Original Research Article

Antioxidant Activities of Aqueous Leaf Extract of *Anacardium occidentale* (Cashew) on Lead acetate-Induced Cerebellar Toxicity in Wistar Rats

Abstract

Aim: The aim of the present study was to investigate the antioxidant effect of aqueous leaves extract of *Anacardium occidentale* (*A.O*) on lead-induced toxicity in the cerebellum of wistar rats.

Methods: Thirty wistar rats were randomly divided into six groups of five rats each. Group A: Normal control received 0.5ml normal saline; group B received 50mg/kg body weight (bwt) lead acetate (Pb) only (28 days); groups C and D: 150mg/kg bwt and 300mg/kg bwt of *A.O* respectively for 14 days and 50mg/kg bwt of Pb next 14 days; group E: 150 mg/kg bwt*A.O* and 50 mg/kg bwt Pb (28 days); group F: 300mg/kg bwt *A.O* and 50 mg/kg bwt Pb (28 days). At the end of the experiment, animals were sacrificed by cervical dislocation. One hemisphere of the cerebellum was homogenized for estimation of tissue Superoxide Dismutase (SOD), Glutathione peroxidase (GPx) and Malondialdehyde (MDA) levels, and the second hemisphere was processed for histological studies.

Results: Histological examination of lead only treated groups, showed alterations in cerebellar architecture. Biochemical estimations showed significant decrease in SOD, GPx levels (*P*=.03 and .04 respectively) and significant increase in MDA levels (*P*=.04), indicative of oxidative stress. Pretreatment and co-treatment with *A.O* showed dose dependent preservation of cerebellar architecture, significant increase of antioxidant parameters (SOD and GPx) (*P*=.03) and significant (*P*=.02) decrease in MDA.

Conclusion: This study suggests that *Anacardium occidentale* especially at a higher dose (300mg/kg) exhibits antioxidant activities against lead-induced oxidative stress in the cerebellum of rats.

Keywords: Cerebellum, Lead toxicity, Antioxidant, *Anacardium occidentale*

Introduction

Lead (Pb) is a heavy metal and ubiquitous environmental neurotoxin known to induce oxidative stress by increasing the generation of reactive oxygen species (ROS), such as hydroxyl radicals, lipid peroxides, superoxide radicals, and hydrogen peroxide [1]. Lead has unique properties such as high density, ductility, malleability and ability to resist corrosion and has been very useful in making building materials, pigments to glaze ceramics, water pipes, glass, paints, protective coatings, and gasoline additives [2, 3]. Exposure of man to Lead and its derivatives in day-to-day life is unavoidable [4]. The transport and distribution of lead from major emission sources, both fixed and mobile are mainly through air. It can enter the human body via uptake of food (65%), water (20%) and air (15%) [2]. Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage. Lead poisoning remains one of the oldest and the most widely studied occupational and environmental hazards [5] and has been implicated as the cause of up to 1.5% deaths annually in the world [6]. Occupational exposure to Pb is linked to several health consequences, such as cognitive impairment, reproductive disorders, hypertension, motor dysfunction, cancer, hepatotoxicity, nephrotoxicity, and mortality [7,8]. As the nervous system is a common target organ for lead-induced toxicity, Pb toxicity has long been linked with impaired motor function, particularly deficits in visuomotor coordination among adult industrial workers and children exposed to Pb [9, 10].

There has been great advancement and increase in the use of plant based medicine in the past few decades owing mainly to the discovery that extracts from plants are pharmacologically safe and contain a diverse array of secondary metabolites with antioxidant potential against heavy metal poisoning [11]. There are approximately 4 major classes of secondary compounds (antioxidants) that are significant to humans. The classes are the alkaloids, phenylpropanoids, flavonoids and the terpenoids [12]. These antioxidants help inhibit peroxidative damage caused by environmental toxicants and also prevent damages to cell membrane due to cellular oxidative processes that may result in diseases [13, 14].

The cashew (*Anacardium occidentale*) is a tree in the family of the flowering plant Ancardiaceae which has its roots in different parts of the world [15]. The local name of the fruit is kaju in yoruba, kasu in Hausa and kashuu in Igbo. Cashew is a useful tree as different parts of it are used either individually or collectively to treat several diseases. A research study has shown that the

stem-bark of *Anacardium occidentale* has anti-inflammatory effects [16]. The roots, leaves, stems and fruits extract of *Anacardium occidentale,* have been reported to display hypoglycemic effects in folk medicine [17]. Fresh or hot water extract of different plant parts is used orally as aphrodisiac, anti-dysenteric, anti-hemorrhagic and externally as anti-inflammatory [18]. Research studies on the hydroethanolic extract of *Anacardium occidentale* leaves have shown the inhibition of gastric lesions induced by HCl/ethanol in female rats [19]. *Anacardium occidentale* leaf extracts have also shown efficient antimicrobial activity against P. gingivalis & P. intermedia [20]. The present study attempts to characterize biochemical and histological alterations induced by lead in the cerebellum of wistar and evaluate antioxidant effect of aqueous leaves extract of *Anacardium occidentale*.

MATERIALS AND METHODS

Experimental Animals

Thirty wistar rats, weighing between 200-240g were obtained from a breeding stock maintained in the animal house of the department of Pharmacology and Toxicology, University of Nigeria, Nsukka. They were housed in cages in the animal house of the Department of Anatomy, University of Nigeria, Enugu Campus, under controlled conditions of 12-hour light/ 12- hour dark cycles. The rats were allowed to acclimatize for 14days before the commencement of the experiment and had free access to clean water and standard livestock pellets (Guinea Feed Nigeria Limited) ad libitum.

Chemicals and Reagents

Lead acetate (C4H6O4Pb_H2O) with molecular weight of 379.33 was purchased from M&B chemicals, England. All other biochemical reagents and chemicals were of analytical grade.

Plant Material

The fresh leaves of *Anacardium occidentale* free from insect infestation were collected from the cashew plantation area in Opi Nsukka, Enugu state, Nigeria. Its botanical identity was

authenticated by a botanist at the Herbarium of University of Nigeria, Nsukka with Herbarium voucher specimen number 240a.

Preparation of Leaf Extract

Anacardium occidentale leaves were washed with tap water, dried at room temperature for a period of 14days and ground into powder. 500g of plant powder sample was extracted in 2L of distilled water by maceration method for 24 hours. The mixture was stirred after every 8 hour using a sterile glass rod. The macerated sample was filtered using muslin cloth at room temperature. The filtrate was concentrated using rotary evaporator and finally dried on the water bath in an evaporating dish until the extract became completely dry. The dried extract was weighed to be 49.1g yielding 9.8%. The extracted sediment was stored at 4°C in a refrigerator.

Preliminary phytochemical screening of crude extract of *Anacardium occidentale* leaves

One gram of the extract of *Anacardium occidentale* leaves was dissolved in distilled water (100ml) used to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening as described by [21].

Acute toxicity study

Acute toxicity studies on *Anacardium occidentale* leaves was carried out using 12 rats.

In the first phase, the rats were randomly divided into 3 groups of 3 rats each and each group was treated with 10mg, 100mg and 1000mg per kg of extract orally, respectively. They were observed for 24 hours for any gross behavioral changes and deaths.

In the second phase, a total of 3 rats were randomly divided into 3 groups of one rat each. Each group received 1500mg, 3000mg and 5000mg per kg of extract orally respectively. The number of deaths was recorded after 24 hours of extract administration.

Experimental Design

Thirty wistar rats used for this study were randomly divided into six groups of five rats each such that the weight difference between and within groups did not exceed ±20 of average weight of

the sample population. Rats in group A served as normal control, received 0.5ml normal saline for 28 days; group B was administered 50mg/kg lead acetate (Pb) only; groups C and D (protective groups) received aqueous leaf extract of *A.O* (150mg/kg and 300 mg/kg respectively) for a period of 14 days and (50mg/kg) Pb for next 14 days; groups E and F (ameliorative groups) received (150 mg/kg *A.O*, followed by 50 mg/kg Pb; 300 mg/kg *A.O* followed by 50 mg/kg Pb, respectively) for a period of 28 days. All administration was by oral gavage.

Experimental Protocol

Twenty-four hours following the completion of treatments, the animals were sacrificed by cervical dislocation. The brain was quickly excised, cerebellum removed and separated into two hemispheres. One hemisphere was homogenized in phosphate-buffered saline (PBS) for biochemical estimation while the other hemisphere was processed for subsequent histological analysis.

Histological Studies

All the groups were subjected to histological studies at the end of 28 days. The cerebellar tissue excised was processed for routine H&E []. Sections was cut on a rotary microtome at 5µm thickness. Photomicrographs were taken with a JVC colour video digital camera mounted on a light microscope.

Microscopy

For light microscopic studies, the cerebellar tissue sections on glass slides were captured using Olympus binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0 MP Amscope Camera (Amscope Inc, USA.)

Preparation of Cerebellum Tissue for Assay

Samples of the cerebellum tissue were collected from each animal after cervical dislocation. 10% (w/v) homogenate was immediately prepared using a homogenizer by weighing the sample and

homogenizing with appropriate volume of ice-cold phosphate buffer 0.1M, pH 7.0. The homogenate obtained was centrifuged to obtain the supernatant which was used to evaluate the activity of oxidative stress markers in the cerebellum.

Evaluation of antioxidant enzymes and Lipid peroxidation

SOD, GPx and Lipid peroxidation, evaluated on the base of MDA level, was determined using assay kits for each. Detailed procedures for the above measurements were carried out according to the kits' protocol.

Data Analysis

The data obtained were analyzed statistically with one-way analysis of variance (ANOVA) followed by student's t-test with the aid of SPSS (V20; USA). Data were presented as means ± SEM (standard error of mean). *P* value ($P < .05$) was considered statistically significant.

RESULTS

Result of phytochemical analysis

Anacardium occidentale aqueous leaf extract in the present study, showed flavonoids and phenolics to be abundantly present, steroids, alkaloids, and saponins were moderately present while triterpenes and tannins was present in trace amount (Table 1).

Table 1: Result for preliminary phytochemical screening

Phytochemicals	Aqueous extract of *A.O* leaves
Saponins	++
Alkaloids	++
Flavonoids	+++
Phenolic	+++
Tannins	+
Steroids	++
Triterpenes	+

**KEY: + = present in trace amount; ++ = moderately present; +++ = abundantly present*

Result for acute toxicity study

The result of the present study shows that *Anacardium occidentale* aqueous leaf extract has a lethal dose above 3000mg/kg body weight in wistar rats (Table 2).

Table 2: Result for acute toxicity test of aqueous leaf extract of *Anacardium occidentale*

PHASE	Treatment	Number of deaths
ONE	10mg/kg	0/3
	100mg/kg	0/3
	1000mg/kg	0/3
TWO	1500mg/kg	0/1
	3000mg/kg	0/1
	5000mg/kg	1/1

Physical Observations

During the acclimatization period and at the beginning of the experiment, all the animals looked healthy. On the administration of lead-acetate, varying gradations of toxicity such as staggering, muscle tremors, irritability, shedding of fur and decreased food intake were observed. These signs however, were not observed in all the groups administered *A.O* aqueous leaf extract.

Antioxidant effect of aqueous leaf extract of *Anacardium occidentale* on lead-induced toxicity in cerebellum of Wistar rats.

Significant decrease in SOD, GPx (P=03 and 0.04 respectively), and significant (P=.04) increase in MDA tissue levels were observed in group B when compared to normal control. Administration of *A.O* especially at high doses (300mg/kg) in pre-treatment and co-treatment groups, showed antioxidant activity, indicated by a significant increase in SOD, GPx tissue

levels (*P*=.03) and significant decrease in MDA tissue levels (*P*=.02) when compared to the lead-only treated group (Fig 1).

Figure 1: Effects of *A.O* on antioxidant levels and lipid peroxidation levels in cerebellum of wistar rats. (Mean ± SEM).

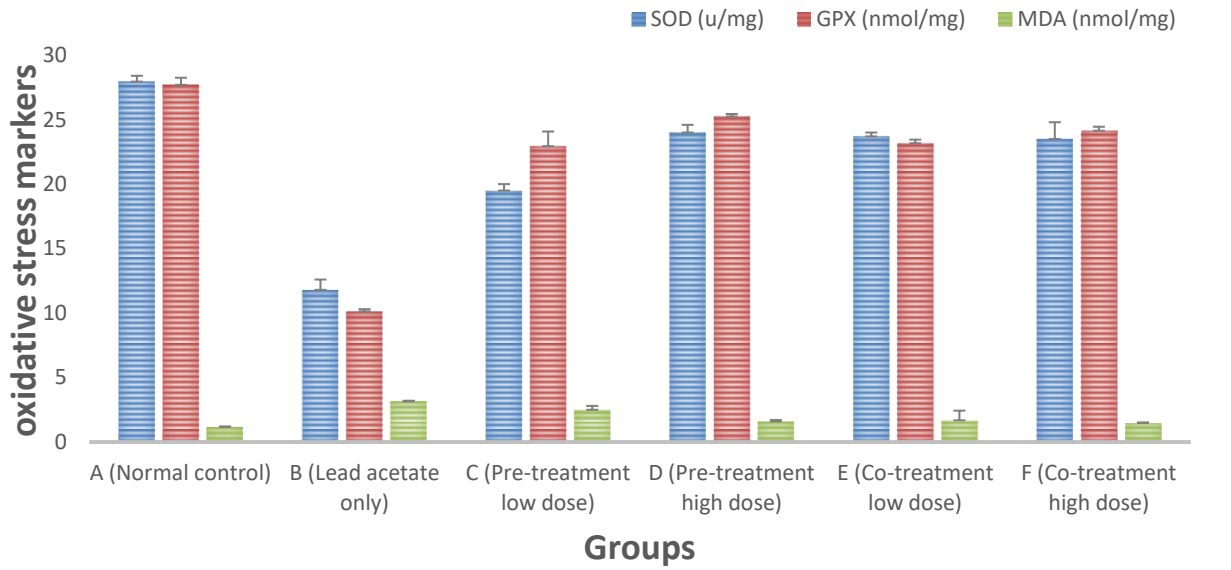

FIG. 1: EFFECTS OF *A.O* ON ANTIOXIDANT AND LIPID PEROXIDATION LEVELS IN CEREBELLUM OF WISTAR RATS (MEAN ± SEM)

Histological Findings

Histological studies on the normal control group showed normal cerebellar architecture (fig. 2).The Group B rats induced with lead acetate without any treatment showed variable degeneration and necrosis of the cerebellar morphology(fig 3). The histological structure of the animals pre-treated before induction with lead acetate (figs. 4 &5) and also those co-treated with the toxic agent (figs. 6 & 7) were better preserved and showed less signs of toxicity when compared with lead only treated group.

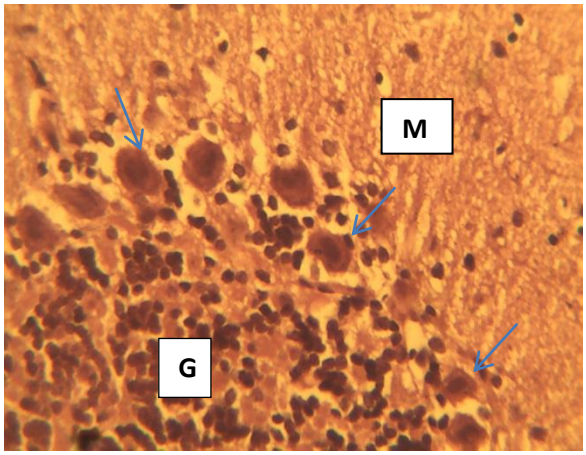

Fig.2 group A (Normal Control):showing normal histo-architecture of the cerebellar layers i.e molecular layer (M), granular layer (G). The Purkinje cells (arrow) are normal in cellular morphology, with visible dendrites in the molecular layer. And the granular cells in the granular layers were normal. **H&E X400**

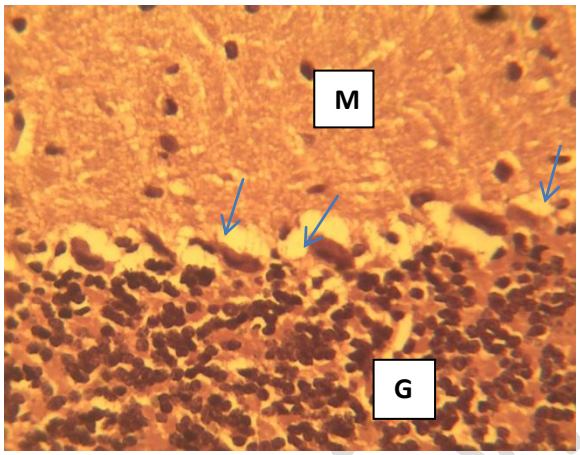

Fig. 3 group B (Lead acetate only):The Purkinje cells showed variable degeneration and necrosis. Purkinje cell dendrites in the molecular layer are relatively inconspicuous. Molecular layer (M); Granular layer (G); Purkinje cells (arrow).**H&E X400**

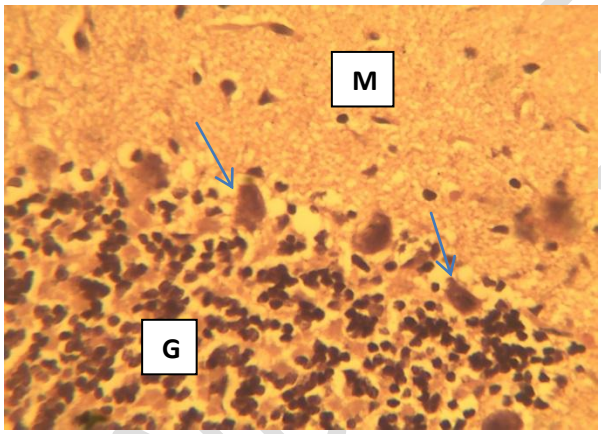

Fig.4 groupC(Pre-treatment low dose):tissue section shows normal histo-architecture of the cerebellar layers. Purkinje cells showed slight degenerative changes (arrow). **H&E X400**

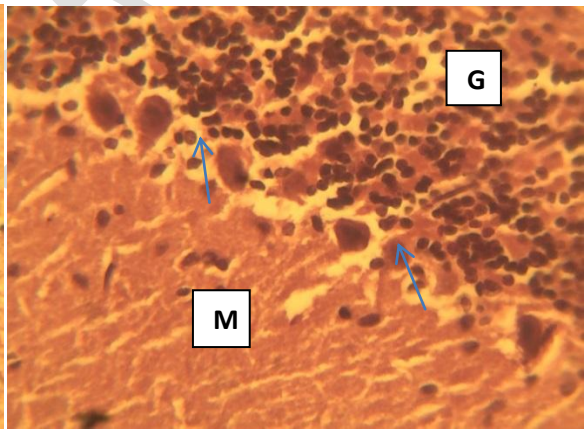

Fig.5 groupD (Pre-treatment High dose);tissue section shows normal histo-architecture of the cerebellar layers. The Purkinje cells (arrow) appeared relatively normal. **H&E X400**

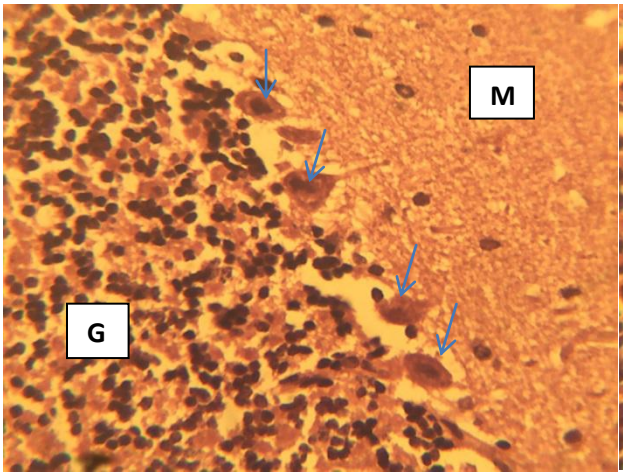

Fig.6 group E (co-treatment, low dose**):** shows normal histo-architecture of the cerebellum and very slight necrosis of purkinje cells (arrow).
Molecular layer (M), Granular layer (G), Purkinje cells (arrow). **H&E X400**

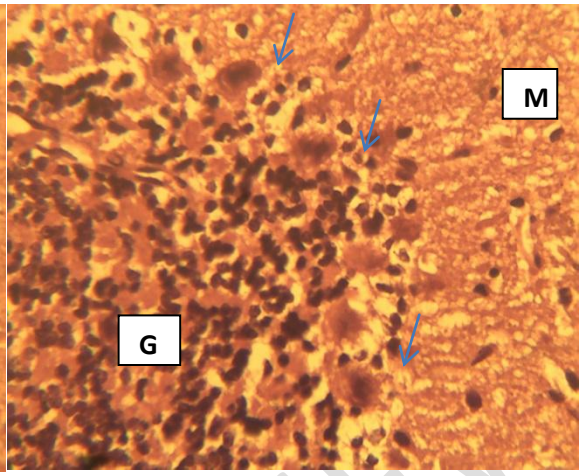

Fig.7 group F (co-treatment, high dose);shows normal histo-architecture of the cerebellum. The Purkinje cells (arrow) are normal in cellular morphology with visible dendrites in the molecular layer. **H&E X400**

DISCUSSION

Previous reports on lead-induced toxicity have presented Lead as a known cause of oxidative damage in various soft tissues by bringing about imbalance in the generation and removal of reactive oxygen species [22], [23] with morbidity on almost all organs, the brain, kidney, and liver serving as primary targets [24], [25], [26]. The cerebellum is a delicate structure that is vulnerable to intoxication and poisoning. The Purkinje cells of the cerebellum are particularly susceptible to injury after exposure to environmental toxins such as Pb [27].

The generation of reactive oxygen species (ROS) such as superoxide ions and hydrogen peroxides or by-products of lipid peroxidation such as lipid hydroperoxides and lipid aldehyde [28], [29] have been implicated in lead-induced toxicity. The body antioxidant defense system however, plays a significant role in scavenging generated ROS thereby protecting the cells of the body against their toxic effects [30]. Studies [31], suggests that exposure to heavy metals such as lead may alter the integrity and selective permeability of cell membranes, thereby raising its susceptibility to lipid peroxidation.

The results of the present study showed that lead acetate exposure (group B) significantly increased activity of antioxidant enzyme (GPx and SOD) (P=03) compared to the normal control (group A), indicating a rise in oxidative stress. Also, there was significant decrease in the activity of the membrane enzyme (MDA) (P=02) compared to the normal control (group A), revealing a rise in lipid peroxidation. The reason for increased lipid peroxidation and oxidative stress may be due to the combined inhibitory effects of the various antioxidants enzymes (SOD and GPx) as observed in our results. Histological analysis of lead exposed animals showed altered cerebellar cytoarchitecture with marked degeneration of purkinje cells and necrosis of cells in the molecular layer. This agrees with previous work documented by [32], who observed similar rats' response to lead treatment.

Phytochemical screening of plant extracts provides insight to their therapeutic properties that may help to mitigate a wide spectrum of human ailments. In the present study, *A.O* showed presence of flavonoids and phenolics in abundance, while steroids, alkaloids, saponins, triterpenes and tannins were present in moderate to trace amounts. This was consistent with documented report by [33] that *A. occidentale* extracts revealed a variety of rich phytochemicals such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils [34]. Flavanoids, saponins and phenolic plant phytochemicals, are well known [35] for their antioxidant activities and have the ability to inhibit peroxidative damage caused by environmental toxicants.

The significant increase in MDA enzyme activity observed in group D and also in groups E&F when compared with the control group was as a result of an inhibition in lipid peroxidation (P=04). The reason why *A.O* can actively modulate lipid peroxidation in cerebellar tissue assays might be related to its antioxidant activities. We observed in this study that administration of *A.O* in groups C, D, E and F significantly decreased the activity of antioxidant molecules (SOD and GPx) in rats of lead-induced toxicity (P=03 and 04 respectively). This was perhaps due to presence of high concentration of some biologically active compounds like flavonoids in *A.O* which has potent free radical scavenging properties thereby protecting the cerebellar tissue against the biochemical alterations of reactive oxygen species. This result, is in consonance with earlier reports suggesting that flavonoids can protect against reactive oxygen species induced by heavy metals in wistar rats [36, 37]

Histological analysis from this study, revealed that in the pre-treatment groups (C and D), the animals were well able to tolerate the pathological effect of lead acetate in their system much longer. Especially at a higher dose (300mg/kg bwt), *A.O* preserved the tissues from the usual degeneration of neural cells in cerebellum, common in lead toxicity. The tissue section of animals in groups (E and F) co-treated with lead acetate and *A.O* extracts at low and high doses, showed no sign of histological alteration in their cerebellar tissue. They had normal purkinje cells and pyramidal cells of the cerebellar layers. By these observations, one may deduce that at both dose levels in the pre and co-treatment groups *A.O* produced protective effect on histological structure of the cerebellum against lead acetate-induced toxicity. High concentration of the natural antioxidants, flavonoids and saponins in the aqueous leaf extract of *A.O* may perhaps, account to the potent antioxidant activity of the plant and the neuroprotective activities observed across the treatment groups. This is in accordance to the reports of previous researchers [32,] who documented that substances with anti-oxidant properties would ameliorate to a large extent against the effects of lead toxicity in cerebellum of wistar rats.

Conclusion

In conclusion, we evaluated the antioxidant activities of aqueous leaf extract of *anacardium occidentale* (cashew) on lead acetate-induced cerebellar toxicity in wistar rats.Aqueous leaf extract of *Anacardium occidentale* has shown potent antioxidant activities in this study which may be due to the high concentration of some natural antioxidants like flavonoids, phenolics and saponins in the plant extract. The protective and antioxidant effect of *A.O* was also supported by histological observations, which suggest that *A.O* especially at a higher dose (300mg/kg bwt) exhibits antioxidant activities against lead-induced cerebellar toxicity in wistar rats.

Ethical Approval

Ethical clearance was obtained from the Research Ethics Committee of the College of Medicine, University Nigeria, Enugu Campus with protocol number UN/CM/004/001/2021.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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