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Original Article

Psidium Guajava Leaves Ameliorates Mercuric Chloride Induced Neurodegeneration in the Cerebral Cortex of Adult Male Wistar Rats

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ABSTRACT

Background: The protective activity exerted by *Psidium guajava* on the nervous system has been suggested to be via its antioxidant composition; however, its role in mercury-induced neurotoxicity remains elusive. This study investigated the ameliorative activity of ethanolic extract of *P. guajava* leaves on mercuric chloride-induced toxicity in the cerebral cortex of male Wistar rats.
Methods: Thirty-five (35) male Wistar rats were separated into seven groups with five rats each. Group, I served as control, Group II received 41.5 mg/kg of mercuric chloride ($HgCl_2$), Group III received 1000 mg/kg bwt of EEPGL, Group IV received 41.5 mg/kg of $HgCl_2$ and distilled water, Group V received 41.5 mg/kg of $HgCl_2$ and 500 mg/kg bwt of EEPGL, Group VI received 41.5 mg/kg of $HgCl_2$ and 1000 mg/kg bwt of EEPGL, while Group VII received 41.5 mg/kg $HgCl_2$ and 1190 mg/kg bwt of Vitamin C. At the end of the administration, the brains of the Wistar rats were excised, oxidative stress markers quantified and brain tissues were fixed in Bouin's fluid, processed, and stained for histological studies.
Results: The results revealed a significant decrease in body weight gain and oxidative stress markers, weak staining of Nissl substance and cytoarchitectural distortion of the cerebral cortices of Wistar rats in mercuric chloride only treated groups when contrasted to the Control and the Groups co-administered mercuric chloride and increasing doses of EEPGL.
Conclusion: Ethanolic extract of *P. guajava* leaves was able to ameliorate neurotoxicity induced by mercuric chloride exposure by mitigating against oxidative stress, preventing weight loss and distortion in the cytoarchitecture of the cerebral cortex.
Keywords: Mercuric chloride, *Psidium guajava*, Cerebral cortex, Neurodegeneration, Antioxidants

1. Introduction

Degenerative diseases of the nervous system, for example, Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) among others are major reasons for death and disability in the elderly people

globally [1]. 60% of around 50 million individuals having dementia live in low and middle-income countries [2]. Although the causes of neurodegenerative diseases remain elusive, several studies have reported contributions from genetics and environmental neurotoxins.

Mercury (Hg) as one of these environmental neurotoxins is among the ten chemicals classified as a significant general wellbeing concern by the World Health Organization (WHO). Mercury has no safe level of exposure via any biological routes [3]. For most people, Hg exposure arises via Hg released from dental amalgams, mining leakage, and pollution, during early pregnancy, and through occupational exposure among others [4]. Exposure to different types of Hg can prompt adverse impacts in numerous organ systems. These, however, are not restricted to, the cardiovascular, gastrointestinal, neurological, hepatic, and renal systems with the central nervous system being its main target [5].

Albeit the mechanism associated with Hg instigated neurotoxicity remains unclear, the chief component currently involved in its neurotoxicity is accepted to be linked to oxidative stress and ionic mechanism [6]. Xu et al. [7] reported that exposure to low grades of mercury triggered neurodegenerative disease and nerve injury through interference with the activity of NMDA receptors. Accumulation of Hg in the motor cortex has also been reported to cause oxidative stress, severe deficits in memory acquisition in rats, and a decrease in the number of neurons and astrocytes [8].

Despite regulations reducing the use of heavy metals in products in developed countries, mercury usage in agriculture, industries, and consumption of contaminated aquatic animals are on the rise in developing countries [9, 10] like Nigeria thereby predisposing human and animal populations to Hg toxicity.

Although chelation and pharmacological antioxidant approach have been reported as promising restorative ways to forestall neurodegeneration [11, 12], they possess side effects and are expensive to acquire. The antioxidant property of naturally occurring plant extract has been reported to have a significant role in curing neurodegenerative diseases [13]. Therefore, the discovery of new antioxidants from natural sources, which are less harmful and cheap than the available engineered drugs, might be basic for its therapy.

Psidium guajava L. (*P. guajava*) is a tree that belongs to the Myrtaceae family. It is local to the subtropical and tropical areas of, Africa, America, and Asia [14]. Different parts of the tree are broadly utilized as food and in traditional medication around the globe [15]. The protective activity exerted by *P. guajava* on the nervous system has been suggested to be via its antioxidant property that is its ability to scavenge for free radicals. However, its role in mercury-induced neurotoxicity remains elusive. Therefore, this study investigated if ethanolic extract of *Psidium guajava* leaves could ameliorate neurotoxicity induced by mercuric chloride in the cerebral cortex of adult male Wistar rats.

2. Materials and Methods

2.1. Experimental animals

Thirty-five healthy adult male Wistar rats weighing between 150g to 175g were acquired from the Animal House, Department of Pharmacology and Therapy, Ahmadu Bello University, Zaria, Nigeria. The rats were confined in standard rat cages, fed with rat chow and water ad libitum for two weeks acclimatization before the study.

2.2. Plant material

Fresh leaves of *P. guajava* were bought from a nearby homestead in Samaru, Zaria, Kaduna State, Nigeria. The leaves were identified and authenticated by comparing with the existing specimen in the Herbarium of Botany, Biological Sciences Department, Faculty of Sciences, Ahmadu Bello University, Zaria, with a Voucher Specimen Number of 3253.

2.3. Ethanol extraction of *Psidium guajava*

The *P. guajava* leaves obtained were dried at room temperature and the maceration technique as described by Seo et al. [16] was utilized for the preparation of ethanolic extracts. One thousand grams (1000 g) of guava leaves were soaked in 1.5 Litres of pure ethanol for 4 days at room temperature. After 4 days, the solution was sifted using a filter and funnel. The filtrate was allowed to settle for a while, followed by decantation of the supernatant. The supernatant was heated to dryness in an evaporating dish over a water bath at 80 ˚C.

2.4. Acquisition of Mercuric chloride and Vitamin C

Mercuric chloride made by May and Baker Chemical Laboratory Limited Dagenham England wit-N202 and Vitamin C made by Sam pharmaceuticals Limited, Ilorin, Kwara State, Nigeria were purchased the study.

2.5. Experimental Protocol

Based on the median lethal dose (LD_{50}) of EEPGL for Wistar rats, 10% (500mg bwt) and 20% (1000mg bwt) of the LD_{50} were used for the study. The reported LD_{50} of Mercuric Chloride ($HgCl_2$) which is 166 mg/kg body weight [17], 25% of the LD_{50} (41.5 mg/kg bwt) was administered in the study. In view of the reported LD_{50} of ascorbic acid (Vitamin C) which is 11,900mg/kg body weight [18], 10% of the LD_{50} (1190 mg/kg) was used.

2.6. Experimental Design

Thirty-five (35) adult male Wistar rats were obtained and categorized into seven (7) groups of five (5) rats each. The rats in Group I served as control and was administered 2mL/kg bwt distilled water from day 1 to day 42, Group II was administered 41.5mg/kg bwt of $HgCl_2$ from day 1 to day 21, Group III was administered 1000mg/kg bwt of ethanolic extract of *P. guajava* leaves (EEPGL) from day 1 to day 21, Group IV was administered 41.5 mg/kg bwt of $HgCl_2$ from day 1 to day 21 and 2 mL/kg bwt of distilled H_2O from day 22 to day 42, Group V was administered 41.5 mg/kg bwt of $HgCl_2$ from day 1 to day 21 and 500 mg/kg bwt of EEPGL from day 22 to day 42, Group VI was administered 41.5 mg/kg bwt of $HgCl_2$ from day 1 to day 21 and 1000 mg/kg bwt of EEPGL from day 22 to day 42, Group VII was administered with 41.5 mg/kg bwt of $HgCl_2$ from day 1 to day 21 and 1190 mg/kg bwt of Vitamin C from day 22 to day 42. All administrations were done via oral gavage using an oropharyngeal cannula and 1 mL syringe.

2.7. Body Weight Assessment

In each group, the body weight was taken at the beginning, weekly during the study and at the completion of the study before sacrifice using a digital weighing balance. Weight change of the animals in each group was studied from recorded body weights.

After the experimental period, the rats in each group were weighed and anaesthetized by intraperitoneal administration of 75 mg/kg bwt of Ketamine. Afterwards, a mid-sagittal incision through the skull was made to harvest the brains. The harvested brains were freed from adherent tissues and cut along their longitudinal fissure to separate them into halves. One half was weighed, minced and homogenized in

cold phosphate buffer. The resultant supernatant was stored in plain bottles under cold temperature for estimation of oxidative stress markers. From the other half, the cerebrum was dissected out, fixed in Bouin's fluid, processed routinely and stained with H and E and Cresyl violet stain as described by Bancroft and Gamble [19].

2.8. Oxidative Stress Markers

2.8.1. Catalase activity assay

Catalase (CAT) enzyme activity was assessed based on formation of a yellow complex with molybdate and hydrogen peroxide in plasma which was expressed with 1, 1, 2, 2,-tetramethoxypropane using a spectrophotometric test [20].

2.8.2. Glutathione activity assay

Glutathione activity was assessed following the method of Ellman [21] as described by Rajagopalan et al. [22] . This assay was based on the reaction of reduced glutathione (GSH) with 5, 5'-dithiobis nitrobenzoic acid (DNTB).

2.8.3. Superoxide dismutase activity assay

Activity of superoxide dismutase (SOD) was determined by the technique of Fridovich [23]. This assay was based on the inhibition activity of superoxide dismutase to auto oxidation of adrenaline at pH 10.2.

2.8.4. *Malondialdehyde assay*

Lipid peroxidation was determined following the method of Ohkawa et al. [24]. Lipids upon cleavage release peroxide intermediates such as malondialdehyde (MDA). MDA reacts with thiobarbituric acid to form thiobarbituric-acid reactive substance (TBARS). Hence MDA was determined by measuring TBARS at 532 nm using a spectrophotometer.

2.9. Photomicrography

After staining with H and E and Cresyl violet, photomicrographs were taken under the microscope at X250 magnifications using MD900 Amscope® digital camera.

2.10. Statistical Analysis

Data obtained were reported as Mean±SE (Standard error) and analysed using IBM SPSS 22. One-way analysis of variance (ANOVA) was used to deduce mean differences between and within the groups followed by Tukey's post-hoc test where p-value was significant. P-value less than 0.05 was considered statistically significant.

3. Results

3.1. Body Weight Change

The result of body weight assessment revealed a significant weight gain ($p<0.05$) in Wistar rats in the Control group compared to Group II and Group IV respectively. Significant weight gain ($p<0.05$) was observed in Groups IV, V, VI and Group VII, respectively, when compared with Group II. There was also a significant weight gain ($p<0.05$) in Groups V, VI and group VII respectively when compared with group IV as shown in Table 1.

Table 1. Effect of exposure to Mercuric chloride, ethanolic extract of *Psidium guajava* leaves and Vitamin C on the weight of adult male Wistar rats

	Initial Weight (g)	Final Weight (g)	Weight Change (g)
Group I	132.33±18.37	206.00±12.53	73.67± 5.84^{ab}
Group II	152.33± 24.36	179.67±18.94	26.67± 1.45^{acde}
Group III	153.33± 27.64	216.67±18.94	62.0± 1.45
Group IV	167.00± 17.21	200.00±17.56	33.00± 3.61^{bde}
Group V	163.33± 26.71	204.67±19.78	40.67± 6.96^{c}
Group VI	172.67± 16.75	225.67±15.21	53.00± 4.04^{d}
Group VII	152.67± 21.11	208.00±15.50	56.67± 5.61^{e}

Values reported as mean ± SE. One way ANOVA followed by Tukey's post hoc test. Cells carrying the same superscript "$^{a, b, c, d, e}$" across the same column are significantly different at $p<0.05$

3.2. Oxidative Stress Markers

The result of oxidative stress markers revealed a significant reduction in the level of CAT in the brain tissues of rats in Groups II and IV respectively when compared with the Control group ($p<0.05$) (Fig. 1). The level of glutathione (GSH) in the brain tissues of rats in Group II and Group IV was significantly decreased in comparison to the Control group and other treatment Groups ($p<0.05$) (figure 2). The result showed a significant decrease in SOD concentration in the brain tissues of Wistar rats in Groups II and IV when compared to the Control group and other treatment groups ($p<0.05$). The SOD concentration in the brain tissues of rats in Group III was significantly increased when compared to the Control group and other treatment Groups ($p<0.05$) (Fig. 3). There was also a significant increase in Malondialdehyde (MDA) concentration in the brain tissues of Wistar rats in Groups II and IV when compared with the Control group and other treatment groups ($p<0.05$) (Fig. 4).

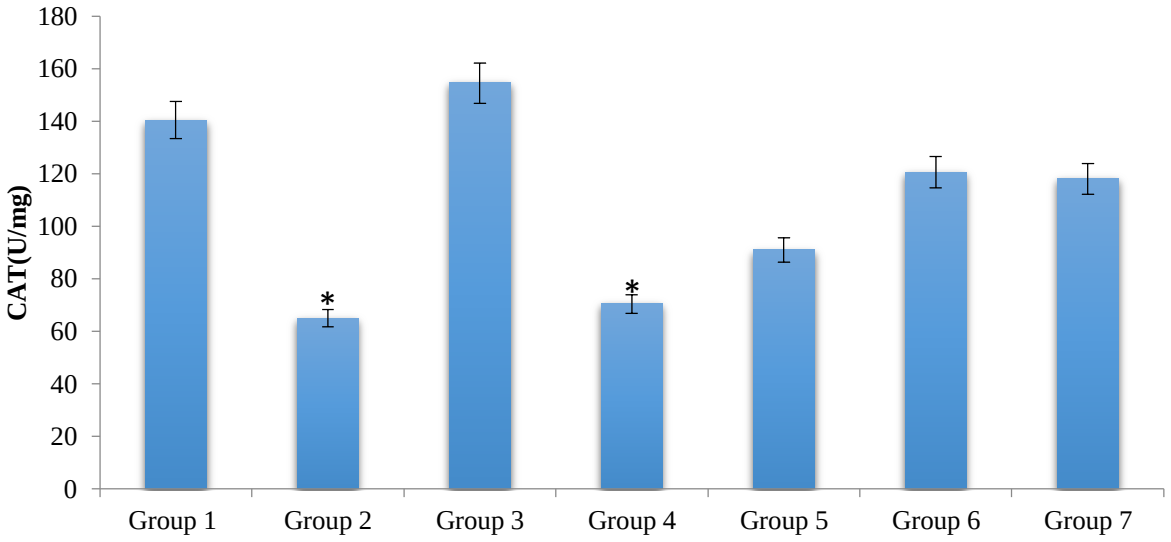

Figure 1. CAT concentration in the brain tissues of adult male Wistar rats after exposure to mercury chloride, ethanolic extract of *Psidium guajava* leaves and Vitamin C

Values reported as mean ± SE. One way ANOVA followed by Tukey's post hoc test. *= Significant difference. CAT = Catalase.

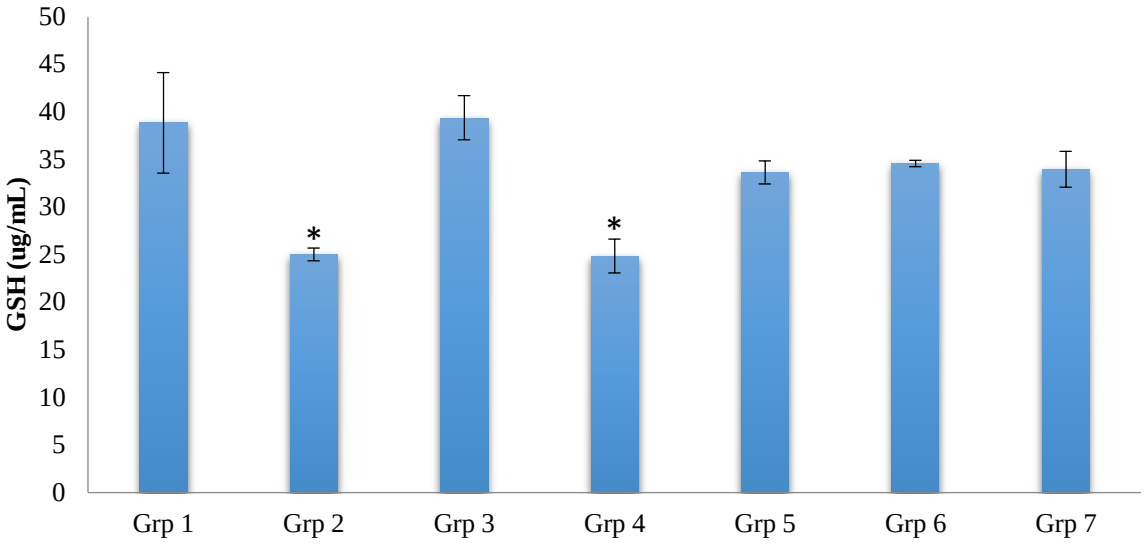

Figure 2. GSH concentration in the brain tissues of adult male Wistar rats after exposure to mercury chloride, ethanolic extract of *Psidium guajava* leaves and Vitamin C

Values reported as mean ± SE. One way ANOVA followed by Tukey's post hoc test. *= Significant difference, GSH= Glutathione.

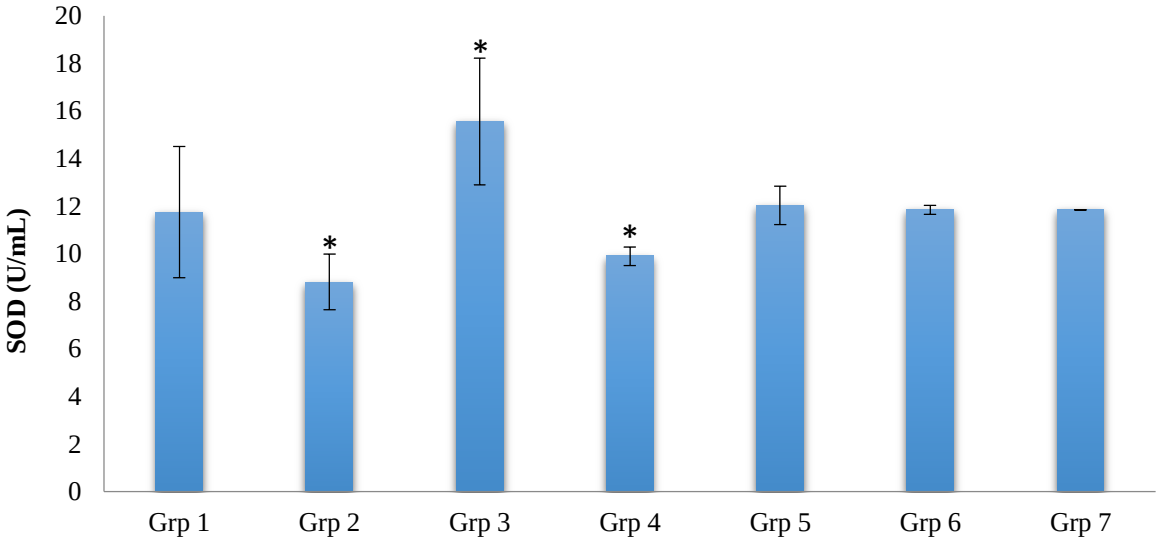

Figure 3. SOD concentration in the brain tissues of adult male Wistar rats after exposure to mercury chloride, ethanolic extract of *Psidium guajava* leaves and Vitamin C

Values reported as mean ± SE. One way ANOVA followed by Tukey's post hoc test. *= Significant difference. SOD= Superoxide Dismutase.

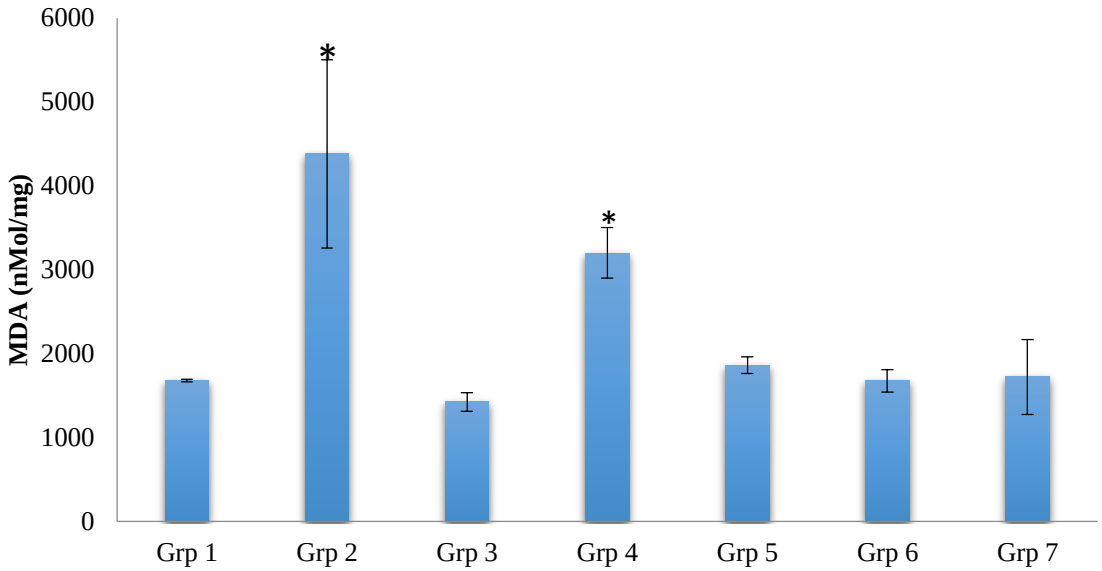

Figure 4. MDA concentration in the brain tissues of adult male Wistar rats after exposure to mercury chloride, ethanolic extract of *Psidium guajava* leaves and Vitamin C

Values reported as mean ± SE. One way ANOVA followed by Tukey's post hoc test. *=Significant difference. MDA = Malondialdehyde.

3.3. Histological Observation

The results from observations of the histology of the cerebral cortex of adult male Wistar rats in the Control group revealed typical histoarchitecture of the cerebral cortex, characteristic appearance of neurons arranged into six layers, the layer contrasting in neuron morphology, size and population density. Particularly the internal pyramidal layer (Layer V) revealed intact vasculature (arteries and vein), normal cytoarchitecture and appearance of the large pyramidal (Betz) cell (Fig. 5). Group II revealed the presence of degenerating pyramidal cells and perineuronal vacuolation in comparison to the Control and other treatment Groups (III, IV, V, VI, and VII). Group III revealed a cytoarchitecture similar to what was observed in the Control group. The cerebral cortex of Wistar rats in Group IV showed the presence of cell loss and cytoplasmic vacuolation similar to what was observed in Group II; however, it was dissimilar when compared with Control group and other treatment groups. Observations from cerebral cortices of rats in Groups V, VI and VII revealed better cytoarchitecture when compared with Group II and IV with more improved architecture observed in Groups VI and VII (Fig. 5).

The ribosomes in the cell body of the pyramidal cells (Nissl substance) of layer V of the cerebral cortex of the Control group and Group III stained intensely after Cresyl violet staining. The pyramidal cells in Groups II and IV showed chromatolysis with weak staining of the ribosomes while Groups V, VI and VII showed stronger staining of Nissl substances when compared with Groups II and IV with improved staining observed in Groups VI and VII (Fig. 6).

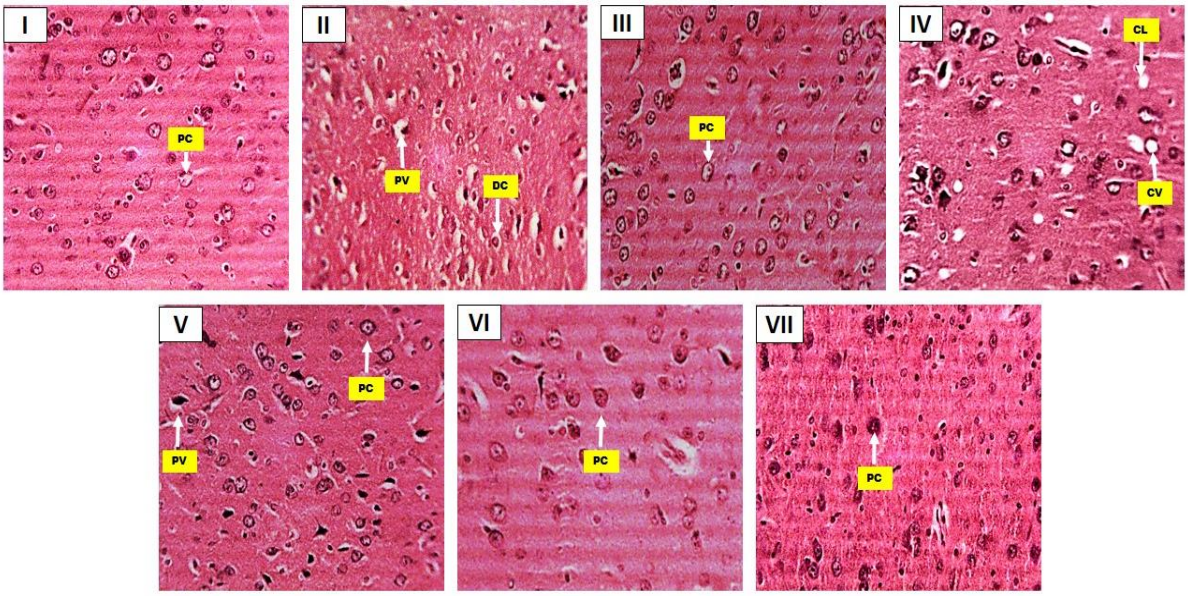

Figure 5. Photomicrograph of the layer V of the cerebral cortices of adult male Wistar rats (H and E Magx250). PC- Pyramidal cells, PV- Perineuronal vacuolation, DC- Degenerating pyramidal cell, CL- Cell loss, CV- Cytoplasmic vacuolation

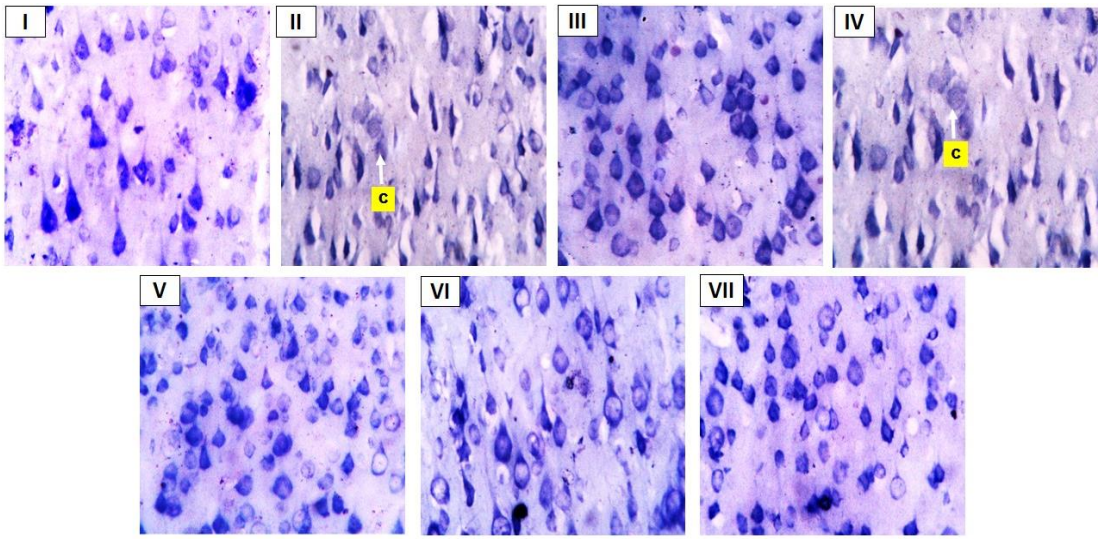

Figure 6. Photomicrograph of the layer V of the cerebral cortices of adult male Wistar rats (Cresyl violet Magx250). C- Chromatolysis

4. Discussion

It has become increasingly clear that neurodegeneration is multifactorial in origin as they are produced by hereditary, environmental and endogenous factors [25]. These environmental factors include, but not limited to, exposure to environmental toxins such as lead, cadmium, arsenic and mercury.

Body weight change serves as a profound clue of overall wellbeing status of an animal. In the present study we observed a progressive increase in the mean body weight of the Wistar rats in the Control group and the treatment groups. However, the degree of weight gain by Wistar rats in Groups treated with $HgCl_2$ alone for 21 days and $HgCl_2$ alone for 21 days followed by treatment with distilled

water from day 22 to day 42 was significantly lower when compared with the weights of Wistar rats in the Control group and Groups treated with $HgCl_2$ for 21 days followed by treatment with vitamin C and increasing doses EEPGL from day 22 to day 42. The reduction in weight gain of rats treated with $HgCl_2$ could be attributed to decrease in the ingestion of food (anorexia) incited by $HgCl_2$ ingestion. Body weight gain is, in any event partially, controlled by the hypothalamus via secretion of several peptides; therefore, it is possible that mercury chloride exposure affected the expression of these neuropeptides as Ferrer et al. [26] reported disruption of these neuropeptides after administration of methyl mercury, hence alteration in body weight gain. This is similar to the work of Animoku et al. [27] who reported substantial reduction in body weight of rats after exposure to mercuric chloride for eight weeks when contrasted with the control group. The marked increase in weight gain in groups co-administered mercuric chloride and increasing doses EEPGL suggests ameliorative activity of ethanolic extract of *P. guajava* leaves on reduction in body weight caused by mercuric chloride exposure and could be ascribed to its antioxidant properties.

Oxidative stress occurs when generation of pro-oxidants exceed the capacity of antioxidant defence mechanisms [28]. A significant decrease in the concentration of CAT, GSH and SOD in brain of Wistar rats in groups treated with $HgCl_2$ alone for 21 days and $HgCl_2$ alone for 21 days followed by treatment with distilled water from day 22 to day 42 was observed when compared with the Control group and groups treated with $HgCl_2$ for 21 days followed by treatment with vitamin C and increasing doses EEPGL from day 22 to day 42. The decrease in endogenous antioxidants in groups treated with mercuric chloride could be due to the affinity of mercury for the sulfhydryl groups present in antioxidants as well as its ability to inactivate the antioxidant enzymes by supplanting zinc ions which serve as vital co-factors for these enzymes [29]. The increase in CAT, GSH and SOD in groups' co-administered mercuric chloride and increasing doses of EEPGL could be due to the antioxidant properties of *P. guajava* as EEPGL has been reported to contain tannins, saponins, flavonoids, alkaloids [15]. This is in line with the work of Tandon et al. [30] who reported a significant restoration in the activities of CAT, SOD and GSH in Wistar rats exposed to arsenic after treatment with *Psidium guajava* leaves. The results from this study also indicated that lipid peroxidation, determined by measuring the MDA levels, was amplified in the brain of Wistar rats after exposure to mercuric chloride when compared with the Control group and groups' co-administered mercuric chloride and increasing doses of EEPGL and Vitamin C. This increment in MDA levels supports our result on decrease in antioxidant levels observed in groups treated with mercuric chloride since a decrease in antioxidant level means an increase in oxidative stress thus more lipid peroxidation and cell disruption. This is in agreement with the work of Bubber [31] who reported significant increase in lipid peroxidation in the brain of Wistar rats after exposure to 0.25 mg/kg of mercury chloride for 21 days. The decrease in MDA levels observed in groups co-administered mercuric chloride and increasing doses of EEPGL and Vitamin C means a decrease in lipid peroxidation and cell disruption and may be attributed to the antioxidant property of EEPGL against mercury induced toxicity. This is in agreement with Tandon et al.'s [30] study who reported a significant decrease in MDA level in Wistar rats exposed to arsenic after treatment with *Psidium guajava* leaves. In sum, more reduction in MDA level and

increase in CAT, GSH and SOD was observed in Groups co-administered mercuric chloride and higher dose of EEPGL. This observation was similar to what was observed in the group treated with standard drug vitamin C although not significant ($p<0.05$). This may suggest that the antioxidant activity of EEPGL is dose dependent with higher doses eliciting better antioxidant and ameliorative activity in mercuric chloride induced neurotoxicity.

In the present study, the cerebral cortex of adult male Wistar rats in the control group revealed typical arrangement of the cerebral cortex. This result differed significantly in the groups treated with $HgCl_2$ alone for 21 days and $HgCl_2$ alone for 21 days followed by treatment with distilled water from day 22 to day 42 which revealed presence of degenerating pyramidal cells, cell loss, perineuronal vacuolation, and cytoplasmic vacuolation. Mercury exposure may have caused this distortion by triggering generation of superoxide radicals in the cell which can obstruct the electron transport chain within the mitochondria leading to a debilitation of energy production and generation of oxidative stress [32].

Perineuronal and cytoplasmic vacuolation observed might be as a result of disruption of ATP production and failure of the ATP dependent sodium pump thus leading to subsequent entry of water into the cell [33]. These degenerative changes observed may lead to deficit and progressive decline in the activity of the cerebral cortex. These result also lends credence to the findings of previous worker on the capacity of heavy metals, to induce nervous tissue damage [34, 35].

The cerebral cortices of Wistar rats in groups co-administered mercuric chloride and increasing doses of EEPGL and Vitamin C revealed relatively normal cytoarchitecture with mild distortion when compared with control and standard drug vitamin C. This shows that administration of EEPGL could ameliorate distortions observed in mercuric chloride alone treated group due to its free radical scavenging capacity. The similarity in the effects observed in groups treated with EEPGL at both 500 mg/Kg and 1000 mg/Kg doses to that of vitamin C, may imply that EEPGL may have exerted its protective capabilities via a mechanism that is related to that of Vitamin C. Improved maintenance of the histoarchitecture of the cerebral cortex observed in the group treated with higher doses of the extract, coincides with our result on increased level of antioxidant production at higher doses of the extract and posits that higher doses of EEPGL has more ameliorative effects against mercury induced neurotoxicity.

Ribosomes and rough endoplasmic reticulum in the cell body of neurons appear as basophilic granular areas with Cresyl Violet staining in light microscopy. Nissl substances are the centres of intense protein synthesis important in the activity of neurotransmitters. The pyramidal cells in the groups treated with $HgCl_2$ alone for 21 days and $HgCl_2$ alone for 21 days followed by treatment with distilled water from day 22 to day 42 showed chromatolysis with weak staining of the ribosomes compared with the control and other treatment groups. The degeneration of the Nissl substance in mercuric chloride treated groups may lead to inadequate synthesis of neurotransmitters. This is consistent with a previous study [36], reporting loss of Nissl substance in cerebellar neurones following mercury induced toxicity. The improved staining observed in groups treated with EEPGL may suggest that EEPGL was able to protect again Nissl substance degeneration.

5. Conclusion

This study investigated the ameliorative effect of ethanolic extract of Psidium guajava leaves on mercuric chloride induced neurotoxicity in the cerebral cortex of adult male Wistar rats. From this study, it can be inferred that ethanolic extract of *Psidium guajava* leaves was able to ameliorate neurotoxicity induced by mercuric chloride exposure. This is marked by its ability to maintain body weight possibly by retaining the neuropeptides responsible for the activity of the hypothalamus as well as restoring the body's antioxidant system by increasing the levels of endogenous antioxidants therefore preserving the cytoarchitecture of the cerebral cortex and thus its activity.

Conflict of Interests

The authors have not declared any conflict of interests

Consent for publications

All authors have read and approved the final manuscript for publication.

Availability of data and material

All authors declare that all data derived from the study was embedded during creation of the manuscript.

Authors' contributions

Samuel Sunday Adebisi and Augustine Oseloka Ibegbu were resourceful in designing and proof reading the entire work. They actively supervised all the laboratory work and ensured that every test was completed qualitatively and timely. Ochai Joy carried out the research work, data analysis and was responsible for writing the draft of the manuscript and all authors commented on previous version.

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Ethics approval and consent to participate

Ethical approval was obtained from Ahmadu Bello University Committee on Animal Use and Care with approval number: ABUCAUC/2021/052.

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