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Doi: doi.org/10.4314/jeca.v21i2.29

Original Article

Ameliorative effect of *Vernonia amygdalina* on lead-induced neurotoxicity on the premotor cortex of adult Wistar rats

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Abstract

BACKGROUND AND AIM: Lead toxicity affects different body systems including the nervous system, resulting in brain damage, neuromuscular weakness, and impaired cognitive functions. This study evaluated the ameliorative effect of aqueous leaf extract of *Vernonia amygdalina* (AEVA) on lead acetate-induced neurotoxicity of the premotor cortex of adult Wistar rats.

METHODOLOGY: Thirty adult male Wistar rats were divided into six groups of five each. The rats were accessed for neurobehavioral studies using a foregrip strength test apparatus. Group I received 1ml/kg body weight of distilled water. Group II received only 120 mg/kg body weight of lead acetate. Group III received 120 mg/kg body weight of lead acetate for 1 week and 2 weeks of natural recovery. Group IV received 120mg/kg body weight of lead acetate and 10mg/kg body weight of Succimer. Group V and VI received 120mg/kg body weighr of lead acetate with 1000mg/kg, and 1500mg/kg body weight of AEVA respectively. Administration lasted for 21 days. At the end of the experimentation, the rats were euthanized and brains harvested for oxidative stress, and histological (H&E) assessments.

RESULTS: A decrease in muscular strength of Wistar rats treated with lead acetate was observed, however, improvement was seen in all the treatment groups, although, it was statistically significant Oxidative stress was induced in rats exposed to lead acetate as indicated by decreased superoxide dismutase, and catalase respectively. However, 1500mg/kg AEVA treatment ameliorated these changes with a significant increase. The results revealed histopathological changes such as karyorrhexis, cytoplasmic vacuolations, pyknosis and karyolysis in the premotor cortices of lead acetate-exposed rats which were ameliorated when treated with AEVA at 1000 and 1500 mg/kg body weight.

CONCLUSION: This study therefore concludes that aqueous leaf extract of *Vernonia amygdalina* ameliorated lead-induced neurotoxicity of the premotor cortex of adult Wistar rats, and may be beneficial to people living in lead endemic areas.

Keywords:
Antioxidant; Neurobehavioural; Lead acetate; Succimer; *Vernonia amygdalina*

Submitted: 13th November, 2024
Revised: 28th November, 2024
Accepted: 30th November, 2024
Published: 31st December, 2024

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INTRODUCTION

Motor coordination involves all areas of the nervous system, from the cortex to the spinal cord, especially in vertebrates (Grillner and El Manire, 2020). The premotor cortex is involved in planning and organizing movements. Neuronal activity in prefrontal and premotor areas precedes activation of the primary motor cortex and neurons in the spinal cord during planning and organization of movement (Daroff and Aminoff, 2014). The premotor cortex is anterior to the primary motor cortex (Daroff and Aminoff, 2014; Fine and Hayden, 2021). The major function of the premotor cortex is presumed to set the stage for the motor cortex by potentiating certain motor plans and depotentiating others (Fine and Hayden, 2021). Humans and some nonhuman primates can stand and walk bipedally. However, the bipedal gait ability can be impaired by biological, and environmental factors such as ageing, chemicals, and heavy metals (Edobor, *et al*., 2021; Jobson, *et al*., 2021; Ilochi and Chuemere, 2021; Takakusaki, 2023).

Exposure to lead affects the nervous system (Brent, 2006). Lead's neurotoxic properties include its ability to pass through the blood-brain barrier, lead-binding proteins, cellular scavengers, redox enzyme systems, and interactions with other micronutrients (Sidhu and Nehru, 2004).

How to cite this article: Muhammed, A.G.; Bauchi, Z.M.; Tanko, M.; Omoniyi, A.A.; Muhammed, M.S. *et al.* Ameliorative Effect of *Vernonia amygdalina* on Lead-induced Neurotoxicity on the Premotor Cortex of Adult Wistar rats. J Exp Clin Anat 2024; 21(2): 358-366.
https://dx.doi.org/10.4314/jeca.v21i2.29

Lead poisoning is a potential factor in brain damage, mental impairment, severe behavioural anomalies, neuromuscular weakness, decreased hearing, and impaired cognitive and motor functions in experimental animals (Flora *et al*., 2007).

The quest for natural therapeutic products that enhance cognitive performance and neuroprotection via antioxidant activation is of great interest, as many age-linked disturbances and neurodegenerative disorders are triggered by elevated oxidative stress (Edobor *et al*., 2021). *Vernonia amygdalina* commonly called bitter leaf due to its bitter taste is a widely used local plant in Nigeria for both therapeutic and dietary purposes. The bitter taste is due to phyto-chemicals such as alkaloids, saponins, tannins and glycosides. (Song *et al*., 2005). The macerated leaves of the plants are consumed as vegetables and condiments while the water extract serves as tonic for prevention of certain illnesses (Izevbigie *et al*., 2003). Bitter juice has been used over centuries to treat fevers especially those related to malaria (Kokwaro *et al*., 2009). *Vernonia amygdalina* also provides anti-oxidant benefits. (Iwolakun *et al*., 2006), thus they help to reduce the risk of serious diseases associated with oxidative stress like cancer, heart disease, stroke, ageing, diabetes, Parkinson's diseases, Alzheimer's autoimmune diseases, cognitive decline and eye conditions like macular degeneration. The study evaluated the ameliorative effect of aqueous leaf extract of *Vernonia amygdalina* on lead acetate-induced neurotoxicity of the premotor cortex of 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 approval number ABUCAUC/2022/Human Anatomy/027.

Plant material purchase

Fresh leaves of *Vernonia amygdalina* were gotten from the Botanical Garden of Ahmadu Bello University, Zaria. The leaves were identified by a Taxonomist (Mr Namadi Sunusi) in the Herbarium Section of the Department of Botany, Faculty of Life Science, Ahmadu Bello University, Zaria after which a voucher number ABU0973 was assigned.

Chemicals and Drugs

Lead acetate (500 g) (the neurotoxicant) in the form of a white crystalline powder, manufactured by British Drug Houses (BDH) Laboratory Chemicals Division, Poole, England, was obtained from a reputable chemical store in Zaria and used as a neurotoxicant for the experiment. Succimer (meso-2,3-Dimercaptosuccinic acid), was obtained from Ibro Hadad Nigeria limited, Lagos Nigeria, a subsidiary of Sigma Aldrid, Germany. It was used in this research as the chelating agent Chloroform (500 ml) in the form of a colorless volatile solvent, manufactured by British Drug Houses (BDH) Laboratory Chemicals Division, Poole, England, was obtained from a reputable chemical store in Zaria and used as an anaesthetic agent for the experiment.

Neurobehavioral Studies: Forelimb grip strength

Forelimb grip strength was conducted to assess balance and coordination in rats. This is a measure of the muscular strength in the forelimbs (Olopade *et al*., 2012). Forelimb grip strength was assessed using a modified procedure described by Olopade *et al*. (2012). The rat's forepaws will be placed on a horizontally suspended metal wire 2.5 mm in diameter, 1 m in length and place 1 m above a landing area filled with soft bedding. The length of time each rat was able to stay suspended before falling off the wire will be recorded (Tamashiro *et al*., 2000). A maximum of 2 minutes was given to each rat.

Dosage determination

Based on the LD_{50} of lead acetate which was 600 mg/kg body weight according to Sujatha *et.al.* (2011), 120 mg/kg body weight (20% of the LD_{50}) was used in this study. The LD_{50} aqueous extract of Vernonia amygdalina was greater than 5000 mg/kg body weight. 1000 mg/kg body weight, and 1500mg/kg body weight doses were used in this study.

Experimental Procedure

Thirty apparently healthy adult male Wistar rats with weight range between 120g and 140g were procured from the Animal House Facility of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, 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, Ahmadu Bello University, Zaria, and allowed to acclimatize for two (2) weeks prior to the commencement of the experiment. All animals were given pelletized feed and water ad libitum.

The animals were divided into six (6) groups of five rats each. Group I (Control) received 1ml/kg body weight of distilled water for 21 days (3weeks). Group II was administered with 120 mg/kg body weight of lead acetate for 21 days (3 weeks). Group III was administered with 120 mg/kg body weight of lead acetate for seven days (1 week), afterward distilled water was administered for 14 days (2 weeks), this group is to study if there will be any natural recovery that would take place after the administration of lead acetate when compared to Group 2. Group IV was administered with 120 mg/kg body weight of lead acetate and 10 mg/kg body weight of Succimer as standard treatment drug for 21 days (3week). Group V was administered 120 mg/kg body weight of lead acetate and 1000 mg/kg body weight of aqueous extract of *Vernonia amygdalina* for 21days (3weeks). Group VI was administered 120 mg/kg body weight lead acetate and 1500

mg/kg body weight of aqueous extract of *Vernonia amygdalina* for 21 days (3weeks). Oral administrations were performed daily, with all treatment given concurrently.

Animal Sacrifice and Tissue Collection

Twenty-four hours after the last administration, rats were anaesthetized under chloroform. The brain of each rat was dissected out of the cranial cavity using forceps and dissecting scissors. Each harvested brain was weighed using a digital weighing scale (Acculab VICON, VIC-303, USA, 0.001 g) after which, some were sectioned into two (2) hemispherical halves, one hemisphere was fixed immediately in 10% Neutral buffered formol saline for histological, analysis, and the other half was then homogenized in 0.1M phosphate-buffered saline (PBS) (pH 7.4) (1g tissue/ 4 ml of PBS (Ige *et al*., 2011)) for biochemical analysis.

Biochemical studies

Each homogenized brain tissue was centrifuged at 4000 revolution per minute for 10 minutes and aliquots of the supernatant were obtained for biochemical analysis. Superoxide dismutase (SOD) and catalase (CAT), was determined using brain homogenate at the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University (ABU), Zaria.

Superoxide dismutase activity was analyzed using Rat Superoxidase Dismutase (SOD) ELISA Kit (WKEA Med Supplies Corp, China) as stated by the manufacturer. The procedure was based on the method reported by Oke and Ayo (2015). Catalase activity was determined using Rat Catalase (CAT) ELISA Kit (WKEA Med Supplies Corp, China) according to the manufacturer's instruction. The procedure used was based on the method reported by Okey and Ayo (2015).

Histological Studies

The fixed brain samples were processed through histological techniques, including fixation, dehydration, clearing, infiltration, and embedding in paraffin wax, for examination under a light microscope. Processed histological paraffin sections were stained with Hematoxylin and Eosin (HandE) to demonstrate the general histoarchitectural features of the brain regions of interest (premotor cortex) using The Rat Brain in Stereotaxic Coordinates (Paxinos and Watson, 2007).

Data Analysis

Data obtained were analyzed using GraphPad Prism (version, 9.5.1). Results obtained were expressed as mean ± standard error of mean (S.E.M), comparison between groups was determined using one-way Analysis of Variance (ANOVA) and presence of significant differences among means was determined using Tukey's honestly significant difference (HSD) as post-hoc test for significance. Paired sample t-test was employed for the comparisons of means pre and post exposure within subjects of the same group. Values were considered statistically significant when $p<0.05$.

RESULTS

Neurobehavioural Studies: Foregrip strength test

This test reflects muscular strength and balance in the animals. The foregrip strength performance to latency fall time showed a decrease in all the groups when initial trial was compared to the final trial, although there were significant decrease in the succimer, and the extract 1500mg/kg groups (Figure 1a). However, there was no significant difference in the foregrip final trial across the group, although, the 120 mg/kg lead acetate treated group showed decreased latency time when compared between the groups (Figure 1b).

Oxidative Stress Markers

A significant decrease ($p<0.05$) in Superoxide dismutase (SOD) level was observed for rats treated with only 120 mg/kg lead acetate (1.67±0.15) when compared to the control group (4.50±0.50). Also, there was significant increase in the group treated with 1500 mg/kg AEVA (3.50±0.23) when compare with the 120 mg/kg lead acetate only treated group (Figure 3A).

Tissue CAT activity levels in AEVA (1000 mg/kg and 1500 mg/kg) treatment group showed a significant increase ($p<0.05$) (3.25±0.09 and 3.56±0.22) when compared to the 120 m/kg lead acetate (1.75±0.43), and Natural recovery group (1.70±0.43). Also, the Succimer (1.80±0.06) group, 120 m/kg lead acetate (1.75±0.43), and Natural recovery group (1.70±0.43) showed a remarkable decreased CAT activity level when compared to the control group (3.84±0.17) (Figure 3B).

Histological features of prefrontal cortex

The premotor cortex of the rats in the control (untreated) group 1 showed normal cytoarchitecture of the cortex; the characteristic appearance of the neurons was arranged into six (6) layers, differing in neuron morphology, size and population density. Particularly the ganglionic layer (layer V) reveals large pyramidal (ganglion) cells of the motor cortex and stellate cells. Unlike layer (III) which is composed of medium-sized pyramidal cells. The premotor cortex of Wistar rats in the control group revealed normal cytoarchitecture of the cortical layers (III and V) with distinctive appearance of the pyramidal, neuronal and glial cells in layer III and V of the cerebral cortex. The premotor cortex of the rats in group 2 administered with lead acetate showed distortion in the histoarchitecture of the cortical layers (III and V); neuronal degenerative changes such as pyknosis, gliosis and cytoplasmic vacuolations were seen. Examination of the premotor cortex of rats in group 3 administered lead acetate with distilled water revealed distorted histoarchitecture such as; degenerating

pyramidal cells, gliosis, cytoplasmic vacuolations and karyorrhexis. The premotor cortex of rats in group 4 administered lead acetate and 10mg succimer showed mild distortion of the cytoarchitecture of the cerebral cortex (III and V). Gliosis, pyknosis vacuolation, and karyorrhexis were also seen. The premotor cortex of rats in group 5 administered lead acetate and 1000mg AEVA showed mild restoration in the cytoarchitecture of the cortical layers (III and V). However, neuronal degenerative changes such as gliosis, pyknosis and karyolysis were seen. The premotor cortex of rats in group 6 administered lead acetate and 1500mg AEVA showed moderate restoration in the cytoarchitecture of the prefrontal cortex (III and V), such as cytoplasmic vacuolation, and karyorrhexis (Plate I and II).

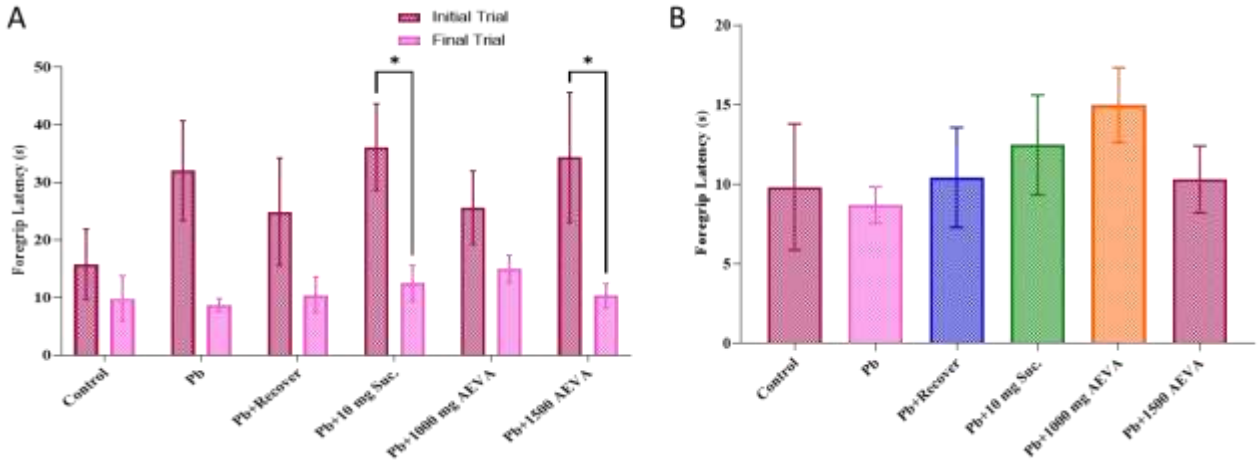

Figure 1: Foregrip latency of adult Wistar rats induced with lead acetate and treated with AEVA and Succimer.
n=6, mean± SEM. (A) paired sample t-test, *=p<0.05 when final trial was compared to initial trial. one=way (B) ANOVA, p>0.05. **Pb**= lead, **Suc**= Succimer, **Recover**=Natural recovery, **AEVA**= Aqueous extract of *vernonia amygdalina*

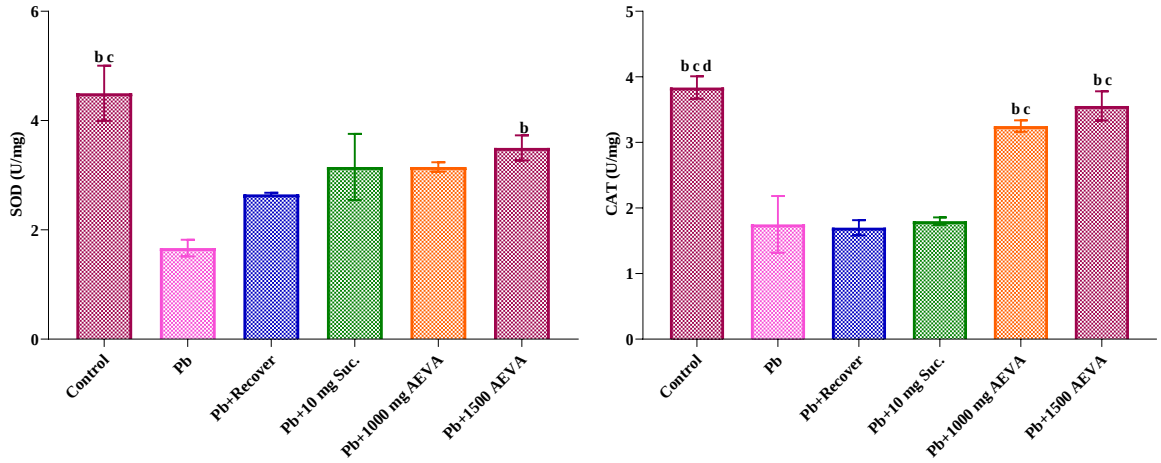

Figure 3: Effect of AEVA on (A) Superoxide dismutase (SOD) activity [left] and (B) Catalase (CAT) activity [right] of Wistar rats
n=6, mean± SEM. one=way ANOVA, *Tukey post hoc* test b=p<0.05 when compared to lead group; c=p<0.05 when compared to Pb + Natural recovery group; d=p<0.01 when compared to the Pb + Succimer group.
Pb= lead acetate, **Recover**=Natural recovery, **Suc**= Succimer, **AEVA**= Aqueous extract of *vernonia amygdalina*

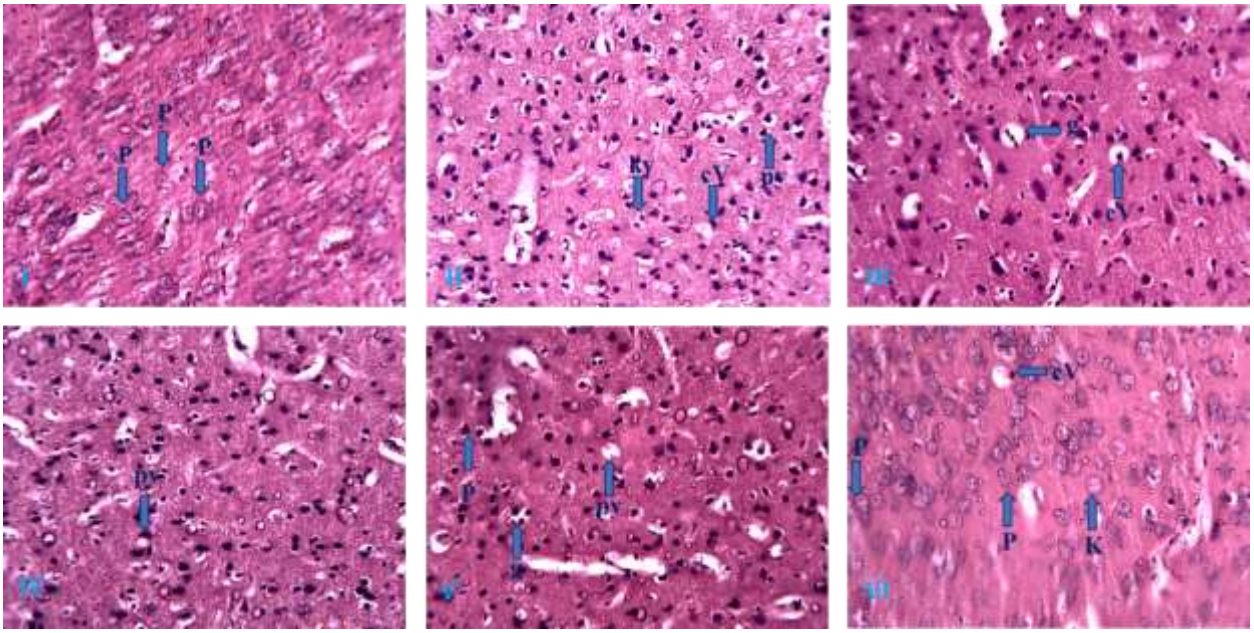

Plate I: Photomicrograph of premotor cortex (Layer III) of Wistar rats (H&E stain 250×)

I: Control group (H_2O) with normal histoarchitecture P=pyramidal cell; II: Group II (lead acetate) with distorted histoarchitecture, py=pyknosis, cV=cytoplasmic vacuolations, ky=karyolysis; III: Group III (lead acetate followed by 1-week natural recovery) with distorted histoarchitecture, g=gliosis, cV=cytoplasmic vacoulations; IV: Group IV (Pb^{2+}+ 10 mg Succimer) with mild distortion in the histoarchitecture, py=pyknosis; V: Group V (Pb^{2+}+ 1000 mg AEVA) with mild restoration in the histoarchitecture: P=pyramidal cell, py=pyknosis; VI: Group VI (Pb^{2+}+ 1500 mg AEVA) with moderate restoration in the histoarchitecture: P=pyramidal cell, cV=cytoplasmic vacoulations, K=karyorrhexis

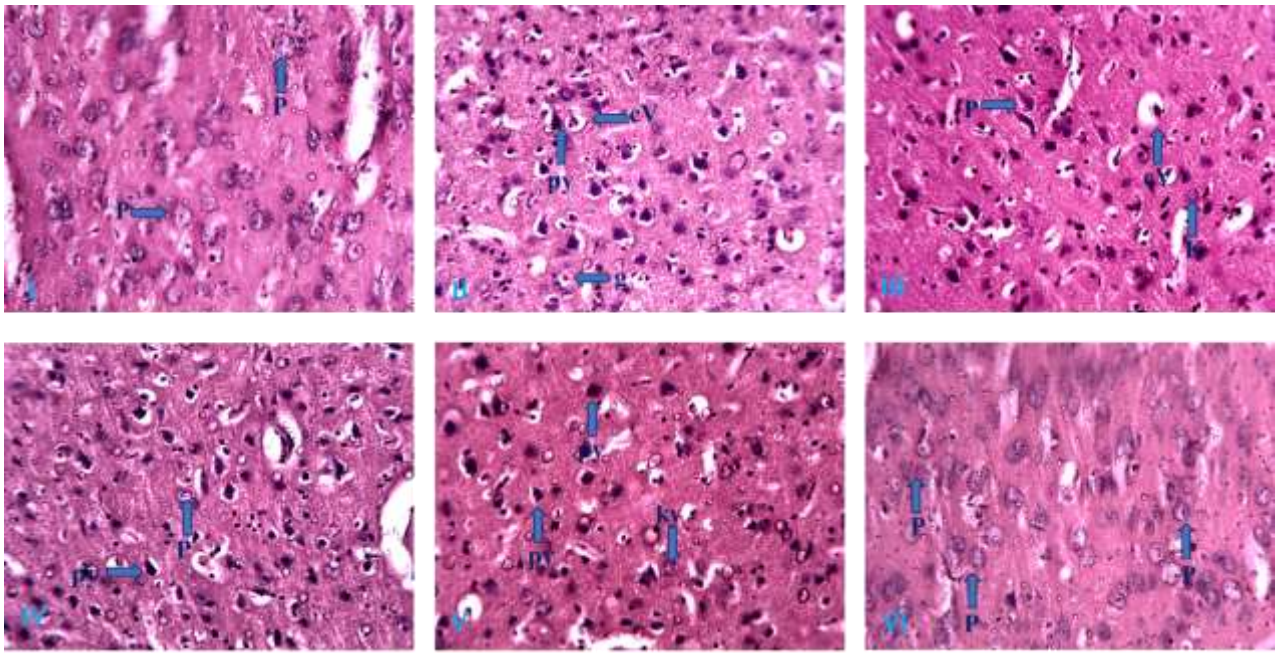

Plate II: Photomicrograph of premotor cortex (Layer V) of Wistar rats (H&E stain 250×)

I: Control group (H_2O) with normal histoarchitecture, P=pyramidal cell; II: Group II (lead acetate) with distorted histoarchitecture, py=pyknosis, g=gliosis, cV=cytoplasmic vacuolations; III: Group III (lead acetate followed by 1-week natural recovery) with distorted histoarchitecture, P=pyramidal cell, g=gliosis, cV=cytoplasmic vacoulations, k=karyorrhexis; IV: Group IV (Pb^{2+}+ 10 mg Succimer) with relatively normal histoarchitectural features, P=pyramidal cell; V: Group V (Pb^{2+}+ 1000 mg AEVA) with mild restoration in the histoarchitecture, py=pyknosis, ky=karyolysis; VI: Group VI (Pb^{2+}+ 1500 mg AEVA) with moderate restoration in the histoarchitecture, P=pyramidal cell

DISCUSSION

The present study attempted to evaluate the ameliorative effect of aqueous leaf extract of *Vernonia amygdalina* (AEVA) on lead-induced neurodegenerative changes in the premotor cortices, sensory and motor coordination, oxidative stress markers, and histological studies of adult Wistar rats.

Muscular strength is an important motor control function of the motor cortex, which is also responsible for sensory-motor integration and higher-order cognitive motor movement (Sanes and Donoghue, 2000). The forelimb grip strength latency was used to assess the muscular strength of Wistar rats. In this study, forelimb grip strength latency was observed to decline across the group. This is consistent with the report of Saritha *et al*., (2014); Lazarus *et al*. (2018), who reported a decline in foregrip strength latency with heavy metal intoxication. Treatment with the aqueous extract of *Vernonia amygdalina* at dose 1500mg/kg and the drug Succimer showed a significant ($p<0.05$) improved foregrip strength latency when compared with the control and lead acetate treatment group. However, there was no significant difference in the foregrip strength latency across the group.

Oxidative stress is considered an imbalance between prooxidant and antioxidant species within the body, which results in molecular and cellular damage. Oxidative stress plays a crucial role in the development of age-related diseases. Reactive oxygen species (ROS) are generated within the biological system to modulate cellular activities such as cell survival, stressor responses, and inflammation (He and Zuo, 2015). Oxidative stress is recognized as accountable for redox regulation involving ROS and reactive nitrogen species (RNS). The role of oxidative stress is key for the modulation of critical cellular functions, notably for neurons astrocytes, and microglia, such as apoptosis program activation and excitotoxicity, the two main causes of neuronal death. Because they have a reduced capacity to detoxify ROS, neurons are particularly vulnerable to increases in ROS levels (Sanders *et al*., 2009). Elevation of ROS has been associated with the onset and progression of neurodegenerative diseases through oxidative damage and interaction with mitochondria (Lôpez-Otin *et al*., 2013; Hajam *et al*., 2022). Due to their reactivity, high concentrations of ROS can lead to oxidative stress by disrupting the balance of antioxidant and prooxidant levels (Zuo *et al*., 2015). The excessive generation of ROS causes oxidative deoxyribonucleic acid (DNA) damage. ROS can react with the nucleic acids by attacking the nitrogenous bases and the sugar-phosphate backbone, thus inducing single- and double-stranded DNA breaks, which are also associated with premature ageing (Hajam *et al*., 2022).

The biological system protects itself against the damaging effect of activated species by several means. Oxidative stress is an indicator of decrease in cellular antioxidant defenses such as SOD, CAT and GSH levels. The cellular antioxidant defense system consists of SOD an important maker in oxidative damage as it scavenges the superoxide anion to form hydrogen peroxide, by so doing helps to diminish the toxic effects of the superoxide anion to form hydrogen peroxide (Khan *et al*., 2013). In the absence of these enzymes, this reaction becomes very slow. A decrease in SOD activity plays a role in enhancing oxidative stress in lead acetate-treated Wistar rats (Flora *et al*., 2012; Kabeer *et al*., 2019). Complex-I of the mitochondrial respiratory chain is the major source of superoxide radicals through inhibition of the electron transport chain (Kudin *et al*., 2004).

In this study marked decreased SOD activity was observed in the lead acetate-treated group when compared to the control. A significant increase in SOD was observed in the AEVA (1500 mg/kg) treated group when compared with the lead acetate treated group. This decreased SOD activity following lead acetate treatment might be due to the inactivation of SOD by ROS, which facilitates the increase in the production of superoxide radicals (Balakrishnan *et al*., 2018). Administration of AEVA (1500 mg/kg) at high dose was beneficial in restoring significantly ($p<0.05$) the SOD activity due to its antioxidant and free radical scavenging ability. This is consistent with the report by Ayoola *et al*. (2008) who reported that the efficacy of bitter leaf increased with increasing doses. Odewusi and Tope-Ajayi (2017) also reported that administration of aqueous extract of *Vernonia amygdalina* was effective enough in restoring SOD activity in lead poisoned rats.

Catalase is an antioxidant enzyme responsible for catalyzing the decomposition of hydrogen peroxidase (H_2O_2) produced as a result of superoxide dismutation. The maintenance of normal metabolism of reactive oxygen species in the body is essential for proper cell functioning (Bellissimo *et al*., 2001; Pong *et al*., 2002). In this study, tissue CAT activity was reduced in the lead acetate-treated groups compared to the control. This is similar with prior studies by Flora *et al*. (2012) who found that lead lowers catalase activity, adding to oxidative stress. Loss of catalase activity results in oxygen intolerance and triggers several deleterious reactions such as protein and DNA oxidation, and cell death (Halliwell and Gutteridge, 2015) which is indicative of enhanced oxidative stress. However, administration of AEVA ameliorated the activity of lead acetate by reversing the activity of CAT significantly in AEVA-treated rats (group V and VI). This is consistent with the work Akinpelu *et al*. (2024) who reported improved CAT activity following AEVA administration. Administration of AEVA was beneficial in restoring the SOD activity due to its antioxidant and free radical scavenging ability.

In this study, the light microscopic examination of histological sections routinely (Haematoxylin and Eosin, (HandE) stained histological sections of Wistar rat's regions: premotor cortex-layers III and V revealed pathological features in the lead acetate-induced group compared to the control group. Pathological changes have been associated with neurodegeneration in

different regions of the brain in animal models resulting from exposure to environmental neurotoxicants (Abon *et al*., 2021). Neurodegeneration is a process involved in both neuropathological conditions and brain ageing (Kumar and Khanum, 2012). Histoarchitectural distortions of neural tissue manifesting as neuronal degenerative changes are indicative of neurotoxicity in the central nervous system (He *et al*., 2023).

In this study, degenerative changes were observed such as pyknosis, gliosis, Cytoplasmic vacuolation, karyolysis, cortical neuronal shrinkage and loss of pyramidal neuron process in the premotor cortex (layers III and V) of lead acetate-treated rats' group when compared to the control. This is in concordance with the findings of Lazarus *et al*. (2018) who reported lead and other heavy metals can induce neuronal damage. Enogieru and Egbon, (2022) also reported degenerating pyramidal cells in the cerebrum of lead-treated rats. Neuronal injury may result in irreversible cell damage, which has been reported to lead to cell death (Faust *et al*., 2009). It is known that pathological or accidental cell death results from extrinsic or intrinsic insults to the cell (Natale *et al*., 2004). The prime factors for inducing this cell destruction in neuronal degeneration are neurotoxic substances present in either small or large amounts in the environment or even naturally occurring chemicals.

However, treatment with AEVA after the administration of lead acetate as a neurotoxin revealed preserved histoarchitecture of the premotor cortex (layers III and V) and mild cytoarchitectural distortions of pyramidal cells as well as neuronal processes. This could infer that AEVA has a therapeutic effect against lead-induced neurotoxicity. Natural agents with antioxidant properties have been reported to be beneficial in attenuating drug-induced oxidative stress in biological systems thereby preventing neurodegeneration (Musa *et al*., 2012; Bauchi *et al*., 2016). The AEVA antioxidant capabilities can be attributed to the presence of phenolic compounds such as phenols and flavonoids. These compounds have been found to possess strong ROS scavenging and metal ions chelating activities, therefore the use of *Vernonia amygdalina* leaf aqueous extracts were suggested as an immune booster and its usefulness in health and disease conditions (Oriakhi *et al*., 2014; Alara *et al.,* 2021).

In conclusion, results obtained from the present study indicates that aqueous leaf extract of *V. amygdalina* at a dose of 1500mg/kg has ameliorative effect on lead induced changes in the premotor cortex, motor and motor coordination and balance, and oxidative stress biomarkers of adult Wistar rats.

REFERENCES

Agbon, A. N., Kwanashie, H. O., Hamman, W. O., Ibegbu, A. O., Sule, H., Yahaya, M. H., Henry, R. and Ivang, A. E., (2021). Microscopic assessments of the effect of phoenix dactylifera L. in a rat model of mercury-triggered cerebral M1 changes. *Annals of Tropical Pathology*, 12(2), 43-52.

Alara, O. R., Abdulrahman, N, H., Mudalipa, SKA., Olalere, O.A. (2021). Phytochemical and pharmacological properties of Vernonia amygdalina. Journal of chemical Engineering and Industrial Biotechnology. 2, 80-90.

Ayoola, G., Coker, H., Adesegun, S., Adepoju-Bello, A., Obaweya, K., Ezennia, E. and Atangbayila, T. (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research. 7(3): 1019–1024.

Balakrishnan, R., Tamilselvam, K., Sulthana, A., Mohankumar, T., Manimaran, D., and Elangovan, N. (2018). Isolongifolene attenuates oxidative stress and behavioral impairment in rotenone-induced rat model of Parkinson's disease. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, *8*(2), 53-58.

Bauchi, Z. M., Kizito, D., Alhassan, A. W., Akpulu, S. P., and Timbuak, J. A. (2016). Effect of aqueous seed extract of Nigella sativa on lead-induced cerebral cortex toxicity in long evans rats. *Bayero Journal of Pure and Applied Sciences*, *9*(1), 48-52.

Bellissimo, M. I., Amado, D., Abdalla, D. S., Ferreira, E. C., Cavalheiro, E. A., and da Graça Naffah-Mazzacoratti, M. (2001). Superoxide dismutase, glutathione peroxidase activities and the hydroperoxide concentration are modified in the hippocampus of epileptic rats. *Epilepsy research*, *46*(2), 121-128.

Brent, J. A. (2006). Review of medical toxicology. Journal of Clinical Toxicology, 44, 355-356.

Daroff, R. B., & Aminoff, M. J. (2014). *Encyclopedia of the neurological sciences*. Academic press. Pg.586

Edobor, H. D., Musa, S. A., Umana, U. E., Oderinde, G. P., and Agbon, A. N. (2021). Neuroprotective effect of phoenix dactylifera (date palm) on paraquat triggered cortico-nigral neurotoxicity. The Journal of Neurobehavioral Sciences, 8(3), 199-208.

Enogieru, A. B., and Egbon, F. O. (2022). Neurobehavioural and Histological Alterations in Lead Acetate-Exposed Rats Pretreated with Aqueous Leaf Extract of Vernonia amygdalina. African Journal of Biomedical Research, 25(2), 205-213.

Faust, K., Gehrke, S., Yang, Y., Yang, L., Beal, M. F., and Lu, B. (2009). Neuroprotective effects of compounds with antioxidant and anti-inflammatory properties in a Drosophila model of Parkinson's disease. *BMC neuroscience*, *10*, 1-17.

Fine, E.J., Ionita, C.C., and Lohr, L. (2002). The history of the development of the cerebellar examination. Seminars in Neurology. 22 (4), 375–84.

Fine, J. M., and Hayden, B. Y. (2021). The whole prefrontal cortex is premotor cortex. *Philosophical Transactions of the Royal Society B*, *377*(1844), 20200524.

Flora, G., Gupta, D. and Tiwari, A. (2012). Toxicity of lead: a review with recent updates. Interdisciplinary Toxicology, 5(2), 47–58

Flora, S. J. S, Saxena, G. and Mehta, A. (2007). Reversal of lead-induced neuronal apoptosis by chelation treatment in rats: role of reactive oxygen species and intracellular Ca2+. Journal of Pharmacology and Experimental Therapeutics, 322(1): 108–116.

Grillner, S., and El Manire, Z. (2020). Current principles of motor control, with special reference to vertebrate locomotion. *Current Opinion in Neurobiology*, 60, 1-7.

Hajam, Y. A., Rani, R., Ganie, S. Y., Sheikh, T. A., Javaid, D., Qadri, S. S., Pramodh, S., Alsulimani, A., Alkhanani, M. F., Harakeh, S. and Hussain, A., (2022). Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. *Cells*, 11(3), 552.

Halliwell, B., and Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.

He, F., and Zuo, L. (2015). Redox roles of reactive oxygen species in cardiovascular diseases. *International journal of molecular sciences*, *16*(11), 27770-27780.

He, F., Liu, R., Tian, G., Qi, Y., and Wang, T. (2023). Ecotoxicological evaluation of oxidative stress-mediated neurotoxic effects, genetic toxicity, behavioral disorders, and the corresponding mechanisms induced by fluorene-contaminated soil targeted to earthworm (Eisenia fetida) brain. *Science of the Total Environment*, *871*, 162014.

Ilochi, O. N., and Chuemere, A. N. (2021). Efficacy of unripe Musa sapientum whole fruit on neuromuscular strength and olfactory response linked to cerebro-cerebellar ionized calcium, muscle creatine kinase and plasma acetylcholinesterase. *Asian Journal of Advances in Medical Science*, 3(1), 156-161

Ilochi, O., and Chuemere, A. (2021). Progressive changes in gait, kinematic, horizontal and vertical activities in response to oleic acid and heavy metal exposure. Asian Journal of Advances in Medical Science, 358-367.

Iwolakun, B. A., Odukoya, O., and Adesanya, S. A. (2006). Antioxidant and antimicrobial activities of some Nigerian medicinal plants. *African Journal of Biotechnology*, 5(13), 1196-1202.

Izevbigie, E. B., and Afolabi, A. O. (2003). The influence of Vernonia amygdalina on reproductive hormones in male rats. *Journal of Ethnopharmacology*, 85(1), 135-140

Jobson, D. D., Hase, Y., Clarkson, A. N., and Kalaria, R. N. (2021). The role of the medial prefrontal cortex in cognition, ageing and dementia. Brain communications, 3(3), fcab125.

Kabeer, A., Mailafiya, M. M., Danmaigoro, A., Rahim, E. A., and bu Bakar, M. Z. A. (2019). Therapeutic potential of curcumin against lead-induced toxicity: A review. *Biomedical Research and Therapy*, *6*(3), 3053-3066.

Khan, M. A., Chen, H. C., Wan, X. X., Tania, M., Xu, A. H., Chen, F. Z., and Zhang, D. Z. (2013). Regulatory effects of resveratrol on antioxidant enzymes: a mechanism of growth inhibition and apoptosis induction in cancer cells. *Molecules and cells*, *35*, 219-225.

Kokwaro, J. O., Ochieng, J., and Wanyonyi, J. (2009). Ethnobotanical study of medicinal plants used by the Abagusii community in Kenya. *Journal of Ethnopharmacology*, 124(1), 12-21.

Kudin, A. P., Bimpong-Buta, N. Y. B., Vielhaber, S., Elger, C. E., and Kunz, W. S. (2004). Characterization of superoxide-producing sites in isolated brain mitochondria. *Journal of Biological Chemistry*, *279*(6), 4127-4135.

Kumar, G. P., and Khanum, F. (2012). Neuroprotective potential of phytochemicals. *Pharmacognosy reviews*, *6*(12), 81.

Lazarus, S. S., Samuel, S. A., Yusuf, T., Abel, N. A., and Michael, N. B. (2018). Histological and histochemical assessements on the effect of ethanol fruit extract of Phoenix dactylifera L.(Date Palm) on cerebral cortex of lead acetate treated wistar rats. *African Journal of Cellular Pathology*, *10*(1), 1-9.

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. *Cell*, *153*(6), 1194-1217.

Lorke, D. (1983). A new approach to practical acute toxicity testing. Archives of Toxicology, 54, 275–87.

Musa, S. A., Omoniye, I. M., Hamman, W. O., Ibegbu, A. O., and Umana, U. E. (2012). Preventive activity of ascorbic acid on lead acetate induced cerebellar damaged in adult wistar rats. Med Health Sci J, 13, 99-104.

Musa, S., Edobor, H., Umana, U., Oderinde, G., Olatomide, O., Zachariah, R., Usman, I. and Agbon, A., 2022. Histological Assessment of the Effect of Phoenix Dactylifera (Date Palm) on Cortico-ponto-cerebellar Structures in Paraquat Model of Neurotoxicity: Date and Cortico-ponto-cerebellar Structures. *Journal of Medical and Basic Scientific Research*, 3(2), 105-116.

Natale, J. E., Knight, J. B., Cheng, Y., Rome, J. E., and Gallo, V. (2004). Metallothionein I and II mitigate age-dependent secondary brain injury. *Journal of neuroscience research*, *78*(3), 303-314.

Odewusi, O.O., Tope-Ajayi, A.A. (2017). Antioxidant and blood lowering effect of aqueous Vernonia amygdalina extract. American journal of biomedical sciences. 9(4): 193-199

Oke, S. M., and Ayo, J. O. (2015). The role of co-administration of ascorbic acid and zinc gluconate on brain biochemical changes in Wistar rats during the hot humid season. *European Journal of Biotechnology and Bioscience*, *3*(1), 46-52.

Olopade, F. E., Shokunbi, M. T., and Sirén, A. L. (2012). The relationship between ventricular dilatation, neuropathological and neurobehavioural changes in hydrocephalic rats. Fluids and Barriers of the CNS, 9, 1-10.

Oriakhi, K., Oikeh, E. I., Ezeugwu, N., Anoliefo, O., Aguebor, O., and Omoregie, E. S. (2014). Comparative antioxidant activities of extracts of Vernonia amygdalina and Ocimum gratissimum leaves. *Journal of Agricultural Science*, *6*(1), 13.

Oyeyemi, I. T., Akinlabi, A. A., Adewumi, A., Aleshinloye, A. O., and Oyeyemi, O. T. (2018). Vernonia amygdalina: A folkloric herb with anthelminthic properties. *Beni-suef University Journal of basic and applied sciences*, *7*(1), 43-49.

Pong, K. (2003). Oxidative stress in neurodegenerative diseases: therapeutic implications for superoxide dismutase mimetics. *Expert opinion on biological therapy*, *3*(1), 127-139.

Sanders, T., Liu, Y., Buchner, V., and Tchounwou, P. B. (2009). Neurotoxic effects and biomarkers of lead exposure: a review. *Reviews on environmental health*, *24*(1), 15-46.

Sanes, J. N., and Donoghue, J. P. (2000). Plasticity and primary motor cortex. Annual review of neuroscience, 23(1), 393-415.

Saritha, K., Reddy, K. S., and Kumar, A. (2014). The influence of heavy metal exposure on foregrip strength in humans. *International Journal of Environmental Research and Public Health*, 11(3), 3892-3903.

Sidhu, P., and Nehru, B. (2004). Lead intoxication: histological and oxidative damage in rat cerebrum and cerebellum. *The Journal of Trace Elements in Experimental Medicine*, *17*(1), 45-53.

Song, Y., Wang, Y., and Zhang, Y. (2005). The anti-cancer activity of Vernonia amygdalina. *Journal of Ethnopharmacology*, 100(1-2), 1-10.

Sujatha, K., Srilatha, C., Anjaneyulu, Y., Rao, T. S., Sreenivasulu, D., and Amaravathi, P. (2011). Ameliorative efficacy of Ocimum sanctum leaf extract in lead acetate induced toxicity in Wistar albino rats: a pathomorphological study.

Takakusaki, K. (2023). Gait control by the frontal lobe. Handbook of clinical neurology, 195, 103-126.

Tamashiro, K. L., Wakayama, T., Blanchard, R. J., Blanchard, D. C., and Yanagimachi, R. (2000). Postnatal growth and behavioral development of mice cloned from adult cumulus cells. Biology of Reproduction, 63(1), 328-334.

Wise, S. P., Boussaoud, D., Johnson, P. B., and Caminiti, R. (1997). Premotor and parietal cortex: corticocortical connectivity and combinatorial computations. *Annual review of neuroscience*, *20*(1), 25-42.

Zuo, L., Zhou, T., Pannell, B. K., Ziegler, A. C., and Best, T. M. (2015). Biological and physiological role of reactive oxygen species–the good, the bad and the ugly. *Acta physiologica*, *214*(3), 329-348.