ASSESSMENT OF PROTECTIVE ROLES OF AQUEOUS SEED EXTRACT OF *ANNONA MURICATA* IN CEREBELLUM FOLLOWING CADMIUM-INDUCED NEUROTOXICITY OF ADULT WISTAR RATS

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ABSTRACT

Cadmium, a deleterious heavy metal that pervades the environment, has the potential to accumulate in the body and cause health complications. In the domain of traditional medicine, medicinal plants have been employed to combat the toxicity of heavy metals and treating of many diseases in traditional medicine.

AIM: To access the protective impact of *Annona Muricata* seed extract on cadmium-triggered neurotoxicity in the cerebellum of albino wistar rat.

PLACE OF STUDY: Department of Anatomy, Faculty of Basic Medical Sciences Olabisi Onabanjo University, Ogun State between June 2022 and June 2023

METHODOLOGY: After a period of 14 days of acclimatization, 40 healthy male wistar rats were randomly allocated to four groups, group1(distilled water only), group2 cadmium only(2g/kg SC), group3 (2g/kg cadmium + 100mg/kg extract) and group4(2g/kg cadmium + 200mg/kg extract) The rats were subjected to cadmium subcutaneously followed by the oral administration of aqueous seed extract of *Annona Muricata* for fifteen consecutive days.

RESULT: The impact of the extract on antioxidant enzymes activities of the cerebellum, cerebellar weight, as well as the histology of the cerebellum were scrutinized. The data revealed that the aqueous seed extract of *AnnonaMuricata*, in a dose-dependent manner, augmented the levels of superoxide dismutase (SOD) (P=0.005) and catalase (CAT) (P=0.027) in contrast to the cadmium-only group. The relative weight of the cerebellum exhibited a significant increasein the treated groups compared to the cadmium-only group.

The histology of the cerebellum delineated pathological changes arising from the exposure to cadmium, while *Annona Muricata* brought about regenerative changes.

CONCLUSION: In summary, the study posits that owing to the presence of phytochemicals in *Annona Muricata* aqueous seed extract, it was efficacious in mitigating the neurotoxicity induced by cadmium in the cerebellum of male rats.

Keywords: Neurotoxicity, Cerebellum, Annona Muricata, Cadmium,

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INTRODUCTION

Cadmium, denoted by the symbol Cd, is a lustrous, pliable, and ductile metallic element that belongs to the d-block series of the periodic table, thereby qualifying as a transition metal. The element occurs naturally in trace amounts in the earth's crust and is present in several minerals such as greenockite, otavite, and cadmium oxide. The industrial significance of cadmium is evident from its widespread applications, including its use in batteries, pigments, coatings, and plastics. However, it is imperative to note that cadmium and its compounds are highly toxic, as demonstrated by an extensive body of literature (Genchi *et al*., 2020), and are associated with a gamut of health issues. The atomic number and weight of cadmium are 48 and 112.411, respectively. The element exhibits a melting point of 321 °C and a boiling point of 767 °C, with a density of 8.65 g/cm3. Cadmium has the potential to form several compounds, among which cadmium oxide (CdO), cadmium sulfide (CdS), and cadmium chloride (CdCl2) are noteworthy examples.Cadmium oxide, a brownish-black powder, finds its usage as a pigment and in the production of batteries. In contrast, cadmium sulfide, a yellow-orange compound, is utilized in pigments, solar cells, and as a semiconductor. Cadmium chloride, a white crystalline solid, serves as a catalyst in chemical reactions (Fang *et al*., 2014). The toxic nature of cadmium, a highly toxic metal, can result in a range of health issues, including cancer, kidney damage, and bone damage. The toxicity of cadmium is attributed to its capacity to bind to proteins, enzymes, and other biological molecules, thereby disrupting their normal functions (Bernhoft *et al*., 2013). Cadmium exposure can occur through inhalation of cadmium fumes or dust, ingestion of contaminated food or water, or through skin contact with cadmium-containing materials. The primary sources of cadmium exposure include tobacco smoke, industrial emissions, and contaminated food and water (Rahimzadeh *et al*., 2017).

The primary adverse impacts of cadmium are attributed to its capability to attach to sulfhydryl (-SH) groups in proteins and enzymes, thereby inhibiting enzyme activity. Consequently, cellular metabolism is disrupted, and cell death may ensue. Furthermore, cadmium can attach to DNA, inducing mutations

and ultimately leading to cancer (Edwards and Ackerman, 2016). One of the most widely recognized health consequences of cadmium exposure is the damage it causes to the kidneys.

Medicinal plants have been employed for their therapeutic properties for centuries. These botanicals have been utilized to treat a plethora of maladies, ranging from minor discomforts to grave illnesses. The utilization of such vegetal agents has been recorded in archaic transcripts and through archaeological corroboration. The scope of medicative flora is all-encompassing, spanning any plant that has been employed for medicinal purposes. These plants can be utilized in their innate form or processed into herbal remedies. Notable examples of frequently utilized medicative flora include chamomile, ginger, garlic, annonamuricataand ginkgo biloba (Hosseinzadeh et al., 2015).The historical record has documented the customary usage of medicinal plants in diverse

Annona Muricata, commonly known as soursop, is a tropical fruit indigenous to Central and South America. It belongs to the Annonaceae family and shares a close botanical relationship with the cherimoya and custard apple. The *Annona Muricata* tree is an evergreen, small-sized plant that can grow up to a height of 10 meters. Its broad-spreading canopy is adorned with shiny, dark green leaves. It's fruit is oval-shaped and has a green exterior covered with spines. It has a sweet and sour flavor, and its interior is made up of white, creamy flesh and large, black seeds (Patel and Patel, 2016).

Annona Muricata can commonly be found in tropical and subtropical regions worldwide, including Africa, Central and South America, and Southeast Asia. This particular species exhibits a preference for moist, well-drained soil and is capable of withstanding both drought and flooding. Following maturation, the fruit of *Annona Muricata* can be harvested and consumed in either its fresh form or processed into various products, such as juices, jams, and other related items (Thang *et al*., 2013). It boasts a rich history of traditional medicinal usage and is reputed to possess anti-inflammatory, anti-cancer, and antioxidant properties. In traditional medicine, the fruit is utilized to manage a broad range of conditions, such as fever, colds, and digestive problems. Additionally, it is believed to be beneficial in treating skin conditions such as psoriasis and eczema (Coria-Téllez *et al*.,

2018).Thepharmacological effects of *Annona Muricata* are predominantly attributed to its phytochemical constituents, which comprise alkaloids, tannins, saponins, flavonoids, terpenoids, and phenolic acids. These compounds are believed to be responsible for the fruit's anti-inflammatory, anti-cancer, and antioxidant properties.

The cerebellum, situated in the posterior aspect of the skull beneath the cerebral hemispheres, constitutes approximately 10% of the total brain volume. The cerebellum's convoluted surface greatly increases its surface area. Its primary functions include movement coordination, balance and posture regulation. Anatomically, the cerebellum consists of three parts: the flocculonodular lobe, the vermis, and the lateral hemispheres. The flocculonodular lobe, the most ancient part of the cerebellum, governs eye movements and balance. The midline vermis coordinates movements of the trunk and limbs. The lateral hemispheres, the cerebellum's largest component, coordinate limb movements.The cerebellum, a vital component of the brain, is interconnected to the brainstem via three pairs of cerebellar peduncles: the superior, middle, and inferior cerebellar peduncles. Specifically, the superior cerebellar peduncles establish connections between the cerebellum and the midbrain, whereas the middle cerebellar peduncles facilitate connections between the cerebellum and the pons. Lastly, the inferior cerebellar peduncles mediate connectivity between the cerebellum and the medulla oblongata (Marzbanet al., 2015; Guell and Schmahmann, 2020). The cerebellum plays a crucial role in movement coordination and timing. It receives inputs from a wide range of sensory systems, including the visual, vestibular, and somatosensory systems, as well as the cerebral cortex. Subsequently, the cerebellum processes these inputs and transmits output signals to the motor cortex and brainstem, leading to the adjustment of movements and the maintenance of balance and posture (Roostaeiet al., 2014; D'Angelo, 2018).Damage or dysfunction of the cerebellum may lead to an array of motor and balance issues. Ataxia, a term utilized to denote the absence of coordination of movements that may emanate from cerebellar damage, may be evident among patients. Those with ataxia may encounter

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difficulties with balance, walking, and fine motor tasks. Dysmetria, or difficulty with the precision of movements, is another common manifestation of cerebellar damage.

Environmental toxins refer to hazardous substances that exist in our surroundings, which can negatively impact human health. These toxins may include pollutants, pesticides, heavy metals, and industrial chemicals. Exposure to such toxic substances can result in detrimental effects on numerous organs in the body, including the brain. In particular, the cerebellum is highly susceptible to the harmful effects of environmental toxins due to its elevated metabolic rate and high concentration of receptors for various environmental toxins. Consequently, exposure to environmental toxins can lead to cerebellum damage, which can manifest as a range of neurological symptoms (Meyer et al., 2013).The exposure of humans to cadmium, a toxic heavy metal, has become an increasing environmental concern. Cadmium has the potential to induce ataxia, tremors, and impairments in balance and coordination, as well as impairments in cognitive function, attention, and memory (Wang and Du, 2013). Its entry into the food chain through soil contamination, industrial emissions, and cigarette smoke can cause its accumulation in various tissues of the body, including the brain. The cerebellum, a crucial component of the brain responsible for movement and balance, is particularly vulnerable to the toxic effects of cadmium (Genchi et al., 2020).*Annona muricata*, also known as soursop, is a tropical fruit with medicinal properties that have been shown to have neuroprotective effects. It is believed that the phytochemicals in soursop have antioxidant properties that can protect against oxidative stress, which is known to contribute to the toxic effects of cadmium in the brain (Moghadamtousi*et al*., 2015). However, the effects of *Annona Muricata* on cadmium-induced neurotoxicity in the cerebellum of adult Wistar rats have not been fully studied. Therefore, there is a need for further research to investigate the potential protective effects of *Annona Muricata* against cadmium-induced neurotoxicity in the cerebellum. The findings from this study will contribute to the understanding of the potential use of *Annona Muricata* as a neuroprotective agent against cadmium-induced neurotoxicity in humans.

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Materials and Methods

Methods

Ethical concerns in animal study

All animal experiments and protocols adhered to the guidelines and regulations set forth by the National Research Council in regards to laboratory animal care and utilization (2011). Following the conclusion of experiments, animal carcasses were buried, no less than two feet beneath the natural surface, and covered with lime, disinfectant, and soil.

Animal management

40 healthy adult malewister rats weighing from 150- 250g were procured from Pharmacy department of OlabisiOnabanjo University, Sagamu, Ogun state. The rats were subjected to a standard acclimatization period of two weeks under controlled conditions of 12-hour light/12-hour darkness, with a temperature range of 25±3°C and a mean relative humidity of 50±5%. Before the commencement of the treatment.

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Animal grouping and care

After acclimatization, the rats were divided into 4 groups (10 rats per group): control group, cadmium chloride only group,100mg/kg treatment of aqueous extract of *Annona Muricata*groupand 200mg/kg group. The animals were provided with standard grower feeds that were purchased from Joyful Feeds Sagamu, Ogun State. Additionally, water was provided for drinking throughout both the acclimatization period and experimental procedures. The animals were housed in appropriately ventilated cages that were equipped with feeding troughs and water plastics. It is important to note that this study adhered to established guidelines for the care and use of laboratory animals in biomedical research and teachings that were approved by the Institute of Laboratory Animal Resources, National Research Council (2010).

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List 1. Animal grouping and treatment

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DURATION	GROUP A	GROUP B	GROUP C	GROUP D
WEEKS	Normal control (n=10)	Cadmium only (n=10)	Treatment 1 (n=10)	Treatment 2 (n=10)
0-4	Feed & Water	2mg/kg of cadmium (SC) once weekly	2mg/kg of cadmium (SC) once weekly	2mg/kg of cadmium (SC) once weekly
4-6	Feed & Water	Feed & Water	100mg/kg of extract daily (P.O)	200mg/kg of extract daily (P.O)

S.C: subcutaneous, P.O: oral route of administration

Preparation of the aqueous seed extract of Annona muricata

The *Annona Muricata* fruit was procured from Ikenne market, located in the Ikenne local government area of Ogun state, Nigeria and then authenticated at the Botany department of Olabisi Onabanjo University. The fruit underwent a peeling process to expose the seed, after which the Annona muricata seed was washed with clean water and subsequently air-dried at room temperature. The seed was then milled into a fine powder, whereupon 500g of the powder was macerated in one litre of distilled water for a duration of 24 hours. Following this, the mixture underwent filtration, and the filtrate was then concentrated using a rotary evaporator at a temperature of 60 °C. The resultant concentrated filtrate was collected in a bottle and stored at room temperature until required for usage. The concentrate was

reconstituted in distilled water to obtain the desired concentrations of 100 mg/kg and 200 mg/kg of Annona muricata, which were utilized in the current study.

Preparation of cadmium

One milligram of Cadmium chloride was suspended in 5 ml of 0.9% NaCl.

Administration of treatment

The administration of cadmium was done according to the method described by Gaurav et al., (2010). Cadmium and Annona muricata aqueous seed extract was administered concomitantly for 15 consecutive days.

Qualitative analysis of Phytochemical Constituent of the Aqueous Seed Extract of *Annona muricata*

The qualitative phytochemical constituents of the plant extracts were carried out to determine the presence of bioactive compounds such as saponins, alkaloids, flavonoids, steroids, tannins, phlobatannins, terpernoid, cardiacglycosides, phenols and reducing sugar in the plants following the standard methods described by (chukwuma*et.al*., 2012)

Animal sacrifice and determination of organ weight

The animals were subjected to cervical dislocation six hours subsequent to the expiration of research. The cerebellum was meticulously extracted and the weight of the organ was ascertained per 100 grams of body weight utilizing a kerroBL20001 weighing scale.

Studies on oxidative stress

The study determined the effect of various doses of *Annona Muricata* on oxidative stress in the cerebellum of cadmium-induced neurotoxicity.

Determination of superoxide dismutase (SOD) activity

The rats' SOD activity was evaluated using the method developed by Misra and Fridovich in 1972. The principle behind this method lies in the capacity of superoxide dismutase to hinder the auto-oxidation of epinephrine at pH 10.2, which serves as the foundation for a straightforward assay of this enzyme. The introduction of increasing concentrations of epinephrine resulted in the oxidation of epinephrine to adenochrome produced per superoxide anion introduced, which was generated by xanthine oxidase reaction. These findings propose that the auto-oxidation of epinephrine progresses through at least two distinct pathways, with only one of them being a free radical chain reaction that involves superoxide radical and, consequently, can be inhibited by superoxide dismutase.

Determination of catalase activity

The determination of catalase activity in tissue and serum was carried out using the method developed by Sinha (1972). The underlying principle of this method is based on the reduction of dichromate in acetic acid to chromic acetate upon heating in the presence of H_2O_2, resulting in the formation of an unstable intermediate known as perchromic acid. The chromic acetate produced is then quantified colorimetrically at a wavelength of 570 - 610 nm. As dichromate exhibits no absorbance in this region, its presence in the assay mixture does not interfere with the colorimetric determination of chromic acetate in any way. The catalase preparation is allowed to cleave H_2O_2 for varying durations of time. The reaction is halted at a specific time by introducing a dichromate acetic acid mixture, and the residual H_2O_2 is determined by measuring chromic acetate colorimetrically following reaction heating.

Morphological studies for Haematoxylin and eosin staining method

The rats were weighed in grams using a weighing scale, before the start of the experiment and before their sacrifice.

- **Preparation of Tissues for Histological Examination;** The cerebellar tissues were duly prepared and subjected to histological and histochemical techniques at the Histological Laboratory of the Department of Anatomy, Olabisi Onabanjo University's Sagamu Campus.

- **Fixation;** The tissues were immersed in a formal saline solution comprising of 0.85 parts of NaCl, 90 ml of water, and 10 ml of formaldehyde for a duration of approximately 24 hours, following which the process of dehydration was initiated.
- **Dehydration;** The tissues were dehydrated in the following solution at different stages; 60% alcohol, 70% alcohol, 80% alcohol, 90% alcohol, 95% alcohol, first absolute alcohol and finally second absolute alcohol all at one (1) hour interval each)
- **Clearing**; Clearing was done by using xylene(a hydrophilic clearing agent) to remove the alcohol from the cerebellar tissue, which was changed at one hour interval in first xylene initially and finally, second xylene**.**
- **Infiltration;** The tissues were infiltrated with paraffin wax at a temperature between the ranges of 50-60C for an hour. The tissues were then embedded in a paraffin wax with proper orientation.
- **Embedding;** The tissues were then embedded in paraffin wax with proper orientation. The embedding took place in a LUKAT embedding mild coated with glycerol. The paraffin wax was allowed to solidify forming a visible scum before cooling at a temperature of about 10-15C.
- **Sectioning;** The cassette that contained the embedded tissue was affixed onto the microtome, which was adjusted to a thickness of 5 micrometers. To mitigate the generation of heat, ice blocks were employed during the sectioning process. The initial set of sections was deemed unfit due to block trimming. Notably, both thick and thin sections were produced, yet only the latter were utilized. The thin sections were promptly immersed in a 5% alcohol solution for a duration of five (5) minutes, followed by a transfer into a warm bath for an additional five (5) minutes. It was essential to ensure that the sections were appropriately spread out to facilitate microscope viewing. Fresh slides were coated with egg albumin, immersed in warm water, and then utilized to retrieve the sectioned tissues. The tissues were then air-dried on a hot plate and stained with Haematoxylin and eosin stain.Nuclei stained blue black and cytoplasm, pink.

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Photomicrography

Image acquisition and analysis: A bright light microscope (10 - 40x magnification objective) used. Digital camera - OMAX Toup view 3.7 attached to P.C - HP used. Java Application Software (image J Software) used.

Statistical analysis

The descriptive statistic of mean, standard deviation and inferential statistics were used for this study, the data was subjected to statistical test and analysis with the aid of Statistical Packages for Social Sciences (SPSS) version 21 and Microsoft excel 2021 for windows using T-test method of data analysis. 0.05 was alpha level of significance ($P<0.05$).

4.0 RESULTS

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4.1 Qualitative Phytochemical Constituent of the Aqueous Seed Extract of *Annona muricata*

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Table 1 presents the qualitative phytochemical constituents of the aqueous seed extract of *Annona muricata*. The tabular representation effectively itemizes the various phytochemicals and their presence or absence in the sample. The symbols employed in the table denotes the presence (+) or absence (-) of particular phytochemicals. From the data, it can be seen that the aqueous seed extract of Annona muricata comprises a variety of phytochemicals, namely alkaloids, saponins, tannins, phlobatannins, steroids, cardiac glycosides, phenols, flavonoids, and terpenoids. These bioactive

compounds are extensively utilized in traditional medicine and are commonly found in several medicinal plants.

Table 1. Qualitative Phytochemical Constituent of the Aqueous Seed Extract of *Annona muricata*

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Phytochemical	Sample
Alkaloid	+
Saponins	+
Tannins	+
Phlobatannin	-
Steroids	+
Cardiac Glycosides	+
Phenols	+
Flavonoids	+
Reducing Sugar	-
Terpernoid	+

Present; +; absent -.

Effect of the oral administration of the aqueous seed extract of *Annona muricata* against oxidative stress induced by Cadmium in the brain of adult male wistar rats

The table shows the effect of the oral administration of the aqueous seed extract of *Annona muricata* against oxidative stress induced by cadmium in the brain of adult male wistar rats. The rats were divided into four groups (A, B, C&D) and treated with distilled water only (group A), 2 mg/kg of cadmium (S.C) (group B), 2 mg/kg of cadmium (S.C) and 100 mg/kg body weight of *Annona muricata* (P.O) (group C), 2 mg/kg of cadmium (S.C) and 200 mg/kg body weight of *Annona muricata* (P.O) (group D). The table presents the levels of superoxide dismutase (SOD) and catalase (CAT) in each group. SOD and CAT are both antioxidant enzymes that protect cells from oxidative stress. The values for SOD and CAT are expressed in µmol/ml/min/mg/protein. The mean and standard error of the mean (SEM) are also provided. The results show that the administration of cadmium significantly reduced the levels of SOD and CAT in the brain of rats in group B compared to group A (the control group). However, the administration of *Annona muricata* extract at different doses (groups C and D) significantly increased the levels of SOD and CAT compared to group B, indicating a protective effect

against oxidative stress induced by cadmium. The letter codes (A, B, C, D) indicate statistical significance. A value with a letter code is significantly different from the corresponding value in the group with the letter code indicated. For example, in the SOD column, the value in group B has the letter code A, indicating that it is significantly different from the corresponding value in group A (the control group). The value in group C has the letter code A as well, indicating that it is significantly different from the value in group B. The value in group D has the letter codes B and C, indicating that it is significantly different from the values in groups B and C.

Table 2: Effect of the oral administration of the aqueous seed extract of *Annona muricata* against oxidative stress induced by Cadmium in the brain of adult male wistar rats

Comment [DA22]: This should be Table 4

Groups	A	B	C	D
Treatment	Distilled water only	2 mg/kg of cadmium (S.C)	2 mg/kg of cadmium (S.C) and 100 mg/kg body weight of Annona muricata (P.O)	2 mg/kg of cadmium (S.C) and 200 mg/kg body weight of Annona muricata (P.O)
SOD (µmol/ml/min/mg/pro)	27.16±0.13	3.77±0.33 ^{A}	25.43±0.81 ^{A}	29.35±0.91 $^{B, C}$
CAT (µmol/ml/min/mg/pro)	26.68±0.76	1.85±0.23 ^{A}	18.31±0.88 $^{A, B}$	17.56±3.44 $^{A, B, C}$

Each value is an expression of mean ± SEM. (P <0.05)

^{A} ***indicates that value was significant when compared to A***

^{B} ***indicates that value was significant when compared to B***

^{C} ***indicates that value was significant when compared to C***

4.3:Effect of the oral administration of the aqueous seed extract of *Annona muricata* on the relative weight of the brain in Cadmium induced toxicity in the cerebellum of adult male wistar rats

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The graph shows the effect of Annona muricata extract on the relative weight of the BRAIN in adult male Wistar rats exposed to cadmium-induced toxicity. Group A received only distilled water, group B received cadmium, while groups C and D received cadmium and different doses of the extract. The results showed that cadmium exposure significantly decreased the relative weight of the brain in group B compared to group A. However, treatment with the extract at doses of 100 and 200 mg/kg body weight (groups C and D) significantly increased the relative weight of the BRAIN compared to group B. Overall, the study suggests that Annona muricata extract has potential as a protective agent against cadmium-induced BRAIN toxicity, especially at doses of 100 and 200 mg/kg body weight.

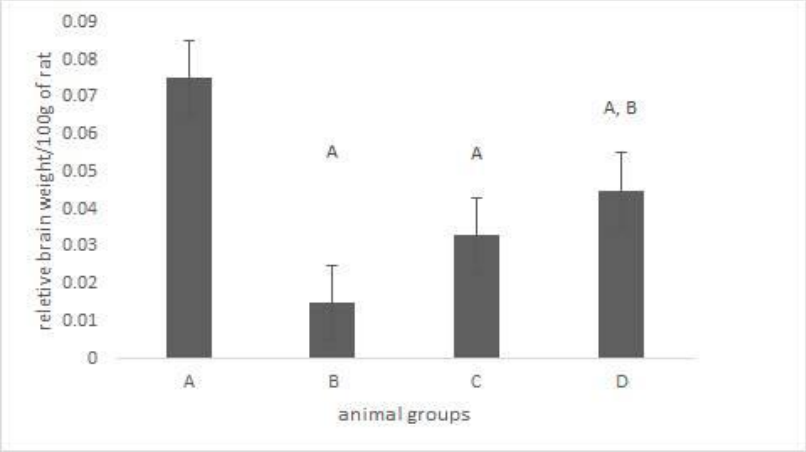

Figure 1.Effect of the oral administration of the aqueous seed extract of *Annona muricata* on the relative weight of the BRAIN in Cadmium induced toxicity in the cerebellum of adult male wistar rats. ***Each bar is an expression of mean ± SEM. (P <0.05) [a] - Values were significant when compared to group A, [b]-Values were significant when compared to group B, [c] -Values were significant at P 0.04 when compared to group c***

4.4: Effect of the oral administration of the aqueous seed extract of *Annona muricata* on weight changes in Cadmium induced toxicity in the cerebellum of adult male wistar rats

The table shows the effect of administering different doses of Annona muricata extract on weight changes in male rats with cadmium-induced toxicity. The groups were categorized based on the

treatment they received. The initial weight and final weight of each group were measured and the values are expressed as mean ± SEM. The significance level is indicated by letters. The results showed that the group treated with cadmium only (group B) had a significant decrease in weight compared to the control group (group A). However, treatment with the extract resulted in an increase in weight in all treated groups, with the highest dose of the extract (group D) showing the most significant increase. The results suggest that Annona muricata extract may have a protective effect against cadmium-induced weight loss in male rats.

Table 3. Effect of the oral administration of the aqueous seed extract of *Annona muricata* on weight changes in Cadmium induced toxicity in the cerebellum of adult male wistar rats

Groups	A	B	C	D
Treatment	Distilled water only	2 mg/kg of cadmium (S.C)	2 mg/kg of cadmium (S.C) and 100 mg/kg body weight of Annona muricata (P.O)	2 mg/kg of cadmium (S.C) and 200 mg/kg body weight of Annona muricata (P.O)
Initial weight (g)	117±3.06	214.67±34.43 A	139±21 B	135.14±14.7 A. B
Final weight (g)	135.33±12.86	178.67±18.58 A	143.33±23.09	139.33±17.9 B

Each value is an expression of mean ± SEM. (P <0.05)

[A] - Values were significant when compared to group A, [B]-Values were significant when compared to group B, [C]- Values were significant when compared to group C, [D] Values were significant when compared to group D,

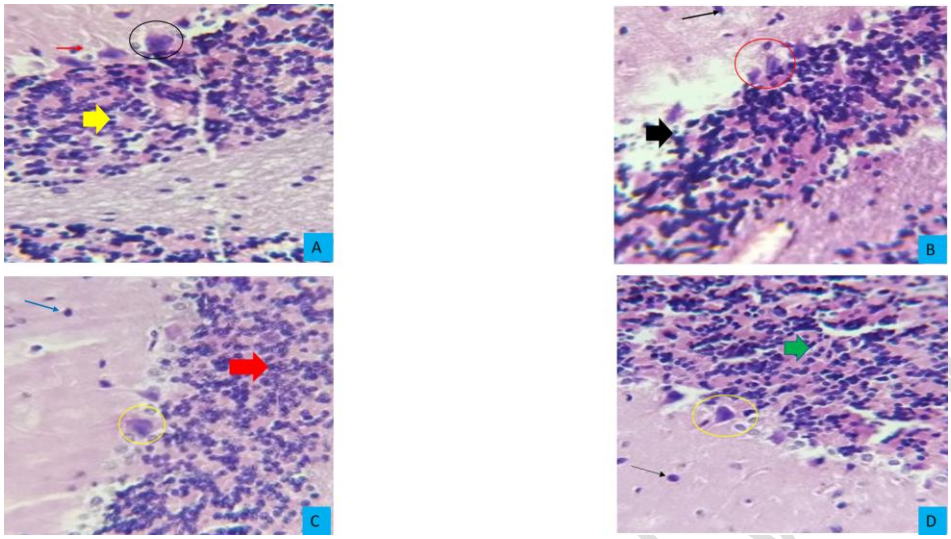

PLATE 1: H & E photomicrograph of the cerebellum(Magnification at x400)

a. Control group showing well differentiated and normal cerebellum histomorphology. The granular cells on the granular layer with (yellow thick arrow), molecular layers(red thin arrow) and purkinje layer containing the pyramidal cells(black circle)

b. Cadmium-induced group shows severe necrotic degeneration and degradation of the purkinje layer with shrunken pyramidal cells(red circle), granular layer cells and hyperchromatic glial nucleus on the molecular layer (black thick arrow).

c. Induced and administered at a dosage of 100mg/kg of aqueous seed extract of *Annona Muricata*, a regenerative alteration is observed in the pyramidal cells of the purkinje layer (yellow circle), granular layer (red thick arrow) and the molecular layer.

d. Induced and treated with 200mg/kg of aqueous seed extract of *Annona Muricata* extract, it shows restorative changes of the pyramidal cells of purkinje layer (yellow circle) with slight hyperplastic changes of the granular layer (green thick arrow)

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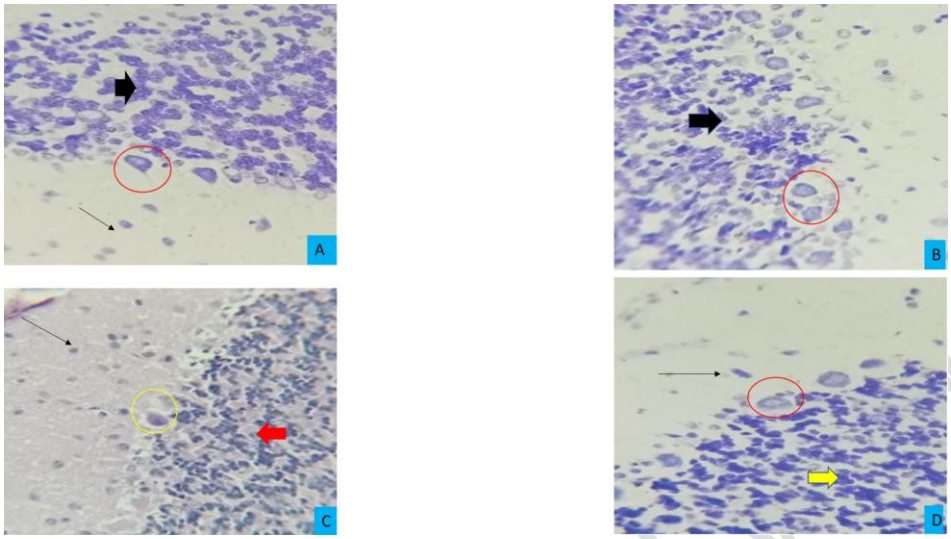

PLATE 2: The photomicrograph of the cerebellum with cresyl violet stain. (Magnification at x400)

a) Control group showing well differentiated granular cells on the granular layer, well differentiated pyramidal cells on the purkinje layer and the molecular layer

b) Cadmium induced shows severe degeneration with hyperplastic granular cells (black thick arrow), purkilocytic pyramidal cells (red circle) and the molecular layer.

c) Induced and treated with aqueous extract of 100mg/kg of Annona Muricata extract shows restorative effect of the molecular layer (black thin arrow), purkinje cells (yellow circle) and the granular layer (red thick arrow)

d) Upon induction and treatment with an aqueous extract of 200mg/kg of Annona Muricata, a mild regeneration of purkinje cells (red circle), granular cells (yellow thick arrow), and the molecular layer (black thin arrow) was observed.

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Discussion

Cadmium, a heavy metal, possesses toxic properties and has been implicated in causing oxidative stress in several tissues, including the cerebellum. The mechanism of cadmium-induced oxidative stress is multifaceted and involves the accumulation of reactive oxygen species (ROS) leading to oxidative damage to cellular components. The generation of ROS, such as superoxide anion (O2-), hydrogen peroxide (H2O2), and hydroxyl radicals (OH), represents a primary mechanism of cadmium-induced oxidative stress (Fang, Zhou, and Dionysiou, 2013; Ozcan and Ogun, 2015). Cadmium's ability to stimulate the production of ROS stems from its capacity to induce the expression of enzymes such as NADPH oxidase, xanthine oxidase, and nitric oxide synthase, which operate in different cellular compartments, including the cytoplasm, mitochondria, and endoplasmic reticulum (Angeli, Pereira, de Oliveira Faria, Stefanon, Padilha, and Vassallo, 2013). Cadmium has the ability to impede the function of enzymes that are involved in the antioxidant defense system, notably superoxide dismutase (SOD) and catalase (CAT). The former converts O2- into H2O2, which is then further neutralized by the latter. The inhibition of these enzymes can occur either directly or indirectly, with the latter being due to a decrease in the levels of antioxidant molecules within the cell, ultimately leading to oxidative stress (Nikolić *et al*., 2016). In our study, we observed this cadmium-induced oxidative stress, as indicated by the results presented in Table 2. Specifically, the exposure to cadmium led to a notable decrease in the activity of SOD and CAT enzymes within the cerebellar tissue.

One of the pathways through which medicinal plants mitigate oxidative stress is via the augmentation of endogenous antioxidants. For instance, plant-based compounds, including flavonoids, carotenoids, and phenolic acids, have demonstrated the ability to upregulate the operation of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). These enzymes are responsible for the conversion of harmful ROS into less injurious molecules, thereby diminishing oxidative stress (Palipoch, 2013). In addition, medicinal plants function by directly scavenging free radicals.Polyphenolic compounds discovered in diverse medicinal plants have demonstrated the

capability to counteract free radicals through the act of donating electrons or hydrogen atoms, as evidenced by Shebisand colleagues in 2013. In addition, Gavamukulya and colleagues in 2014 have previously demonstrated that the examination of the seed extract of Annona Muricata uncovered the existence of certain chemical substances notably, alkaloids, flavonoids, terpenoids, coumarins and lactones, anthraquinones, tannins, cardiac glycosides, phenols, phytosterols, and saponins. These phytochemicals may be accountable for the observed antioxidant and anti-inflammatory transformations in Table 2 following seed extract administration.The animals in Group B, which were solely exposed to Cadmium, exhibited a notable reduction in antioxidant enzyme levels, particularly in CAT and SOD. This decrease may be attributed to the accumulation of superoxide anion radicals and hydrogen peroxide that accentuate peroxidative activity, as reported by Halliwell and Gutteridge in 1985. Conversely, the animals that were exposed to Cadmium and simultaneously treated with aqueous extract of Annona Muricata showed significant increases in antioxidant enzyme levels. These increases were observed in both SOD and CAT activity. *Annona Muricata* extract is recognized for its very potent anti-inflammatory and antioxidant activities, and it has been shown to be rich in eugenol. Plants rich in anti-inflammatory and antioxidant compounds have shown to be useful agents in ameliorating oxidative stress.

Comment [DA28]: In discussion, you will tell us your findings. Thereafter, you will see whether it agreed or disagreed with other authors findings with their citations or references. You will then tell us the possible pathophysiology of your findings.

Conclusion

The current study concludes that exposure to Cadmium chloride had some histological changes on cells of the cerebellum. Meanwhile, *Annona Muricata*, with its known antioxidant properties, was able to protect these cells from such toxic effects which could reflect on motor and balance problems especially for those continuously exposed to such metallic hazards. More emphasis is still needed on its dose and time-dependent protective effects. The study conducted by Adeyemi et al. (2019) has demonstrated that both Superoxide Dismutase (SOD) and Catalase (CAT) enzymes, which function as antioxidants, play a pivotal role in safeguarding cells against the detrimental effects of oxidative stress. In the context of this study, it was observed that the administration of cadmium significantly

attenuated the levels of SOD and CAT in the brain of rats belonging to group B, compared to the control group (group A). However, a noteworthy finding of this study was that the administration of Annona muricata extract at varying doses (groups C & D) significantly elevated the levels of SOD and CAT in the brain tissue of rats when compared to group B. This suggests that the extract may have a protective effect against cadmium-induced oxidative stress. Therefore, it can be concluded that the aqueous extract of *Annona Muricata* exhibits a neuroprotective action against cadmium-induced neurotoxicity in the cerebellum of adult wistar rats.

Recommendation

The use of aqueous seed extract of *Annona muricata* can provide a natural and safe alternative to alleviate the adverse effects of cadmium on the brain. However, further research is needed to explore the extract's long-term effects, optimum dosage and potential side effects.

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Comment [DA29]: Names of Some journal were in italic while some were not in your references. Please see the authors guide to see the journal referencing style.

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