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Ocimum gratissimum leaf ameliorates lead-induced neurotoxic effect on the cerebellar cortex of wistar rats

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Abstract

Lead-induced ataxia is a recognised neurological disorder. This study, therefore, investigated the ameliorative effects of the ethyl-ethanol fraction of *Ocimum gratissimum* leaf (EAFOG) on lead (Pb)-induced neurotoxic effects on the cerebellar cortex of male Wistar rats. The rats were divided into six groups of five each; the 1mL/kg H_2O group received only water for 35 days. The 120mg/kg Pb (1st–3rd weeks) group received 120mg/kg Pb for 21 days. The 120mg/kg Pb + 1mL/kg H_2O group received Pb for 21 days, followed by water administration for another 14 days. The 120 mg/kg Pb + 1,000mg/kg EAFOG group received Pb for 21 days, followed by treatment with 1,000mg/kg EAFOG for 14 days. The 120mg/kg Pb + 1,500mg/kg EAFOG group received Pb for 21 days, followed by treatment with 1,500mg/kg EAFOG for 14 days. The 120mg/kg Pb + 10mg/kg succimer group received Pb for 21 days, followed by treatment with 10mg/kg succimer for 14 days. Footprint and beam walking tests were performed to analyse motor coordination and balance. After experimentation, the rats were anaesthetised, brains excised, and fixed in Bouin's fluid for histological tissue processing. Ataxic behaviour was observed in the lead treatment groups; however, significant improvements in motor coordination and balance were noted after treatment with EAFOG and succimer. Histological examination indicates necrosis and distortion of the Purkinje layer in the 120mg/kg Pb (1st–3rd weeks) group, while treatment with EAFOG and succimer showed varying preservation of the histoarchitecture. Treatment with EAFOG and succimer showed potential in mitigating Pb-induced cerebellar damage in Wistar rats.
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Keywords

Beam walking, Motor coordination, Footprint analysis, Cerebellar cytoarchitecture

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Introduction

Lead is a known toxic heavy metal reported to harm both humans and animals, and the nervous system has been severely implicated. Toxicological and epidemiological studies have linked lead exposure to various ailments, including cardiovascular disease, infertility, increased risk of miscarriage, organ failure and neurodegenerative diseases, among others (Wu *et al*., 2019; Menke *et al*., 2020; D'Souza and Rajan, 2021). Lead exposure occurs through various sources, including contaminated air, soil, water, and consumer products. In 2021, over 18 million people in the United States were reported to be exposed to lead in their drinking water, with the highest levels of exposure in disadvantaged communities (Wu *et al*., 2021).

There has been an increased effort to minimise the use of lead. Lead paint regulations have helped to control the use of leaded paint in residential buildings (Jacobs and Brown, 2023) and protect workers from lead exposure in industries such as construction and manufacturing (Kosnett *et al*., 2023), but environmental contamination continues, as it is used for various industrial and commercial purposes such as lead-acid batteries, cable sheathing, paints, pigments, ammunition and radiation shielding (Ali *et al*., 2024), with a report stating the half-life of lead in the bone to be more than 10 years, 35 days in the erythrocyte and 2 years in the brain (Barkur and Bairy, 2014).

Lead-induced ataxia is a well-documented neurological disorder that has been extensively studied in animal models. Damage and reduction in the number of cerebellar Purkinje cells with increased oxidative stress and inflammation in the cerebellar cortex were reported mechanisms in lead-induced ataxia in animal models (Basha *et al*., 2005; Wang and Du, 2013; Alzahrani *et al*., 2022). Quantity and period of exposure are major determinants of the level of toxicity. The cerebellum is particularly vulnerable to the toxic effects of lead because of its high concentration of calcium channels, which are targeted by lead (Gudadhe *et al*., 2024).

The cerebellum is a highly specialised structure located at the back of the cerebrum, just below the occipital lobes. It accounts for approximately 10% of the brain's total volume and contains nearly half of all the neurons in the brain (Miall, 2021). The cerebellum is a complex and highly specialised structure that plays a critical role in motor coordination, learning, and non-motor functions, such as cognition and emotion regulation. The cerebellum supports motor and cognitive functions; lesions cause motor syndromes and cognitive affective syndromes, affecting emotional and executive skills (Koziol *et al*., 2014; Schmahmann and Pandya, 2019; Sokolov *et al*., 2020; Liu *et al*., 2021).

However, occupational and environmental exposure to lead remains a serious problem in many developing and industrialised countries, including Nigeria (Fabolude *et al*., 2025). Succimer is an oral chelating agent used to treat heavy metal poisoning (Vahabzadeh *et al*., 2021). This organosulphur compound contains two sulfhydryl groups that effectively bind divalent metal ions like lead, cadmium, mercury, and arsenic. Importantly, succimer does not significantly chelate essential metals such as zinc, copper, or iron. Its specificity, safety, and oral bioavailability make it a preferred choice over other chelating agents for treating lead poisoning (Balali-Mood *et al*., 2025). Earlier studies reported severe adverse reactions as a result of succimer administration, including bone marrow and kidney dysfunction resulting in leukopenia, thrombocytopenia, and skin complications, which have prompted the consideration of alternative treatments for lead poisoning (Chandran and Cataldo, 2010; Dorooshi *et al*., 2021). Incessant exposure to lead and related products requires investigations on locally available and affordable modes of treatment. To mitigate the adverse effects of lead toxicity, natural compounds with antioxidant properties are being considered, even when it is not possible to prevent people from lead exposure.

Ocimum gratissimum contains a variety of phenylpropene (eugenol and methyl eugenol), sesquiterpenes (germacrene D and caryophyllene, -muurolene), and monoterpenes (-ocimene) (Ashokkumar *et al*., 2021). Other phytochemicals present include terpenes, tannins, phenols and flavonoids (Edo *et al*., 2023), which are rich in natural antioxidants and offer numerous health benefits (Priyanka *et al*., 2018). Udi and colleagues previously reported the ameliorative effect of aqueous extract of *O. gratissimum* L. leaf on lead-induced toxicity in Wistar rats (Udi *et al*., 2022). The mode of action of *O. gratissimum* also includes anti-inflammatory and anti-oxidative actions, including scavenging or quenching free radicals, chelating metal ions, or inhibiting enzyme systems that produce free radicals (Pizzino *et al*., 2017). In an investigation of the chemical composition and antioxidant properties of different fractions of *O. gratissimum* L. leaf, it was observed that the ethyl acetate fraction exhibited the highest antioxidant activity (Ouyang *et al*., 2013). Hence, there is a need to study the ameliorative effect of the ethyl acetate fraction *of O. gratissimum* L. leaf on lead-induced toxicity in the cerebellum of adult Wistar rats.

Materials and Methods

Ethical approval

Ethical approval for this study was obtained from the Ahmadu Bello University Committee for Animal Use and Care (ABUCAUC) with the approval number ABUCAUC/2020/Human Anatomy/55. The study followed the guidelines for the care and use of laboratory animals set forth by the National Research Council (National Research Council (2011).

Experimental animals

Thirty healthy adult male Wistar rats with an average weight of 140±4.21 g were procured from the Animal House Facility in the Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria. The Wistar rats were transferred and housed in wired cages in the Animal House of the Department of Human Anatomy, Faculty of Basic Medical Sciences, ABU Zaria, and allowed to acclimatise for two weeks before the commencement of the experiment. All animals were given feed and water *ad libitum*. Analytical grade of lead acetate (Best of Chemical (BOC) Science, New York, with batch number CAS 304-55-2) was used in this study as the neurotoxin. Meso-2,3-dimercaptosuccinic acid (succimer) (Sigma-Aldrich, D7881) was used as the chelating agent.

Plant material

Fresh leaves of *O. gratissimum* L. were gotten from the Botanical Garden of ABU Zaria. The leaves were identified in the Herbarium Unit of the Department of Botany, Faculty of Life Science, ABU Zaria, after which a voucher number, 01285, was assigned by comparison with an existing voucher specimen number.

Ethyl fractionation of *O. gratissimum* extract

The fresh leaves of *O. gratissimum* L. obtained were taken to the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU, Zaria, for extraction and fractionation. Solvent-solvent

partitioning was done using the protocol designed by Kupchan and Tsou (1973).
The powdered leaves of *O. gratissimum* (1,000 g) were subjected to extraction using a Soxhlet apparatus with 2.5 L of absolute ethanol for 24 h. Following this, the ethanol crude extract (ECE) was evaporated using a water bath set at a temperature of 40–45°C to remove the solvent. To isolate additional phytochemicals, the ECE was partitioned between water and ethyl acetate using a separating funnel. The ECE was initially dissolved in distilled water and gently heated. Ethyl acetate was then added to the jar containing the dissolved ECE and water, creating two distinct layers in the separating funnel. The aqueous fraction, characterised by a reddish-brown colour, was collected separately, while the ethyl acetate fraction, which was dark green, was also gathered. This extraction process was repeated four times, with additional ethyl acetate added each time until the ethyl acetate fraction appeared light in colour. Both fractions were subsequently evaporated using a water bath. The final ethyl acetate fraction of *O. gratissimum* (EAFOG) leaf was prepared for this study.
One thousand grams of dry powdered *O. gratissimum* leaves yielded 55.72 g of EAFOG. The percentage yield is thus 5.57%.

Experimental design

Thirty adult male Wistar rats were divided into six groups of five rats each. Group 1 received 1 mL/kg body weight of distilled water for 35 days (5 weeks) and served as a negative control. Group 2 was administered 120 mg/kg lead acetate for 21 days (3 weeks), and the rats were euthanised on day 22 of the administration to study the direct effect of lead acetate. This group served as a positive control. Group 3 was administered 120 mg/kg of lead acetate for 21 days and then administered distilled water from day 22 to day 35 (2 weeks) of the administration. Group 4 was administered 120 mg/kg lead acetate for 21 days and then treated with 1,000 mg/kg EAFOG from day 22 to day 35 of the administration. Group 5 was administered 120 mg/kg lead acetate for 21 days and then treated with 1,500 mg/kg EAFOG from day 22 to day 35. Group 6 was administered 120 mg/kg lead acetate for 21 days and treated with 10 mg/kg succimer as the standard treatment drug from day 22 to day 35. Administrations were done orally once per day.
Weights were obtained using a digital weighing scale (Acculab Vicon VIC-511 Precision Balance/Scale, USA, 0.001 g). The body weights of the rats were recorded at the beginning and end of experimentation, and the percentage body weight was calculated:

Neurobehavioural study
Footprint test

The neurobehavioural assessment was conducted using a footprint test to evaluate gait (Carter *et al*., 1999). Each rat was given three consecutive trials on each of three consecutive days of training before the test. During the first few trials, some rats needed encouragement (such as an occasional nudge with a finger) to reach the goal box. However, they soon learned to run rapidly towards the enclosed goal box. The tests were carried out once, weekly throughout the study.
The apparatus consisted of an open-top runway (100 cm long, 10 cm wide, with walls 20 cm high) brightly illuminated from above with an enclosed goal box (20 cm square, with a 4 × 5 cm entrance hole). White paper, non-toxic paints in two contrasting colours (blue and black), and fine paintbrushes were also used.
To obtain footprints, rat paws were painted with the non-toxic paints, and each rat was allowed to walk along a narrow, paper-covered corridor, leaving a track of footprints. Each rat was placed on the end of differently marked sheets of paper opposite the goal box and allowed to walk on the paper. The sheet of paper from the runway was removed, and the footprint patterns were allowed to dry in a well-aerated room for more than an hour before being stored. Once the footprints were dry, measurements were taken from the prints manually. For each step parameter, two values were measured from the middle portion of each runway trial, excluding footprints made at the beginning and end of the trial where the rat initiated and finished movement, respectively, and the mean of each set of two values was used in the analysis. The assessments that were recorded include:

- Stride length was measured as the average distance of forward movement between each stride,
- Front base width was measured as the average distance between left and right front footprints.
- Hind base width was measured as the average distance between the left and right hind footprints, along with the distance between the front and hind footprints on each side.
- The distance between the front and hind footprints on each side.

Beam walking test

The beam walking test is a test of motor coordination and balance in rodents (Carter *et al*., 1999). The test requires a beam balance with one or more narrow beams with varying dimensions and elevated from the ground. The apparatus consisted of an elevated platform connected by a 100 cm-long wooden beam with a width of 3 cm. The beam was placed at an angle of 90°, with one end mounted on a narrow support and the other end attached to an enclosed box. The start point was placed by a bright light source to motivate the rats to traverse the beam. A sawdust-filled box at the base served as protection for the falling rats. Before the test, rats were habituated to the beam-walking apparatus daily for 3 days with 4 trials per day.
Rats were tested once, weekly throughout the duration of the study. The rats were gently placed on the start platform and allowed to walk to the end of the beam. A box at the other end of the beam served as the finish point. Each rat was exposed to two consecutive trials. The time taken to cross the beam was recorded in seconds. The apparatus was cleaned with 70% alcohol before the trial and after each rat was tested so that the succeeding rat to be tested would not perceive the trails of the preceding rat. After each test, the rats were returned to their respective cages. The average value for each of the two

trials was recorded and used for the subsequent analysis.

Animal euthanasia

Twenty-four hours after the last administration, rats were deeply anaesthetised with ketamine hydrochloride (A 1945, Uruguay) at a dose of 75 mg/kg body weight intraperitoneally (Wellington *et al*., 2013). The head was decapitated, and the brain was quickly excised; the cerebellum was separated from the cerebrum. The cerebellum was weighed using an electronic digital scale (Atom A-110c) and was fixed in Bouin's fluid for tissue processing. The cerebellum-body weight ratio was calculated from the formula:

$$\frac{Cerebellum\ weight}{Final\ body\ weight}$$

Histological studies

The cerebellum was processed using histological techniques by making thin sections and staining using haematoxylin and eosin (H and E) stains for demonstration of the cytoarchitecture of the cerebellum in the Histology Unit of the Department of Human Anatomy, ABU, Zaria. Microscopy and micrography (using an optical microscope, HM-LUX, Leitz Wetzlar, Germany, and a digital microscopic camera, MA 500 AmScope®, USA) were conducted in the Microscopy and Stereology Research Laboratory of the same facility.

Data analysis

Data obtained were expressed as mean ± SEM (standard error of the mean). Split-plot ANOVA was used to compare the mean differences for the neurobehavioural studies. One-way ANOVA was also used to compare the mean differences for the percentage body weight change and organ-body weight ratio across the groups. A p-value of <0.05 was considered statistically significant. Results were analyzed using the IBM Statistical Package for Social Sciences (SPSS) software version 23 (IBM, USA).

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Results

Body weight change and organ-body weight ratio

The results of the body weight assessment showed that the percentage body weight change of the rats in the 120 mg/kg Pb (1st – 3rd weeks) treatment group was not statistically significant when compared with the 1 mL/kg distilled water treatment group. However, there was a statistically significant decrease ($p < 0.05$) in the 120 mg/kg Pb (1st – 3rd weeks) treatment group when compared with the 120 mg/kg lead acetate plus 1,500 mg/kg EAFOG treatment group. There was also a statistically significant increase in the percentage body weight of the lead acetate plus 1,500 mg/kg EAFOG treatment group when compared to the 120 mg/kg Pb plus 1 mL/kg distilled water treatment group, the 120 mg/kg Pb plus 1,000 mg/kg EAFOG treatment group, and the 120 mg/kg Pb plus 10 mg/kg succimer treatment group (Table 1).

There was no significant difference in the organ-body weight ratio across all the groups. Meanwhile, there was an increase in the of 120 mg/kg Pb (1st–3rd weeks) treatment group when compared with the 1 mL/kg distilled water treatment group, the 120 mg/kg Pb plus 1 mL/kg distilled water treatment group, the 120 mg/kg Pb plus 1,000 mg/kg EAFOG treatment group, the 120 mg/kg Pb plus 1,500 mg/kg EAFOG treatment group, the 120 mg/kg Pb plus 10 mg/kg succimer treatment group, and the 120 mg/kg Pb plus 10 mg/kg succimer (Table 1).

Table 1: Percentage of body weight change and organ-body weight ratio in adult Wistar rats induced with lead acetate and treated with EAFOG and succimer

Group	Treatment	% Body weight change	OBWR
1	1 mL/kg distilled water	14.41±2.43	0.15 ± 0.01
2	120 mg/kg Pb (1st–3rd weeks)	7.03±2.29[a]	0.23 ± 0.01
3	120 mg/kg Pb + 1 mL/kg distilled water	1.86±1 .34[b]	0.20 ± 0.03
4	120 mg/kg Pb + 1,000 mg/kg EAFOG	9.04±3.49[c]	0.19 ± 0.01
5	120 mg/kg Pb + 1,500 mg/kg EAFOG	21.08±2.42[abcd]	0.16 ± 0.02
6	120 mg/kg Pb + 10 mg/kg succimer	10.94±3.21[d]	0.14 ± 0.02

One-way ANOVA followed by Duncan's post hoc test. Results are expressed as mean ± SEM. Column with the same superscript is statistically significant ($p < 0.05$). Pb: Lead acetate. EAFOG: Ethyl acetate fraction of *O. gratissimum* leaf, OBWR: Organ-body weight ratio.

Neurobehavioural study

Forelimb stride length

The results of the forelimb stride length (FSL) showed that on the last day of the test, the rats had a mean FSL ranging from 10.0 cm to 11.6 cm. There was no significant difference ($p > 0.05$) in the FSL of rats in all the groups throughout administration (weeks 1-5) (Fig. 1).

Hindlimb stride length

The results of the hindlimb stride length (HSL) showed that on the last day of the test, the rats had a mean HSL ranging from 10.6 cm to 11.3 cm. There was no significant difference ($p > 0.05$) in the HSL of rats in all the groups through the duration of administration (weeks 1-5) (Fig. 2).

Front base width

The results of the front base width (FBW) revealed that on the last day of the test, the Wistar rats had a front base width ranging from 1.8 cm to 2.4 cm. The FBW of rats administered 120 mg/kg lead acetate plus 1,000 mg/kg EAFOG, 120 mg/kg lead acetate plus 1,500 mg/kg EAFOG and 120 mg/kg lead acetate plus 10 mg/kg succimer were significantly decreased ($p < 0.05$) at week 5 when compared to week 3 (Fig. 3).

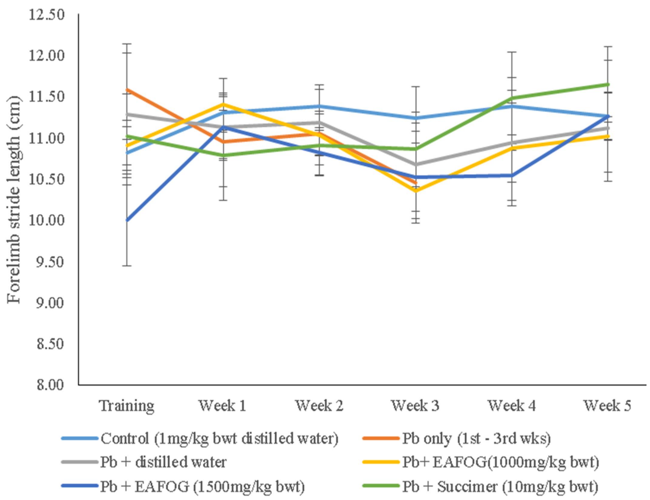

Fig. 1: Forelimb stride length (cm) of adult Wistar rats induced with lead acetate and treated with EAFOG and succimer. Results are expressed as mean ± SEM. Level of significance p<0.05. Pb: lead acetate, EAFOG: Ethyl acetate fraction of *O. gratissimum* leaf.

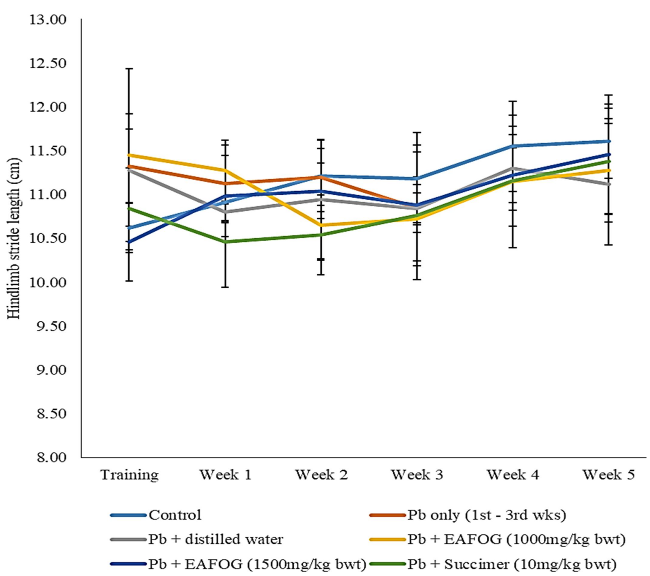

Fig. 2: Hindlimb stride length (cm) of adult Wistar rats induced with lead acetate and treated with EAFOG and succimer. Results are expressed as mean ± SEM. Level of significance: p>0.05. Pb: lead acetate, EAFOG: ethyl acetate fraction of *O. gratissimum* leaf.

Hind base width

The results of the hind base width (HBW) revealed that on the last day of the test, the rats had a hind base width ranging from 3.0 cm to 3.5 cm. There was a significant increase ($p < 0.05$) in the HBW of rats treated with 120 mg/kg lead plus 1,000 mg/kg EAFOG, 120 mg/kg lead acetate plus 1,500 mg/kg EAFOG and 120 mg/kg lead acetate plus 10 mg/kg succimer at week 3 when compared to the last day of training (Fig. 4).

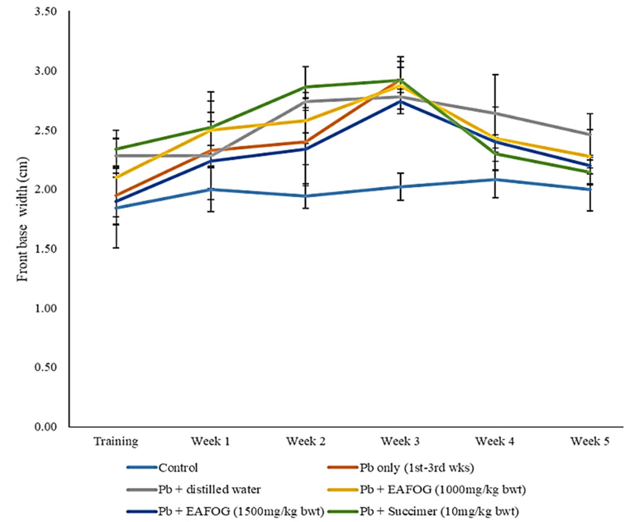

Fig. 3: Front base width (cm) of adult Wistar rats induced with lead acetate and treated with EAFOG and succimer. Results are expressed as mean ± SEM. Level of significance p<0.05. Pb: Lead acetate, EAFOG: Ethyl acetate fraction of *O. gratissimum* leaf.

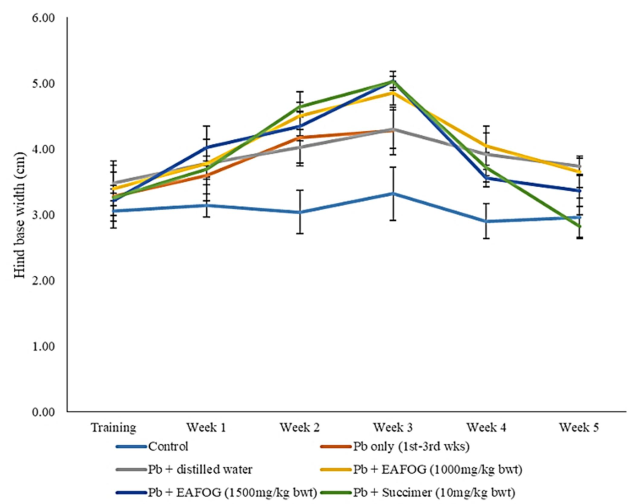

Fig. 4: Hind base width (cm) of adult Wistar rats induced with lead acetate and treated with EAFOG and succimer. Results are expressed as mean ± SEM. Level of significance p<0.05 Pb: Lead acetate, EAFOG: Ethyl acetate fraction of *O. gratissimum* leaf.

Overlap of front and hind paws

The results of the overlap of front and hind paws showed that on the last day of the test, the Wistar rats had an overlap ranging from 0.3 cm to 0.6 cm. Administration of 1000 mg/kg EAFOG, 1500 mg/kg EAFOG and 10 mg/kg succimer after exposure to 120 mg/kg lead acetate resulted in a significant decrease in overlap at week 5 compared to week 3 (Fig. 5).

Beam walking test

At the end of the training, all the rats crossed the beam within five seconds. Throughout the administration, rats in the control group crossed the beam within five seconds. A significant increase ($p < 0.05$) in the time taken to cross the beam for rats treated with 120 mg/kg lead acetate plus 1 mL/kg distilled water, 120 mg/kg lead acetate plus 1,000 mg/kg EAFOG, 120 mg/kg lead acetate plus 1,500 mg/kg EAFOG, and 120 mg/kg lead acetate plus 10 mg/kg succimer was observed at week 3 when compared to the last day of training. At week 5, a significant decrease ($p < 0.05$) in time taken to cross the beam was observed in rats administered 120 mg/kg lead acetate plus 1,500 mg/kg EAFOG and 120 mg/kg lead acetate plus 10 mg/kg succimer when compared to week 3 (Fig. 6).

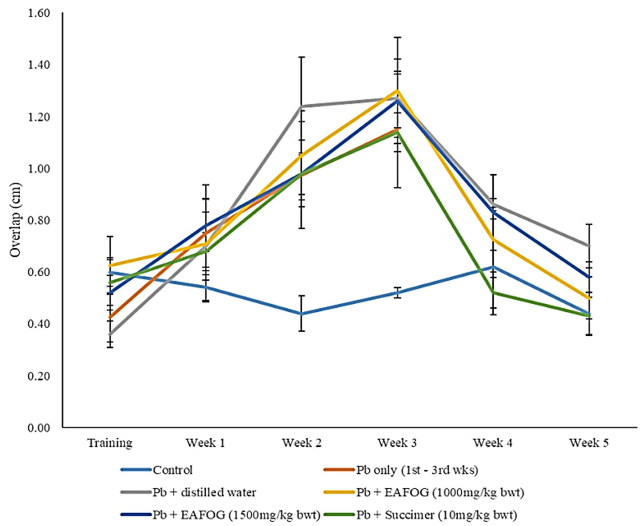

Fig. 5: Overlap of forepaw and hind paw placements of adult Wistar rats induced with lead acetate and treated with EAFOG and succimer. Results are expressed as mean ± SEM. Level of significance $p<0.05$. Pb: Lead acetate, EAFOG: Ethyl acetate fraction of *O. gratissimum* leaf.

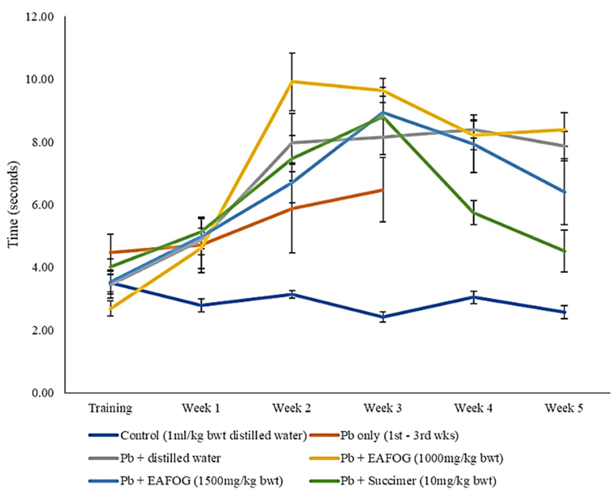

Fig. 6: Mean time (seconds) taken for adult Wistar rats to cross the beam balance during lead acetate administration and treatment with EAFOG and succimer. Results are expressed as mean ± SEM. Level of significance $p<0.05$. Pb: lead acetate, EAFOG: ethyl acetate fraction of *O. gratissimum* leaf.

Histological studies

In this study, a histological examination of the cerebellar cortex of Wistar rats was conducted, and the following features were observed: Sections of the cerebellar cortex of the 1 mL/kg distilled water treatment group showed relatively normal histoarchitectural features of the molecular, Purkinje and granular layers. The cerebellar cortex of the 120 mg/kg Pb-only (1st–3rd weeks) treatment group showed necrosis (karyorrhexis) of Purkinje cells. The Purkinje layer also appeared distorted. Rats in 120 mg/kg Pb plus 1 mL/kg distilled water-treatment group showed degeneration, indicated by the irregular shape of the Purkinje cell body. The cerebellar cortex of the 120 mg/kg Pb plus 1,000 mg/kg EAFOG-treatment group showed the presence of some degenerated Purkinje cells. The cerebellar cortex of 120 mg/kg Pb plus 1,500 mg/kg EAFOG-treatment groups showed preservation of the cerebellar cortex histoarchitecture. The cerebellar cortex of the 120 mg/kg Pb plus 10 mg/kg succimer treatment group showed relatively normal orientation of the molecular and granular layers and a Purkinje layer having some normal and degenerated Purkinje cells (Fig. 7).

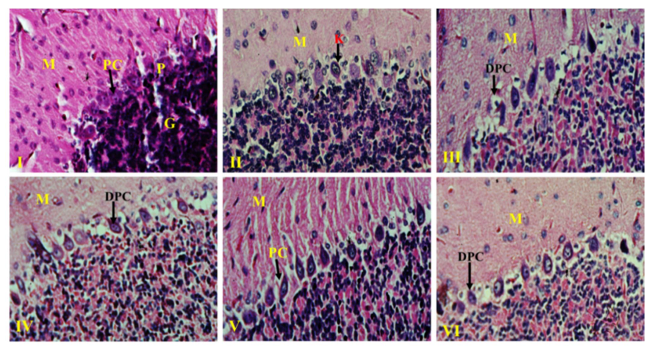

Fig. 7: Micrographs of the cerebellar cortex of Wistar rats

I. 1 mL/kg distilled water-treatment group with a relatively normal orientation of the molecular layer (M), granular layer (G) and Purkinje layer (P) with normal Purkinje cells (PC)
II. 120 mg/kg Pb (1st–3rd week)-treatment group with relative histoarchitectural distortions, and distorted Purkinje layer (P) with karyorrhexis (K) in the Purkinje cells.
III. 120 mg/kg Pb plus 1 mL/kg distilled water treatment group with relative histoarchitectural distortions. Purkinje cells are irregular in shape and degenerating Purkinje cells (DPC).
IV. 120 mg/kg Pb plus 1,000 mg/kg EAFOG-treatment group with Purkinje layer with degenerating Purkinje cells (DPC). M: molecular layer, P: Purkinje layer, G: granular layer.
V. 120 mg/kg Pb plus 1,500 mg/kg EAFOG-treatment group with relatively normal histoarchitecture.
VI. 120 mg/kg Pb plus 10 mg/kg succimer-treatment group with relatively normal histoarchitecture and degenerating Purkinje cells (DPC).

Pb – lead acetate

Discussion

Lead and other environmental neurotoxicants can interfere with many biochemical events present in cells of the body, and they can produce a wide spectrum of alterations in many organs and systems (Gudadhe *et al*., 2024;

Sharma *et al*., 2025). Among alterations induced by lead exposure in adults and children are those involving motor system dysfunction, which represents a common public health problem (Gudadhe *et al*., 2024). The present study investigated the effect of EAFOG on Pb-induced changes in body weight, neurobehavioural study and histoarchitectural changes in the cerebellar cortex of adult Wistar rats. The study assessed body weight changes, organ-body weight ratios, and neurobehavioural effects in Wistar rats treated with Pb and EAFOG. Rats given 120 mg/kg Pb plus 1500 mg/kg EAFOG showed significant weight changes ($p < 0.05$) compared to other groups, excluding the 1 mL/kg distilled water-treatment group. The organ-body weight ratios differences were minimal. Neurobehavioural tests revealed no significant differences in forelimb and hindlimb stride lengths ($p>0.05$). Notable reductions in front base width were seen with certain treatments at week 5. Histological analysis indicated necrosis in Purkinje cells for lead-treated rats, while those with EAFOG or Succimer showed varying levels of preservation and degeneration.

Body weight is a crucial parameter in animal model research, as it can provide important information about the animal's health status, nutritional status, and overall well-being. Changes in body weight can be an indicator of the effects of treatments on the animal model. In this study, the percentage change in body weight was significantly lower in the 120 mg/kg lead acetate groups compared to the EAFOG-treated group.

A major symptom of lead toxicity is weight loss, which could be a result of loss of appetite. Although Benammi *et al*. (2017) reported that lead interferes with glucose metabolism by causing a significant increase in plasma glucose levels, this increase may be induced by circulating adrenaline released from the adrenal medulla, which could affect sympathoadrenal medullary activity and thereby increase body weight. However, Bellinger *et al*. (2018) reported a decrease in body weight in children exposed to lead. Gao *et al*. (2016) also reported a reduction in the weight of broiler chickens after exposure to lead. Consequently, Onaolapo *et al*. (2011) reported a decrease in body weight in the ethanol extract of *O. gratissimum* and streptozotocin treatment groups when compared with the control group. Bako *et al.* (2020) also reported a decrease in body weight of obese-induced rats after treatment with the methanol extract of *O. gratissimum*. The differences observed could be a result of the EAFOG used in this study. Beyond these, lead can affect neurobehavioural patterns.

Neurobehavioural studies have shown that exposure to lead has some profound outcomes, including decreased exploratory behaviour, hyperactivity, and altered motor activity (Moreira *et al*., 2001; Trombini *et al*., 2001). Thus, brain areas involved in motor activity, the cerebellar cortex, cerebral cortex, and basal ganglia, are targets of lead toxicity (Benammi *et al*., 2017). A footprint test was used in this study to assess gait and motor coordination. This behavioural test involves footprints left by animals as they walked or ran on surfaces. The evaluation of these footprints provides information about various aspects of locomotion, such as stride length, stance width, and paw angle, which are indicative of motor deficits or abnormalities (Carter *et al*., 1999). Beam walking is another commonly used neurobehavioural test that assesses motor coordination and balance. The animals are allowed to walk along a narrow beam to a safety platform (Carter *et al*., 1999).

In this study, the lead-treated groups exhibited abnormal widths of the front and hind bases, as well as overlap of the front and hind paws during the initial weeks of experimentation. However, these abnormalities were restored following treatment with varying doses of EAFOG. Additionally, significant improvements were noted in the beam walking test, with a marked reduction in the time taken to traverse the beam at week 5 compared to week 3, likely attributed to the treatments administered. Bazrgar *et al*. (2015) previously observed that lead impaired locomotor performance in rats, but their treatment significantly reduced this motor impairment. Reports have shown that long histories of leaded gasoline exposure cause encephalopathy (Burns *et al*., 1995; Maruff *et al*., 1998). Severe movement abnormalities such as abnormal tandem gait and bilateral palmomental reflexes are characteristic of chronic leaded gasoline encephalopathy (Cairney *et al*., 2004). Additional severe motor abnormalities attributable to cerebellar dysfunction include ataxia, nystagmus and poor hand and foot coordination (Goodheart and Dunne, 1994).

The proteomic profile of the cerebellum is associated with several biological processes responsible for organ maintenance, such as energetic metabolism and neural function, which are targeted in lead exposure, causing impairment of spontaneous locomotion and motor coordination abilities (Leão *et al*., 2020). However, the mechanism of EAFOG against lead-induced locomotion and motor coordination impairment could be attributed to the principal constituents of *O. gratissimum,* such as eugenol and myrcene (Paula-Freire *et al*., 2012). Eugenol and myrcene have the common property of inhibiting the monoamine oxidase B (MAO-B) gene (Tisserand and Young, 2013). MAO-B genes catalyse the catabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues, such as dopamine (Cho *et al*., 2021). Sarral *et al.* (2013), with Tankam and Ito (2014), all reported that *O. gratissimum* shows potential sedative and anxiolytic-like effects and improved locomotor activities in animal models.

Behavioural alterations can be associated with histological alterations in the cerebellum. Our work demonstrated cytoarchitectural distortions in the Purkinje cells in the cerebellum. Morphological alterations of the brain were observed after chronic exposure to lead (Dumková *et al*., 2017). Alterations in the composition of cerebellar tissue can directly compromise the functional activity of the animals (Zhang *et al*., 2015; Leão *et al*., 2020). Meanwhile, in this study, EAFOG mitigated these cytoarchitectural distortions, which is also in support of the report of Udi *et al*. (2022). Ibegbu *et al*. (2015) also reported some neuroprotective potentials of *O. gratissimum* against xenobiotic substances.

Conclusion

In conclusion, this study highlights the detrimental effects of lead exposure on body weight, neurobehavioural functions, and cerebellar histoarchitecture in adult Wistar rats, confirming prior findings on the neurotoxic impact of lead. The observed significant decrease in body weight in lead-treated groups paralleled neurobehavioural deficits, such as impaired motor coordination and altered exploratory behaviour. The ethyl acetate fraction of *O. gratissimum* leaves possesses potential neuroprotective properties against lead-induced neurotoxic changes in the cerebellar cortex of adult Wistar rats. Neuroprotective properties could be attributed to bioactive compounds present in EAFOG. Further research is warranted to elucidate the specific mechanisms by which compounds in *O. gratissimum* exert their protective effects, potentially guiding future therapeutic strategies against environmental neurotoxicants.

Declaration

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Conflict of interest

None declared.

Ethical approval

Ethical approval for this study was obtained from the Ahmadu Bello University Committee for Animal Use and Care (ABUCAUC) with the approval number ABUCAUC/2020/Human Anatomy/55.

Authors' contribution

Conceptualisation: ML, WOH, SAM. Data acquisition: ML, GPO, MSM, AGM. Data analysis: GPO, AGM, MSM. Methodology: ML, WOH, SAM, GPO. Supervision: WOH, SAM. Validation: GPO, MSM. Visualisation: ML, GPO, WOH, SAM. Roles/Writing draft: GPO, ML. Writing, reviewing, and editing: ML, WOH, SAM, MSM, and AGM.

Consent to participate and publish data

Not Applicable.

The use of generative artificial intelligence

Generative AI was not used as a substitute for the author's own understanding. The content generated by AI was used as a grammar verification and rephrasing tool for the writing process, and the author remains responsible for the final content, interpretations, and academic integrity.

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