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Original Article

# The effects of aqueous extract of ocimum gratissimum on the cerebellum of male wistar rats challenged by lead acetate

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SUMMARY

*Objectives:* Lead acetate (LA) is a known toxicant, and its exposure in the environment has been on the increase in recent times, leading to oxidative stress and tissue damage. Based on this background, we investigated the role of the antioxidative properties of *Ocimum gratissimum* (OG), a potent medicinal plant, in ameliorating and protecting the brain from lead acetate-induced cerebellar damage via the assessment of oxidative stress parameters and brain histology.

*Methods:* Thirty-five adult male Wistar rats weighing 145–200g divided into five groups were used for this experimental study. Groups 1, 2, and 3 served as positive and negative controls and were administered sterile water, 252.98 mg/kg body weight of aqueous leaves extract of OG and 120 mg/kg of LA for 21 days. Furthermore, following the administration of LA, rats in groups 4 and 5 were treated with 125 and 250 mg/kg body weight of OG. OG and LA were administered orally for 42 days. Oxidative stress parameters, as well as histoarchitectural investigations following LA and OG treatment, were analysed.

*Results:* Results showed normal histoarchitecture of the cerebellum in control and group 2 animals (OG only). In group 3

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animals that received LA only, we observed severe stages of neurodegenerative features in the Purkinje, granular cell molecular cell layers of the Cerebellum. Also, results from groups 4 and 5 rats that were co-administered with LA and OG revealed regenerative tendencies and an increase in the neuronal cell density of the cerebellum. Nevertheless, we recorded an increase in the brain content of malondialdehyde and decreased antioxidant status (catalase (CAT), glutathione (GSH), and sodium dismutase (SOD)) following LA administration. OG attenuated this heightened MDA activity and depletion of the antioxidant status.

*Conclusion:* Overall, these findings suggest that oral administration of aqueous extract of OG improved cerebellar neurotoxicity and degeneration in rats exposed to LA by modulating oxidative damage.



## 1. Introduction

In Nigeria, exposure to occupational lead is the primary source of lead poisoning [1–3], with the most threatening risk emanating from welding, painting, printing, combustion, as well as copper and zinc smelting [4] thus, posing a significant risk to workers and their families (Shaffer and Gibert, 2017). Lead-exposed individuals can bring lead dust home on clothes or skin to expose others. Occupational lead intoxication occurs via the respiratory route, where absorption is more rapid and complete than other percutaneous, ingestion, and transdermal routes [5]. After lead absorption from the gut or lungs, it enters the bloodstream - its half-life in the blood of an adult is about 28–36 days [6]. About 99% of lead binds to red blood cells (RBC), while the remaining 1% attaches to blood plasma [6]. They are then circulated to the body, where they cause tissue and organ damage.

In the brain, lead exposure mediates neuropathologic conditions by altering cellular metabolism gene transcriptions and by inducing abnormal protein accumulation, and malfunctions in messenger systems as well as apoptosis [7]. Lead transverses the blood-brain barrier (BBB) by substituting for calcium ions and binds to endothelial cells, causing dysfunctions in BBB's tight junction and exchange of molecules across the BBB [8]; Gorkhali, 2017). Several studies have discovered that lead exposure heightens the risk of Alzheimer's disease (AD) by causing amyloid precursor protein (APP) overexpression [9–11]. In addition, patients who reported neurofibrillary tangles were shown to have high levels of lead in the brain [12], while in animal models, lead exposure-induced distortions in hippocampal granule cell neurogenesis as well as hippocampal neuronal degeneration [13], and synaptic injuries [14].

Lead exerts its toxic potential by targeting heme synthesising enzymes, thiol-containing antioxidants, and oxidative enzymes, including CAT, GSH, MAD, and SOD. One of the significant lead markers of

**Table 1**
Experimental design for lead acetate and *Ocimum gratissimum* administration to experimental rats

| Groups | Dosage |
|---|---|
| Group I (control group(n=7) | Animals were administered 0.1 mL orally of 0.9 % sterile water orally for 42 days. |
| Group II (n=7) | Animals were administered 250mg/kg/bwt of OG orally for 21 days. |
| Group III (n=7) | Animals were administered 120mg/kg/bwt of LA orally for 21 days. |
| Group IV (n=7) | Animals were administered 120 mg/kg/bwt of LA orally for 21 days, followed by 125 mg/kg/bwt of OG extract orally for 21 days. |
| Group V (n=7) | Animals were administered 120 mg/kg/bwt of LA orally for 21 days, followed by 250 mg/kg/bwt of OG extract orally for 21 days. |

oxidation is lipid peroxidation, which results in RBC hemolysis, oxidative stress, and tissue degradation [15,16]. In light of this, targeting this redox imbalance may be imperative in mitigating lead-induced deleterious effects. However, due to the recent trends in phytochemicals to combat oxidative damage and neurotoxicity, we employed OG to ameliorate LA-induced toxicity.

*Ocimum gratissimum* (OG), commonly called scent leaf, is a plant employed by different ethnic groups in Nigeria and other parts of the world as a culinary spice and vegetable to prepare soup and stew [17]. It is also applied in folklore medicine to manage and treat several pathologies such as common cold, body aches, pneumonia, diarrhoea, anemia, inflammation, and bacterial and fungal infections [18]. These medicinal potentials of OG are linked to its phytochemical constituents. Previously, we demonstrated that the OG attenuated chronic oxidative damage induced by LA on the spleen, thymus, and blood by reducing MDA levels and increasing antioxidant status [19] and mitigated restrained stress in rat models [20].

The phytochemical screening of the OG plant demonstrated the presence of essential oils called Ocimum oil and non-phlobatannins; Ocimum oil is made up of 48.1% thymol, 12.5% p-cymene, and trace elements [21]. According to [22]; OG contains other potent constituents such as alkaloids, tannins, saponins, polyphenols, flavonoids, oligosaccharides, and eugenols. Flavonoids possess anti-inflammatory, cytotoxic, antidepressant, and antioxidant activities [23]. Apigenin, a flavonoid in the leaves of OG, is used to relieve nerve pains [24], while eugenol, another powerful component of OG, was previously reported to protect neuronal activity cells from excitotoxic and oxidative injury in mouse cortical cultures [25]. Furthermore, it prevented the brain from 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in mouse striatum and provided a neuroprotective effect against hippocampal CA1 neuronal ischemia-induced injury in gerbils. It also proved to protect against acrylamide-induced neuropathy and chlorpyrifos-induced brain toxicity in rats, which may at least in part be attributed to its antioxidative properties.

Nevertheless, due to the numerous applications of OG in ayurvedic medicine, a study modified OG seeds as an orally disintegrating tablet because of their availability, non-toxicity, easy manufacture, and purchase [26]. Concordantly, another relevant question of scientific importance is the side effects of OG. Interestingly, after a thorough literature search, no study has reported the deleterious potentials of OG, but several studies reported promising results in the application of OG in therapeutic medicine [18,19,24,25,27]. Despite these breakthroughs, there is no effective therapy yet that could mitigate the neurotoxic potentials of lead and its pathological manifestations; hence this study seeks to justify the use of OG in ameliorating LA-induced cerebellar oxidative damage. In this study, we evaluated the histoarchitectural, cytometrical analysis, and biochemical changes following OG, LA, and co-administration of LA and OG in the cerebellum of adult Wistar rats in view to assess its ameliorative potential in LA-induced injury.

## 2. Materials and methods

### 2.1. Animal care

Adult male Wistar rats weighing 145g–200g were used for this study. The rats were obtained and bred in the animal house of Delta State University, Nigeria. They were kept in plastic cages in a well-ventilated, disease-free animal core facility with free access to water and pelletised rat chow under controlled light conditions. Rats were acclimatised for two weeks before the commencement of extract administration. All experimental and laboratory procedures were approved by the Research Ethics Committee on Animal use of Delta State University Abraka, Nigeria, with reference number (REF/FBMS/DELSU/18/33) and performed following the Animal Reforms Guidelines and the National Institute of Health Guide for Care and Use of Laboratory Animals.

### 2.2. Source of lead acetate

LA of 100% purity manufactured by BDH Chemical Ltd England was obtained from a chemical shop in Onitsha, Anambra State, Nigeria, with batch number R: 61-33-48/22-62-50. LA was administered at a dose of 120mg/kg/bwt [19].

### 2.3. Plant identification and extraction

Fresh OG leaves were obtained from farmland in Owo, Nigeria, and were identified and authenticated at the Department of Botany, Ekiti State University, Ado-Ekiti, Nigeria, and specimen/herbarium number UHAE 0155 was obtained. OG leaves were extracted following the methods described in our previous study [19], and the dose of OG administered was also based on our previous report. The $LD_{50}$ of OG is 1264.9 mg/kg [28].

### 2.4. Experimental design

This was an experimental study involving thirty-five adult male Wistar rats randomly divided into five groups of 7 animals each. Group, I served as the control group and was administered sterile water throughout the experiment. Group II and III served as the negative treatment group and received 250mg/kg/bwt of aqueous OG extract and 120mg/kg/bwt of LA for 21 days. Further, groups IV and V, which constituted the treatment group, were administered 120mg/kg/bwt of OG for 21 days, followed by 125mg/kg/bwt and 250 mg/kg/bwt of OG aqueous extract, respectively, for an additional 21 days (see Table 1 below). Both LA and OG aqueous extract was given to the rats orally using a 1ml syringe with an in-tube sterile cannular. All administrations were carried out orally once per day between 8:00 am, and 10:00 am daily.

### 2.5. Procedure for animal euthanasia and brain collection

Following the completion of LA and OG administration, the rats were euthanised using sodium pentobarbital. The dose of sodium pentobarbital used was administered according to the bodyweight of the rats (0.1ml/100g of rat). After that, rats for histological assessments were perfused by directly passing phosphate buffer and 4% paraformaldehyde through the heart. They were post-fixed in paraformaldehyde until tissue processing. Also, the brains of other rats were harvested and homogenised for biochemical analysis.

### 2.6. Biochemical analysis

Brain samples from the hippocampus and cerebellum were homogenised in a mortar and pestle with a pinch of acid-washed sand. A total of 5mls of normal saline (0.9%) was added sequentially during the homogenisation process. The homogenates were centrifuged at 350rpm for 5 minutes with a centrifuge. The clear supernatants were collected using a micropipette and transferred into an empty specimen container, and refrigerated until needed for the assays.

#### 2.6.1. Glutathione (GSH) level assessment

Glutathione activity was determined by [29]. The tissues were thawed in PBS Lysis buffer (pH 7.4) containing 320mM sucrose, 1% of 1.0 M Tris – HCL (pH = 8.8), 0.098 mM$MgCl_2$, 0.076 mM EDTA, phosphatase inhibitor cocktail. The tissues were homogenised by ten quick pulses using the hand homogeniser. The homogenates were centrifuged at 14,000× g for 10 minutes to remove cellular debris, and the supernatants were separated to determine the total protein concentration using the BCA method. A detection kit from Arbor assays (K006 – H1) was used to measure concentrations of total reduced and oxidised forms of GSH. Samples were then assayed for GSH and presented in μm/ml.

#### 2.6.2. Malondialdehyde (MDA) level assessment

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), according to the method of Beuge and Aust. 1.0ml of tissue homogenate was added with 2.0ml of the TCA – TBA – HCL reagent (15% (w/v) TCA, 0.37% (w/v) TBA and 0.25N HCL). The contents were boiled for 15 minutes, cooled, and centrifuged at 1000g to remove all precipitates. The absorbance was read at 535 nm, and the MDA concentration of the sample was calculated using the extinction coefficient of 1.56 x $10^5$ $M^{-1}$ $Cm^{-1}$

*2.6.3. Measurement of superoxide dismutase (SOD) activity*

The SOD activity was determined by [30] method. 0.4ml aliquot of the supernatant was added to 5ml of 0.05M carbonate buffer (Ph 10.2) and equilibrated in the spectrophotometer for 2–3 minutes. This reaction procedure is based on the ability of SOD to inhibit the autoxidation of adrenaline.

*2.6.4. Assessment of catalase (CAT) activity*

Cohen et al. [31] method was adopted to assess CAT activity. Aliquots of the homogenate supernatant (0.5ml) were added into ice-cold test tubes, while the blank contained 0.5ml distilled water. The reaction was initiated sequentially at a fixed interval; 5ml of cold 30mm hydrogen peroxide was mixed thoroughly. The samples and the blank were taken one at a time, and 0.7ml of 0.01m potassium permanganate was added, which was mixed twice by inversion and absorbance at 480nm, and it was read for 30–60 seconds. The spectrophotometer standard was prepared by adding 7ml of 0.01ml potassium permanganate to a 5.5ml of 0.05m phosphate buffer with pH 7.0 and 1ml of 6M-tetraoxosulphate (IV) acid solution. The spectrophotometer was zeroed with distilled water, and the enzyme activity was estimated.

*2.7. Histological procedure*

Brain tissues were processed using a standard histological technique described by [32]. It entails fixing the brain tissues in 4% paraformaldehyde, dehydration in ascending grades of alcohol, clearing in xylene, and impregnating tissues in molten paraffin wax. Tissues were embedded using molten paraffin wax and embedding moulds and sectioned using the rotary microtome. Brain slices from the hippocampus and cerebellum of each treated rat were stained with hematoxylin and eosin, a routine histology stain, and cresyl fast violet stain (CFV), which stains for nissl substances in neurons.

*2.8. Cytometry analysis and photomicrography*

Cell diameter was determined using the method described by [33]. The Purkinje cells of the cerebellum were counted using Digitizer image analysis software. Specific fields of the brain slides were snapped at × 100 magnifications with the aid of an MD900am microscope digital camera and uploaded into the image area of the software. A marker tool was used to click on the image on the cells to mark and count the number of each cell, and the sum was automatically displayed in the statistics window. The stained tissue images were captured using a digital microscopic eyepiece, 'SCOPETEK DCM 500, 8.0 MEGA PIXEL', connected to a computer. The stained slides were interpreted with a light microscope and analysed for histological changes across the groups.

*2.9. Statistical analysis*

The biochemical and cytometry analysis data were presented as means ± SEM and analysed using one-way ANOVA. Student Newman Keuls (SNK) post − hoc test for multiple comparisons was carried out to determine mean significant differences between groups with SPSS version 21. Levels of statistical significance were set at $P < 0.05$.

## 3. Results

*3.1. Biochemical evaluations*

*3.1.1. Effect of* Ocimum gratissimum *on the level of MDA in the cerebellum of rats exposed to Lead acetate*

Fig. 1 below shows the effect of OG treatments on MDA levels in the brain regions of rats exposed to lead acetate. Rats treated with extracts of OG (group 2) had significantly lower MDA levels than the controls and all other treated groups. Conversely, exposure to lead acetate alone (group 3) significantly ($P>0.05$) rapidly increased MDA levels compared to the control rats (group 1 and group 2). However, the treatment of lead acetate-exposed rats with high and low doses of OG extract (Groups 4 and 5) brought about a significant ($P>0.05$) decrease in MDA level compared to rats maintained on LA alone

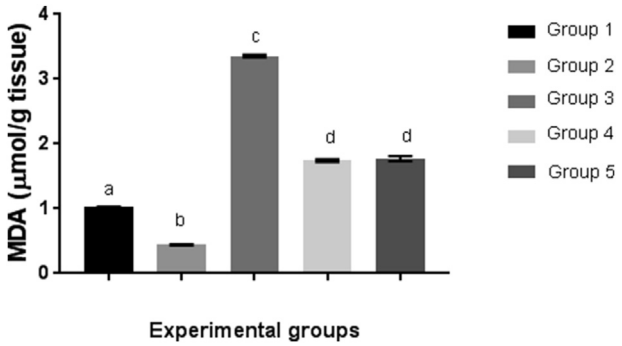


**Figure 1.** Effects of Ocimum gratissimum on MDA level in the cerebellum of rats exposed to Lead acetate Values are presented as Mean ± SEM. n=7. Values with different superscripts differ significantly ($P<0.05$).

(Group 3), though not to a level comparable with the control or rats administered OG extract independently. Thus, the result shows that MDA level in the brain was significantly increased by exposure to LA, but the treatment of lead acetate-exposed rats with high and low doses and high doses of OG ameliorated the lead acetate-induced lipid peroxidation by reducing MDA level.

*3.1.2. Effects of* Ocimum gratissimum *on level of GSH in the cerebellum of rats exposed to Lead acetate*

Fig. 2 below shows the effect of OG treatment on the level of GSH in the cerebellum of lead acetate rats. Rats treated with extract of OG (group 2) had significantly higher GSH levels than the control and other treated groups. Conversely, exposure to lead acetate alone significantly ($P>0.05$) decreased GSH compared to the control groups (group 1 and group 2). The treatment of lead acetate-exposed rats with high and low doses of scent leaf extract (groups 4 and 5) led to a significant ($P>0.05$) increase in GSH level compared to rats administered on lead acetate alone (group 3). Thus, the result shows that the GSH level in the brain was significantly reduced by exposure to lead acetate. Still, the treatment of lead acetate-exposed rats with high and low doses of scent leaf ameliorated the lead acetate-induced decrease in GSH level.

*3.1.3. Effect of* Ocimum gratissimum *on the level of SOD in the cerebellum of lead acetate-exposed rats*

The result in Fig. 3 below shows the effect of OG treatment on the level of SOD in the tissues of the brain of rats exposed to lead acetate. Rats treated with extract of OG (group 2) had significantly higher SOD levels than the control in all the tissues examined. Rats exposed to lead acetate alone (group 3) showed significantly ($P>0.05$) reduced SOD compared to the group 1 and group 2 rats that received OG.

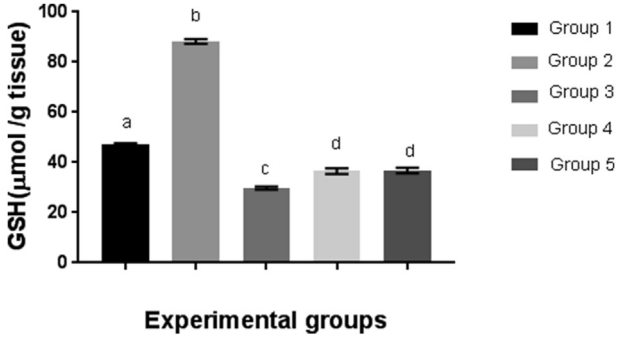


**Figure 2.** *Effect* of Ocimum gratissimum on level of GSH in the cerebellum of lead acetate-exposed rats. Values are presented as Mean ± SEM. n=7. Values with different superscripts differ significantly ($P<0.05$).

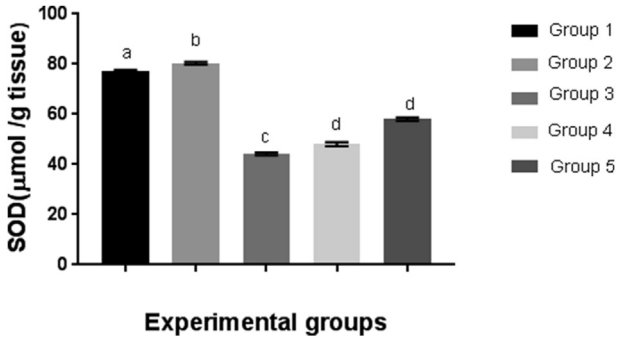


**Figure 3.** Effects of Ocimum gratissimum on the activity of SOD in the cerebellum of rats exposed to Lead acetate Values are presented as Mean ± SEM. n=7. Values with different superscripts differ significantly ($P<0.05$).

Also, treatment of experimental animals with co-administration of LA and OG (groups 4 and 5) showed a significant ($P>0.05$) increase in SOD activities compared to group 3 rats which received LA alone. Thus, the result indicates that the SOD level in the brain was significantly reduced by exposure to LA. Still, treatment with a low and high dose of OG ameliorated SOD reduction induced by LA.

*3.1.4. Effect of* Ocimum gratissimum *on the level of CAT in the cerebellum of rats exposed to Lead acetate*

Fig. 4 below shows the effect of OG leaf treatment on the level of CAT in the brains of rats exposed to lead acetate. Rats treated with leaf extract of OG (group 2) had significantly increased CAT levels compared to the group 1 rat. Group 3 rats were administered LA alone, which showed a significant decline in CAT activity in the cerebellum. Furthermore, treatment of lead acetate-exposed rats with high and low doses of scent leaf extract brought about a significant ($P>0.05$) increase in CAT activity compared to rats administered lead acetate alone (group 3). Thus, the result shows that the CAT level in the brain was significantly reduced by exposure to lead acetate. Still, the treatment of lead acetate-exposed rats with high and low doses of OG restored the lead acetate-induced CAT depletion.

*3.2. Histological effects of Ocimum gratissimum in the histology of the cerebellum*

Fig. 5 shows histoarchitectural changes in the cerebellum following oral administration of OG, LA, and co-administration of LA and OG. Sections of the cerebellar cortex (A and B) shows a grey matter composed of the molecular layer (ML), granular layer, and the Purkinje layer (red arrow). The ML and GL are made up of abundant neuronal nuclei displayed in clusters. At the same time, the PL (red arrow)

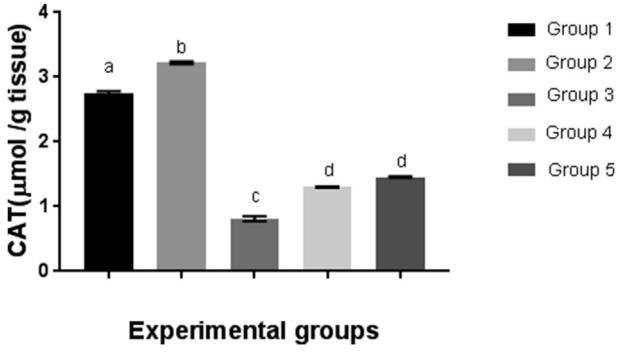


**Figure 4.** Effects of Ocimum gratissimum on the activity of CAT in the cerebellum of rats exposed to Lead acetate. Values are presented as Mean ± SEM. n=7. Values with different superscripts differ significantly ($P<0.05$).

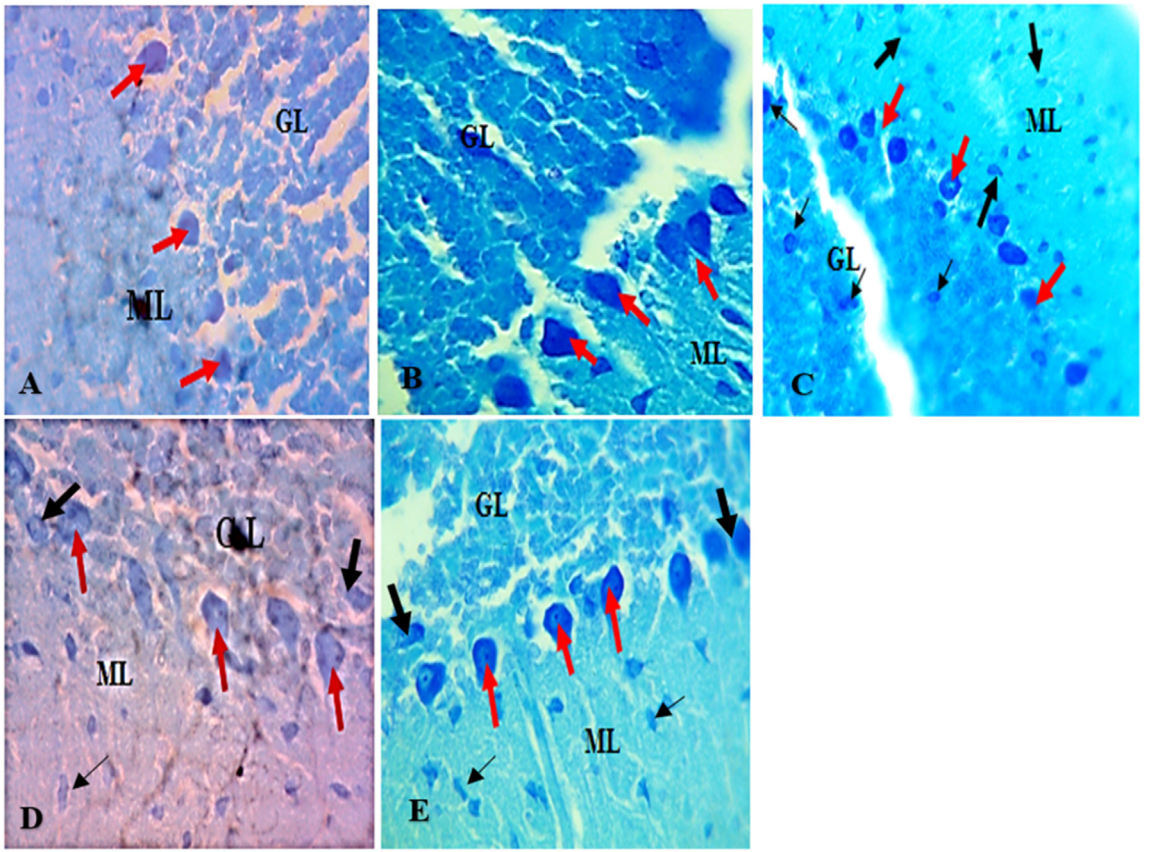


**Figure 5.** Representative photomicrograph of cerebellum of experimental animals stained with CFV at ×400 obj. A and B showed normal cellular morphology, and C showed a severely degenerated cerebellar cortex made up of distorted Purkinje neurons (red arrow) and neurons in the ML when compared to other groups. D and E showed normal morphology with very few distorted neurons than positive (A and B) and negative control groups (C).

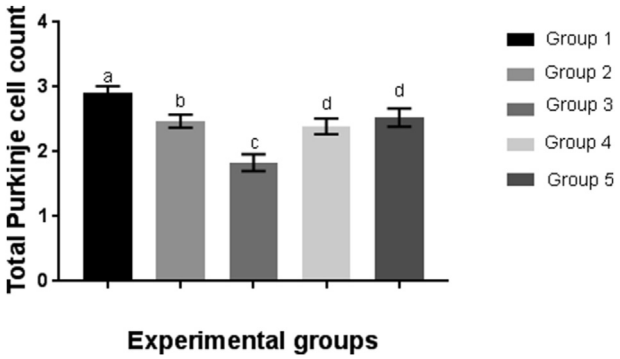


**Figure 6.** Graph showing Purkinje neuron density in the cerebellum of experimental animals. *Values are presented as Mean ± SEM. n=7. Values with different superscripts differ significantly (P<0.05).*

consists of single-layered, deeply basophilic Purkinje neuronal cell bodies of varying diameters. The molecular layer (ML) was made up of abundant neurons. A thick granular layer (GL) comprises deeply stained granulated cells. Cytoarchitectural details showed the molecular layer composed of neurons with basophilic nuclei peripherally located. The Purkinje cells (red arrow) were seen with granulated eosinophilic cytoplasm and a lightly stained round to oval granulated centrally placed nuclei. Abundant neuronal nuclei are seen in the granular layer with a centrally placed nucleus. The cerebellum from group three rats (C) showed a morphologically altered cerebellar cortex with a small Purkinje cell (red arrow) characterised by nuclear edema and cellular shrinkage. Some of these Purkinje cells have been displaced from the Purkinje layer and are seen invaginating into the granular layer (thin arrows). Also seen in C are sparse oedematous neurons (thick black arrows) and spindle-shaped neuronal cell bodies, while within the granular layer (GL) is degenerated granular cells that are tightly packed, clumped, and clustered together. However, sections from D and E also show a three-layered cerebellar cortex with scanty spindle-shaped neurons (thin arrow) in the ML and a few small diameters of Purkinje cell bodies characterised by nuclear chromatolysis. The above histological features in C described degenerated cerebellar cortex while D and E described a restoring architecture compared to normal (A and B).

### *3.3. Neuronal cytometry analysis of the cerebellar cortex purkinje cells in experimental rats*

One way ANOVA test shows (Fig. 6) a significant decrease in the neuronal population of the Purkinje cells in the group 3 animals administered with LA alone. But with a co-administration of LA and OG, there was a significant increase in the number of Purkinje cells compared with neuronal counts from groups 2 and 3. Maximal Purkinje cell population was seen in group 1 rats. This implied that LA induced Purkinje cell degeneration while OG restored and increased the populations of Purkinje cells.

## 4. Discussion

This study demonstrated the effects of oral administration of aqueous extract of OG on LA–induced cerebellar toxicity in adult Wistar rats. Although the exact mechanism underpinning neural regeneration and protection on exposure to plant phytochemicals in the cerebellum has not been unravelled, our study showed that extract of OG attenuated cerebellar degeneration induced by LA by reducing the levels of MDA and increasing the activities of SOD, CAT, and GSH in the cerebellum. These intracellular enzymes (SOD and CAT), alongside non-enzymatic antioxidants (GSH), act as a primary defence line against the harmful effects of reactive oxygen species that may contribute to neurodegeneration in the brain. LA is regarded as a neurotoxin that induces oxidative damage and neurodegeneration via enhancing lipid peroxidation and the depletion of antioxidant status in the brain and other vital organs [19,20].

### 4.1. Ocimum gratissimum *administration abrogates brain lipid peroxidation and depletion of antioxidant status induced by lead acetate*

The present study showed a significant decline in MDA level in the cerebellum of all treated rats administered OG and co-administration of LA and OG compared with rats treated with LA only. This implies that LA is a brain oxidative stressor [34], and elevated levels of lead (Pb2+) were found to induce oxidative damage cognitive and behavioural deficits by inhibiting the N-methyl-D-aspartate receptor (NMDAR) [35]. Inhibition of NMDAR results in a significant reduction of $Ca^{2+}$ entry into the cerebral cells, altering metabolic pathways involved in synaptic development and neurotransmissions in the brain [35]. Nevertheless, our findings revealed that OG administration following LA exposure led to a significant increase in MDA levels and thus implies that OG extract attenuated lipid peroxidation induced by LA in the cerebellum.

The present study also showed a significant reduction in GSH activities in the cerebellum of Wistar rats exposed to LA. At the same time, concomitant administration of LA and OG caused a significant elevation in GSH activities. This implies that oral administration of OG elevated GSH activities which LA depleted. Similarly, we also observed that OG treatment improved SOD and CAT activities in the cerebellum of rats exposed to LA. A similar trend has shown that LA is depleted by SOD and CAT activities in the brain, leading to increased membrane lipid peroxidation [34]. Our finding thus confirmed the ability of LA to induce oxidative stress in cells. LA alters the cellular redox state by inhibiting the activities of antioxidant defence enzymes, including CAT, GSH, and SOD [36], which it does by causing oxidation of sulfhydryl groups, thus leading to the alterations in membrane integrity [36]; Debnath et., 2019). Thus, the increase in MDA following a decrease in the activities of SOD, CAT, and cellular level of GSH observed in this study is not surprising and further elucidates the neurotoxic potentials of LA. This observation corroborates [37] findings, who reported that complex cerebral processes might be altered following lead administration.

Furthermore, this attenuation of LA-induced oxidative stress and depletion of antioxidant status in rats was ameliorated by oral administration of OG. Thus, this finding may indicate that OG extract possesses an antioxidant potential that is potent enough to improve the harmful effects of LA. This agrees with theories on using OG as an antimalarial, anticonvulsant, antibiotics, antifungal, antidiarrhea, antioxidant and anti-epileptic agent in ayurvedic medicine [18]. Considering this, elevated SOD activities may play a protective role in preventing cells from peroxynitrite formation and averting oxidative stress [38,39]. This implies that OG's increase in SOD activity prevented cells from peroxynitrite, which has been implicated in the progression of many pathologies such as neurodegenerative diseases and cancers [38,39]. Another independent study suggested that the oxidative stress linked with deteriorating tissues can be possibly reduced by the administration of OG and thus, improve metabolic activities in the disease [40]. In summary, biochemical analysis of MDA, SOD, GSH, and CAT are imperative in assaying oxidative stress in tissues. An increase in oxidative stress indicates a decrease in the antioxidant defence system. Since oxidative stress contributes meaningfully to the pathophysiology of diseases, substances that overwhelm oxidative stress might be therapeutically beneficial [19,37,41,42].

### 4.2. Ocimum gratissimum *extract ameliorates cerebellar neurotoxicity and loss of cellularity induced by lead acetate*

In time past, numerous studies have confirmed the deleterious effects of free radicals induced by heavy metals on tissues, implicating cellular degeneration [15,16,36]. We hypothesised that LA would induce cerebellar toxicity in rats exposed to LA in the current study. Our study revealed neuronal degenerations in the neurons of molecular, Purkinje, and granular layers of the cerebellum of LA-induced rats. This degeneration in the cerebellar neurons resulted to a decrease in the number and size of Purkinje cells and may thus lead to alterations in the activations of these neurons. The Purkinje neurons are regarded as the output of the cerebellum and play a vital role in control, learning of movement, and equilibrium maintenance. This was supported by [43]; who reported degeneration of Purkinje neurons following chronic lead acetate exposure.

Consequently, since the cerebellum is known to play a crucial role in the coordination of fine movements, its damage by exposure to LA in experimental groups may cause several cerebellar dysfunctions, including hypotonia, ataxia, and intention tremor. Most such abnormalities are apparent during movement, with ataxia characterised as incoordination due to errors in the rate, range, force, and direction of motion (Fredricks, 2011). Voluntary movements may also be affected abnormally, leading to dysmetria [44]. Thus, the outcome of the cerebellar damage depends on the severity and the region involved. Again, we hypothesised that OG extract would rescue the brain from the LA-induced toxicity in experimental rats. Here, we exposed the experimental animals to LA and OG alone and co-administration of LA and OG: and assessed the histoarchitectural and morphometric changes in the cerebellum. Surprisingly, we discovered that OG attenuated neuronal degenerations and loss of cellularity in the molecular, Purkinje, and granular layers of the cerebellum induced by LA. Furthermore, we carried out a cytometric analysis to quantify the level of degeneration in the cerebellum following LA and OG administration. Again, LA led to a significant decrease in the number of Purkinje neurons in the cerebellum compared to the control group. With Purkinje cells known for autorhythmicity and potency in action potential generation, an insignificant decline in their number by LA implies complete degeneration of cerebellar tissues in an almost irrevocable and degenerated fashion; pointer again, to the harmful effect that LA may pose against tissues in the long run. Conversely, in rats administered LA and oral administration of OG, we discovered a significant increase in the number of Purkinje cells compared with the control groups. This implies that OG extract restored the cerebellar architecture from the harmful effect of LA and suggests that OG may possess a regenerative potential that is enhanced by its antioxidative properties. This assertion is in line with several studies that have attributed the protective and restorative effect of this agent to the potent antioxidant compounds present in the plant [19,37,45–49]. Moreover, these therapeutic or protective potentials observed in this study may be attributed to OG's antioxidant and anti-inflammatory properties [19,50]. OG leaf extract has been demonstrated to contain alpha-tocopherol and phenols, potent antioxidant vitamins that fight against oxidative stress-induced cellular damage [50].

It is noteworthy that the possible mechanism for the destruction of cerebellar tissues by LA may be via oxidative stress and the generation of reactive oxygen species (ROS) in the cerebellum. Also, the exact mechanism by which OG exerts its protective effects on LA-induced cerebellar toxicity has not been elucidated. Our findings suggest that OG extract protects the cerebellum from lipid peroxidation by increasing the activities of GSH, CAT, and SOD and reducing MDA levels. This ability of OG to mitigate oxidative stress is ascribed to the antioxidative constituent of OG extract, which was shown to possess protective properties [19,37,45–49]. Antioxidants protect against LA-induced oxidative damage by deactivating free radicals at the molecular level, abrogating ROS formation and lead ion chelation [51,52]. More importantly, in OG extract, flavonoids and phenols, which are phytochemicals, exert their antioxidative potentials by chelating metal ions, scavenging, and deactivating enzyme activities that generate ROS [50]. In addition, the presence of other essential compounds such as saponins, alkaloids, terpenoids, and glycosides may further contribute to its anti-inflammatory and anti-oxidative activities [19,50].

## Conclusion

This study suggests that *Ocimum gratissimum* leaf extract possesses ameliorative effects on lead acetate-induced cerebellar neurotoxicity in rats via mechanisms related to the attenuation of lipid peroxidation, improving antioxidant defence enzymes, and restoring loss of cellularity and Purkinje cells integrity in the cerebellum.

## Author contributions

Udi, Onoriode Andrew; Conceptualization; Data curation, Methodology; Project administration; Resources and investigation, Oyem, John Chukwuma Roles/Writing - original draft; Writing - review & editing, Investigation; Methodology, Ebeye, Oladuni Abimbola Writing - review & editing, Methodology; Project administration. Chris-Ozoko, Lilian Ebite Supervision, Validation; Visualization and Resources, Igbigbi, Patrick Sunday Supervision, Validation; Visualization. Olannye, Donald Uzowulu,

Roles/Writing - original draft; Note: All authors read and approved the final version of the manuscript.

## Data availability

Data will be made available on request.

## Compliance with ethical standards

The research procedures complied strictly with the Faculty of Basic Medical Sciences, Delta State University Abraka, Nigeria Animal Research Ethics Committee (REF/FBMS/DELSU/18/33), which conforms to the "Guide to the care and use of laboratory animals in research and teaching" as prescribed in NIH publications volume 25 No.28 revised in 1996 (NIH, 1996).

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## Financial interests

The authors have no relevant financial or non-financial interests to disclose.

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nutos.2022.06.001.

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