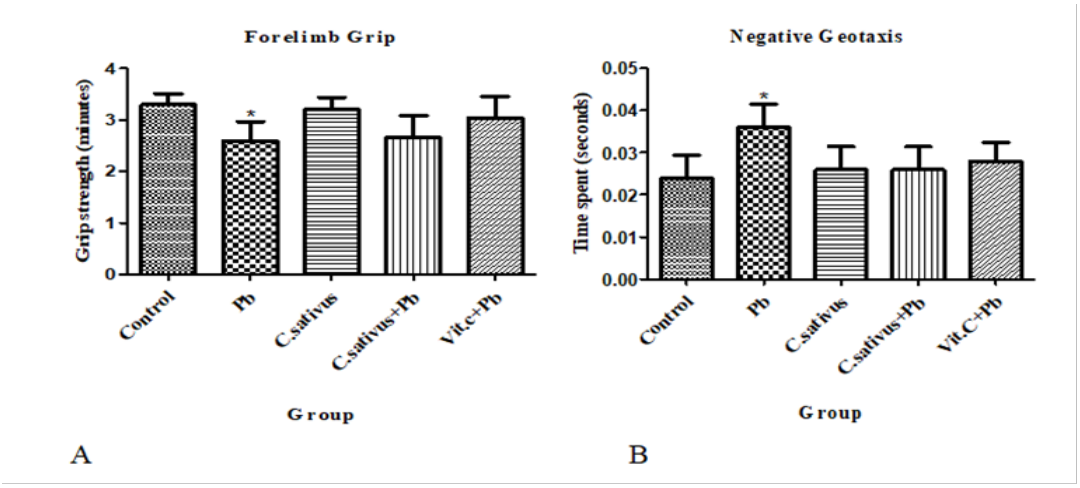

Figure 1(A) Forelimb grip strength (mins) **(B)** Negative geotaxis (secs) of day 21 pups. Values (n=5) are expressed as mean±SD. Pb- lead acetate, *C. sativus*-*Cucumis sativus*, Vit.C- Vitamin C. *p<0.05 vs control.

Effect on oxidative stress markers

Table 2 Oxidative stress markers of the cerebellum (per mg protein) of day 21 pups

Group	LPO	GSH	CAT	SOD	GPx
	µmol/mg	µg/mg	Unit/mg	Unit/mg	µg/mg
Control	0.18±0.03	2.36±0.23	0.88±0.13	0.58±0.07	0.35±0.04
Pb	0.34±0.05[a]	0.23±0.05[a]	0.39±0.08[a]	0.33±0.04[a]	0.15±0.03[a]
C. sativus	0.15±0.04[b]	2.12±0.23[b]	0.89±0.10[b]	0.50±0.03[b]	0.33±0.01[b]
C. sativus+Pb	0.21±0.04[b]	0.98±0.17[a,b]	0.54±0.18	0.48±0.03[a]	0.27±0.01[a,b]
VitC+Pb	0.24±0.03[b]	0.64±0.07[a,b]	0.56±0.13[b]	0.43±0.03[a]	0.25±0.01[a,b]

Values (n=5) are expressed as mean±SD. Pb, lead acetate;*C. sativus*,*Cucumis sativus*;Vit.C, vitamin C; CAT, catalase; LPO, lipid peroxidase; SOD, superoxide dismutase; GSH, glutathione;GPx, glutathione peroxidase; ap<0.05 compared with control, bp<0.05 compared with Pb group.

Effects on the thickness of the External granular layer (EGL) of the cerebellar cortex

There was no significant difference in the thickness of the external granular layer of cerebellar cortex of all of the groups on days 7 and 14 pups at p>0.05 (Table 3, Figures 2 & 3). However, the EGL persisted in the treated groups (thicker in the Pb-and VitC+Pbtreated groups) compared with the control group of day 21 pups (Figure 4).

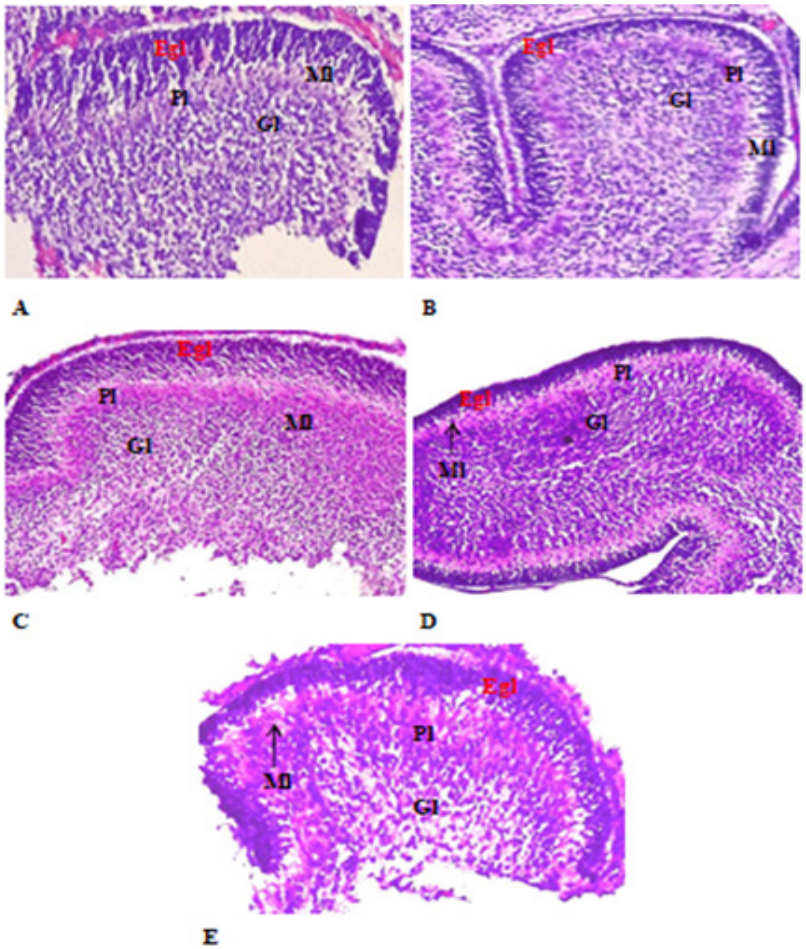

Figure 2 Photomicrograph of the cerebellar cortex of day 7 pups stained with H&E, with magnification x100. There was no significant difference in the Egl thickness in the control and treated groups.

A, Control; B,Pb-treated; C,*C. sativus*; D,*C. sativus*+Pb and E,Vit.C+Pb. Egl, external granular layer; Ml, molecular layer; Pl, purkinje layer;Gl, granular layer

Citation: Imosemi IO, Adu YO, Owoeye O, et al. Lead-induced oxidative stress in postnatal developing cerebellum of Wistar rats: role of aqueous extract of *Cucumis sativus Linn* and vitamin C. *MOJ AnatPhysiol.* 2020;7(4):104–113. DOI: 10.15406/mojap.2020.07.00299

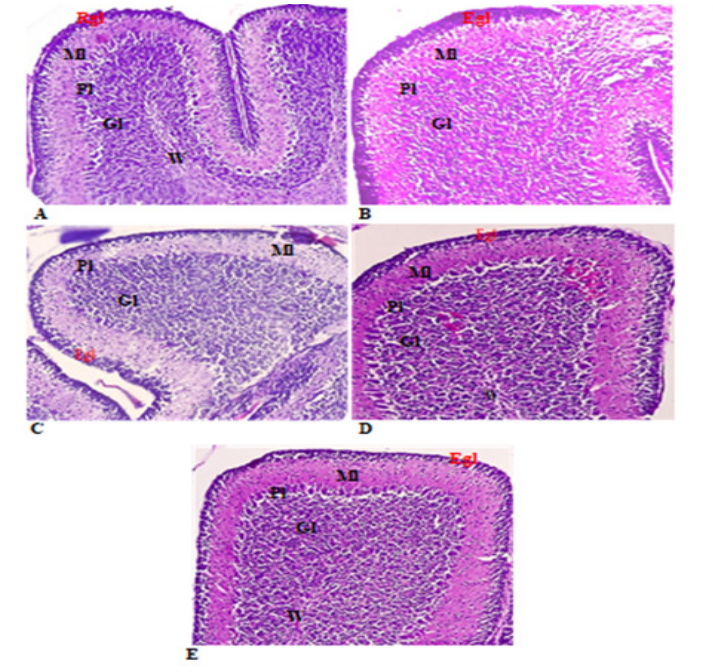

Figure 3 Photomicrograph of the cerebellar cortex of day 14 pups stained with H&E, with magnification x100. There was no significant difference in the Egl thickness in the control and treated groups.

A, Control; B,Pb-treated; C,*C. sativus*; D,*C. sativus*+Pb and E- Vit.C+Pb. Egl, external granular layer; Ml, molecular layer; Pl, purkinje layer;Gl, granular layer;W, white matter

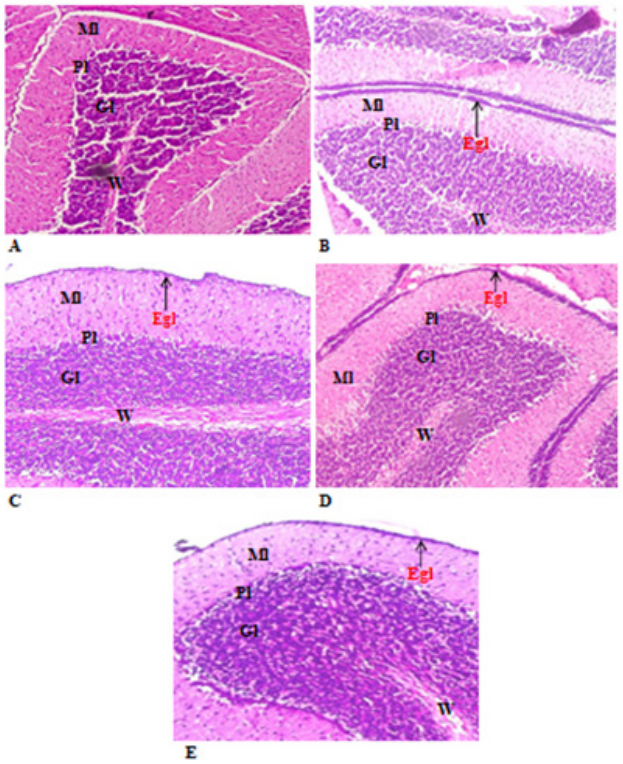

Figure 4 Photomicrograph of the cerebellar cortex of day 21 pups stained with H&E, with magnification x100.

A, Control group with absent Egl; B,Pb group with 3-4 cell-layer Egl; C, *C.sativus*group with 1-2 cell-layer Egl; D,*C. sativus*+Pb group with 2-3 cell-layer Egl; E,Vit. C+Pb group with 2-3 cell-layer Egl;Egl, external granular layer; Ml, Molecular Layer; Pl, purkinje layer;Gl,granular layer;W,white matter

Citation: Imosemi IO, Adu YO, Owoeye O, et al. Lead-induced oxidative stress in postnatal developing cerebellum of Wistar rats: role of aqueous extract of *Cucumis sativus Linn* and vitamin C. *MOJ AnatPhysiol.* 2020;7(4):104–113. DOI: 10.15406/mojap.2020.07.00299

*C.sativus*group with 1-2 cell-layer Egl, D- *C. sativus*+Pb group with 2-3 cell-layer Egl. E- Vit. C+Pb group with 2-3 cell-layerEgl. Egl-External Granular Layer, Ml- Molecular Layer, Pl-Purkinje Layer, Gl- Granular Layer, W- White Matter.

Effect on the thickness of the molecular layer

Decreased molecular layer (ML) thickness was seen in the Pb, *C. sativus*+Pb, Vit.C+Pbon day 28 pups when compared with control and *C. sativus* groups at p<0.05 (Table 4, Figure 5).

Effect on the Purkinje cell density

There was a non-significant decrease in the Purkinje cell (Pc) density of Pb and vit C groups of day 28 pups compared with the control and *C. sativus* groups at p>0.05 (Table 5, Figure 5).

Immunohistochemical evaluation

Astrocyte population: Using the Glial fibrillary acidic protein (GFAP) immunostain, there was increased astrocyte population in the cerebellar cortex of the treated groups but significantly in the Pb group compared with the control group on day 21 (Table 6, Figure 6).

Table 3 Thickness of the external granular layer (mm) of the cerebellum of day 7 and 14 pups

Groups	Control	Pb	*C. sativus*	*C. sativus*+Pb	Vit.C+Pb
Day 7	0.07±0.03	0.05±0.01	0.07±0.03	0.05±0.01	0.04±0.00
Day 14	0.02±0.00	0.03±0.01	0.02±0.01	0.02±0.01	0.02±0.01

Values (n=5) in mm are expressed as mean±SD. Pb, lead acetate;*C. sativus*, *Cucumis sativus*;Vit.C,Vitamin C; p>0.05

Table 4 Thickness of the molecular layer (mm) of the cerebellum of day 28 pups

Groups	Control	Pb	*C. sativus*	*C. sativus*+Pb	Vit.C+Pb
Day 28	0.30±0.06	0.17±0.04[a]	0.28±0.03[b]	0.23±0.03[a]	0.22±0.03[a]

Values (n=5) in mm are expressed as mean±SD. Pb, lead acetate;*C. sativus*, *Cucumis sativus*;Vit.C,Vitamin C. ap<0.05 compared with control, bp<0.05 compared with Pb group.

Table 5 Purkinje cell (Pc) density of day 28 pups of the control and treated groups

Groups	Control	Pb	*C. sativus*	*C. sativus*+Pb	Vit.C+Pb
Pc density/1.3mm	17.6±2.30	15.0±3.16	18.8±3.49	16.0±4.06	14.8±2.77

Values (n=5) are expressed as mean±SD. Pb, lead acetate; *C. sativus*,*Cucumis sativus*;Vit.C,Vitamin C; p>0.05.

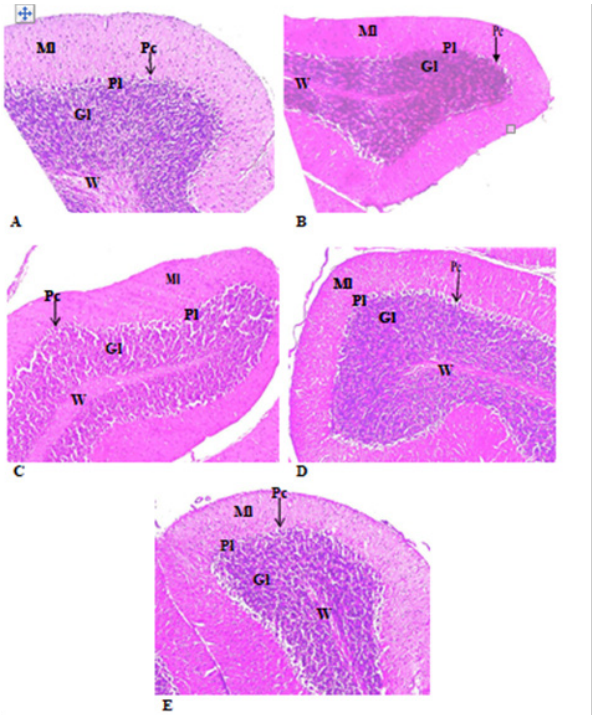

Figure 5 Photomicrograph of the cerebellar cortex of day 28 pups stained with H&E, with magnification x100.

A, Control group; B, Pb group with decreased ML thickness and depleted Pc; C,*C. sativus* group; D,*C. sativus*+Pb group with decreased ML thickness; E,Vit. C+Pb group with decreased ML thickness; Ml, molecular layer; Pl,purkinje layer; Gl, granular layer;W, white matter; Pc,purkinje cell

Citation: Imosemi IO, Adu YO, Owoeye O, et al. Lead-induced oxidative stress in postnatal developing cerebellum of Wistar rats: role of aqueous extract of *Cucumis sativus Linn* and vitamin C. *MOJ AnatPhysiol.* 2020;7(4):104–113. DOI: 10.15406/mojap.2020.07.00299

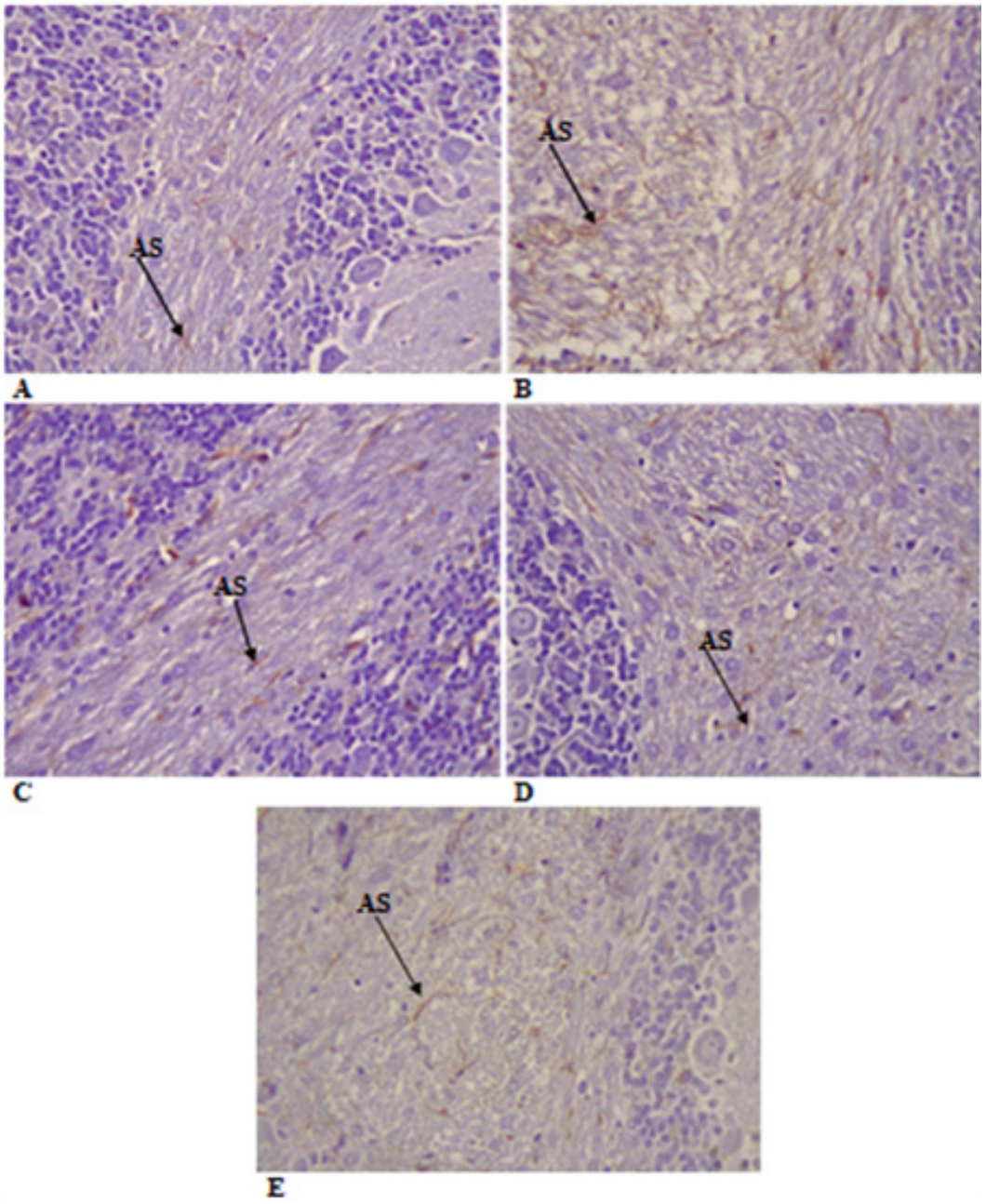

Figure 6 Photomicrograph of the cerebellar cortex showing the astrocyte population of day 21 pups stained with GFAP antibody, with magnification x400.

A, Control group; B, Pb group with increased astrocyte population; C,*C. sativus* group; D,*C. sativus*+Pb group; E,Vit. C+Pb group; AS, Astrocyte

Table 6 Astrocyte count in the cerebellar cortex of day 21 pups

Groups	Control	Pb	*C. sativus*	*C. sativus*+Pb	Vit.C+Pb
Pc density/1.3 mm^2	26.8±7.76	46.2±6.22[a]	37.8±5.12	40.0±7.91	39.4±5.86

Values (n=5) are expressed as mean±SD. Pb, lead acetate; *C. sativus*, *Cucumis sativus*;Vit.C,Vitamin C; ap<0.05 compared with control

Citation: Imosemi IO, Adu YO, Owoeye O, et al. Lead-induced oxidative stress in postnatal developing cerebellum of Wistar rats: role of aqueous extract of *Cucumis sativus Linn* and vitamin C. *MOJ AnatPhysiol.* 2020;7(4):104–113. DOI: 10.15406/mojap.2020.07.00299

Discussion

The naturally occurring sources of antioxidants used in various studies have demonstrated promising outcomes with regard to their effects on metal-induced toxicity.[42–45]The present study demonstrated the ability of aqueous extract of *Cucumis sativus* in protecting or ameliorating oxidative stress induced by lead acetate in the post natal developing cerebellum of rats.

Body weight changes serve as a sensitive indication of the general health status of animals[46] and used as an adverse effect of chemical and drugs.[47] The mean body weight of rats in this study showed reduction in the Pb- treated group of days 14, 21 and 28 pups when compared with control. This finding agrees with the study of Nwokochaet al.[48]who reported that rats that are continuously exposed to heavy metals such as cadmium, arsenic, mercury and lead usually results in reduction in the body weight. The reduction in body weight might be due to anorexia which occurs as a result of exposure to lead.[49]Haouaset al.[50]also reported that lead toxicity causes malabsorption of nutrients and less efficient metabolic processes resulting in decreased weight gain. However, administration of *Cucumis sativus*and vitamin C improved the body weight compared with the Pb-treated group probably due to the antioxidant property of *Cucumis sativus* and vitamin C.

The forelimb grip measures the muscular strength of rat. In this study, Pb group had decreased muscular strength and increased time of negative geotaxis when compared with the control. This decrease could be as a result of oxidative stress, affecting the motor coordination by the cerebellum. The result of the forelimb grips agrees with the study of Owoeye& Ojora[31]on the protective effect of tomato pomace powder on lead-induced oxidative stress in adult Wistar rats where they reported similar result but disagreed with the negative geotaxis in which they reported no significant difference in all the groups. *Cucumis sativus* and vit. C ameliorated the effect of lead acetate on the neurobehavioral study.

Heavy metals, including lead induce oxidative stress by generation of free radicals and this has been the focus of toxicological research in the decade, to evaluate the possible mechanism of the toxicity.[51] Oxidative stress occurs as a result of an imbalance between the productions of reactive oxygen species (ROS) and the cell's ability to reduce ROS, detoxifyreactive intermediates and repair damage that may occur in cellular molecules. This imbalance may occur as a result of increased ROS production, a decrease in defense mechanisms or both.[52] Even though the exact mechanism of lead toxicity is not very well known, however, many researches had reported that it can cause generation of ROS and inhibits the antioxidant enzyme activities in tissues.[53] Our study showed an increased LPO in the cerebellum of day 21 pups exposed to lead, corroborating the reports of El-Masry[54]and Bennet et al.[55]The observed increased in LPO significantly decreased GSH levels, and CAT and SOD activities. The reduced antioxidant activity agrees with the findings of Hatice et al.[56]who reported significant inhibition of SOD, CAT, GPx and glutathione-S-transferase activities and increased malondialdehyde (MDA) concentration (a bye-product of lipid peroxidation in rat brain exposed to lead nitrite and mercuric chloride. El-Sokkary et al.[16]and Wang et al.[57]also reported decreased antioxidant function as a result of oxidative stress due to lead-induced neurotoxicity. There was significant increase in GSH levels, and SOD, CAT and GPx activities with a corresponding reduction in LPO in the developing rat cerebellum when extract of *C. sativus* and vit C were administered to lead-treated rats. This improvement in the antioxidant system could be attributed to the antioxidant property of *C. sativa*and vit. C. Takeota& Dao[58]reported that flavonoids and phenols are antioxidant agents which help in mopping up free radicals and Saidu et al.[59] reported that *C. sativus* contains alkaloid, glycosides, saponins, flavonoids, phenol, tannins and terpenes.

Histology examination of cerebellar cortex section on days 7, 14, 21 and 28 pups, showed no significant difference in the thickness of the external granular layer (EGL) of cerebellar cortex of all the groups on days 7 and 14. There was persistent EGL in the experimental groups compared with the control on day 21. The EGL is the most metabolically active part of the developing cerebellar cortex, whose differentiation gives rise to majority of the cells (outer stellate, basket, Golgi and granule cells) of the cerebellar cortex. The EGL disappeared on day 20 after birth in rats.[60]The persistent EGL observed in the treated groups may be due to delayed differentiation and migration of the cells of the EGL probably as a result of oxidative stress induced by lead. The ML becomes the most superficial layer of the cerebellar cortex after the complete disappearance of the EGL[61,62] and its thickness is determined by the amount of cells and fibres present[63] but mainly by gradual growth of new parallel fibres.[64] There was a significant reduction observed in the thickness of molecular layer in Pb group when compared with control. The mechanism involved in the reduction of the thickness of the ML in the Pb-treated animals is not very clear but neuronal cell death induced by oxidative stress in the GL or delayed parallel fibre formation caused by delayed granule cell formation could have affected the density of unmyelinated parallel fibres in the ML, hence, the reduction. There was non-significant decrease in the number of Purkinje cells in the Pb- and vit. C+Pb-treated groups which may be to oxidative stress induced by Pb exposure. Oxidative stress has been implicated in the loss of Purkinje cells.[65–67]

The developing nervous system is preferentially vulnerable to lead exposure with alteration in neuronal and glial cells of the brain. In the central nervous system (CNS), astrocytes express GFAP which is important in many CNS processes, including cell communication and the functioning of the blood brain barrier, formation of glial scars and repair after CNS injury.[68,69]Glial fibrillary acidic protein expression can be regarded as a sensitive and reliable immunohistochemical marker that labels most, if not all, reactive astrocytes that are responding to CNS injuries.[70] The present study revealed there was a significant increase in astrocyte population in Pb group when compared with control indicating astrogliosis. However, administration of *Cucumis sativus* and vit. C decreased the overexpression of GFAP probably by mopping up free radicals and reducing oxidative stress induced by Pb.

Conclusion

The results obtained from this study showed that rats treated with lead acetate during pregnancy till postnatal day 21, induced oxidative stress causing behavioural and morphological alterations in the developing cerebellum of the pups, with the observed decrease in body weight, reduced muscular strength, increased negative geotaxis, increased LPO, and decreased GSH, SOD, CAT and GPx, reduced ML thickness and Purkinje cells, and astrogliosis. However, the administration of aqueous extract of *Cucumis sativus* and vitamin C ameliorated the damage caused by lead in the developing rat cerebellum.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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