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Afr. J. Biomed. Res. Vol. 26 (January 2023); 119 - 128

Research Article

Aqueous Extract of *Allium Sativum* Linn. Protected the Cerebellum of Adult Female Wistar Rats against Mercury-induced Oxidative Stress

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ABSTRACT

Environmental pollution and work related hazard have exposed humans to various metallic agents including, mercury. The protective effect of *Allium sativum* (*A. sativum*) Linn (Garlic) on mercury-induced oxidative stress in the cerebellum of adult female Wistar rats was studied. Twenty five adult female Wistar rats weighing between 180g and 210g, used for this study were divided into five groups; Group 1 rats received distilled water and served as the control, group 2 rats were administered 3.75mg/kg body weight of Mercury (II) chloride ($Hgcl_2$) only, group 3 rats were administered 50mg/kg body weight of *A. sativum* extract only, group 4 rats were administered 3.75mg/kg $Hgcl_2$ and 50mg/kg of *A. sativum* extract and group 5 were administered 3.75mg/kg $Hgcl_2$ and 100mg/kg vitamin C orally for 28 days. The body weight and neurobehavioral studies were done shortly before sacrifice on day 29. After sacrifice, the brain was dissected out and the cerebellum, processed for biochemical and histolomorphological evaluations. Data were analyzed using ANOVA at $p<0.05$. Results showed signs of general body weakness, decline in food and water intake, and decreased weight gain in the $Hgcl_2$ and A. sativum + $Hgcl_2$ groups compared with the control and other treated groups. There was reduced drop-off time in the forelimb grip strength in the $Hgcl_2$-treated groups compared with the control. An increased lipid peroxidation, and decreased glutathione levels, SOD and GPx activities was seen in the cerebellum of $Hgcl_2$-treated group compared with the control and other treated groups. Histomorphologically, there was reduction in the molecular layer thickness, loss of Purkinje cells and increased astrocytes population in the cerebellum of the $Hgcl_2$-treated group compared with the control and the other treated groups. In conclusion, exposure to mercury affected muscle tone, induced oxidative stress causing morphological alterations. Aqueous extracts of *Allium sativum* and vitamin C decreased the rate at which mercury induced oxidative damage in the cerebellum of adult female Wistar rats.

Keywords: *Environmental pollution, Mercury chloride, Allum sativum, neurobehaviour, cerebellum*

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Received: September 2021; Accepted: March 2022

DOI: 10.4314/ajbr.v26i1.16

INTRODUCTION

Man in his environment has been exposed to potential hazard of heavy metals through bio-accumulation and bio-magnifications which has been transferred to man via food chain as a result of anthropogenic activities (Ghosh and Sil, 2008). Mercury with the formula Hg, is a heavy metal, noted for inducing public health disasters (Environmental Health Department, 2002). Mercury (Hg) is a highly toxic metal causing a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive, and developmental disorders (Risher and Amler, 2005; Senese, 2007). Presently, large population worldwide is exposed to relatively low levels of Hg, especially via its use in medicine as a topical antiseptic and disinfectant, as pesticides in agriculture and of fluorescent light bulbs as well (El-Shenawy and Hassan, 2008; Mahmoud, *et al*, 2014). Mercury exists in several forms: inorganic mercury, which includes metallic mercury and mercury vapor (Hg^o) and mercurous (Hg^{2++}) or mercuric (Hg^{++}) salts; and organic mercury, which includes compounds in which mercury is bonded to a structure containing carbon atoms (methyl, ethyl, phenyl, or similar groups). Exposure to Hg vapor as well as to organic Hg compounds specifically affects the central nervous system (Vahter *et al*., 2000), while kidneys, liver and gastrointestinal tract are mainly targeted by inorganic mercury (Schurz *et al*., 2000; Ghosh and Sil, 2008).

Mercury poisoning can result from inhalation, ingestion, or absorption through the skin, and may be highly toxic and corrosive once absorbed into blood stream. High exposures to inorganic mercury may result in damage to the gastrointestinal tract (Bernhoft, 2012) nervous system (Teixeira *et al*., 2014a), and the kidneys (Joshi *et al*., 2014). The neurotoxicity, nephrotoxicity, reproductive toxicity, gastrointestinal toxicity with ulceration and haemorrhage of mercury are well

established (Rao and Purohit, 2011; Uma *et al*., 2012; Xu *et al*., 2012; Owoeye and Farombi, 2015). Mechanisms proposed for inorganic mercury toxicity include the binding of mercuric ions to sulfhydryl groups resulting in decreased glutathione levels and depletion of thiols leading to an increase of reactive oxygen species (ROS), ultimately leading to oxidative stress and increased neurotoxicity (Rao and Purohit, 2011; Owoeye and Farombi, 2015). Antioxidants, including vitamins A, C, E, carotenoids and polyphenols are substances that neutralize or remove free radicals by donating an electron which helps protect the body from oxidative stress (Farombi and Owoeye, 2011) Vitamin C or Ascorbic acid is an essential nutrient for humans and certain other animal species. In living organisms, ascorbate acts as an antioxidant by protecting the body against oxidative stress (Padayatty *et al*., 2003). Natural products of animals, plants and microbial sources have been used by man for thousands of years either in the pure forms or crude extracts to treat many diseases (Parekh and Chanda, 2007). Medicinal plants such as Curcumin (Rao and Patel, 2013), *Zingiber officinale* (Ezeuko *et al*., 2007), *Tabernaemontana coronaria* (Uma et al., 2012) and *Lycopersicon esculentum* (Farombi and Owoeye, 2015) have been reported to reduce the of toxicity of $Hgcl_2$.

Allium sativum (*A. sativum*) Linn (Garlic) belong to family, Amaryllidaceae (Friesen *et al*., 2006), cultivated throughout the world but originally from Central Asia (Kao *et al*., 2004). The phytochemical constituents of *A. sativum* include alkaloid, terpenoids, flavonoids, steroid, phenol, anthraquinones, saponin, tannin and glycoside (Ali and Ibrahim, 2019). *Allium sativum* contains at least 33 sulfur compounds, carbohydrates, proteins, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, fiber and water. It also contains 17 amino acids including, lysine, histidine, arginine, aspartic acid threonine, swine, glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine (Josling, 2005). The antifungal, antiviral, antibacterial, antihelmantic, antiseptic, anti-inflammatory, anticancer, anti-atherosclerotic and antioxidant properties of *A. sativum* are well documented (Ali and Ibrahim, 2019). Ethno-medical practitioners in Nigeria, have used *A. sativum* in the treatment of abdominal discomfort, diarrhea, otitis media and respiratory tract infections (Jaber and Al-Mossawi, 2007). While in Europe and India, the spice has been used to treat common colds, hay fever and asthma (Timbo *et al*., 2006).

The central nervous system (CNS) is one of the most vulnerable organs affected by mercury toxicity. Within the CNS, two of the most often affected areas are the cerebral cortex (Eto *et al*., 2001; Ferraro *et al*., 2009) and the cerebellum (Fonfria *et al*., 2005; Korogi *et al*., 2011). Exposure to higher levels of mercury is associated with mercurial tremor: fine muscle fasciculation punctuated every few minutes by coarse shaking, severe behaviour and personality changes, emotional excitability, loss of memory, insomnia, depression, fatigue, and in severe cases delirium and hallucination (Bernhoft, 2012). Considering the important functions of the cerebellum in controlling various motor activities, the neurotoxic effects of mercuric chloride and the antioxidant activities of *Allum sativum*, this study aimed at investigating the protective effects of aqueous extracts of *Allium sativum* on mercury-induced oxidative stress in the cerebellum of adult female rats.

MATERIALS AND METHODS

Preparation of aqueous extract of *Allium sativum :* *Allium sativum* was purchased from local market (Bodija, Market, Ibadan, Nigeria), identified and authenticated at the Forestry research Institute of Nigeria (FRIN) with FHI number 112305. The *Allium sativum* bulbs were separated, peeled and washed with distilled water. After drying in a shed, the clean *Allium sativum* bulbs was crushed with an electric grinder and then dissolved in distilled water. The extract was carefully decanted using muslin cloth (Senapati *et al.,* 2000).

Chemical and drugs: Twenty grams of Mercury chloride ($Hgcl_2$) manufactured by May and Bakers Chemical Laboratory Limited, Dagenham, England was purchased from Ad-Folak scientific limited, Ibadan, Nigeria. Standard vitamin C was purchased from Kunle Ara Pharmarcy, Ibadan, Nigeria.

Experimental animals and design: Twenty-five adult female Wistar rats (160-210g) were randomly assigned into five groups (n=5). The rats were acclimatized to the animal house condition (12 hours light/dark cycle) for two weeks with ***ad libitum*** access to feed and water and the grouping was maintained throughout the period of the experiment. All animals received humane care according to criteria outlined in the Guide for the Care and Use of Laboratory Animals (prepared by the National Academy of Science and published by the National Institutes of Health).

The animals were randomly divided into five groups as follows: Group 1 (control rats received distilled water); Group 2 (given 3.75mg/kg $Hgcl_2$, p.o) (Oriquat *et al*., 2012); Group 3 (given 50mg/kg, *Allium sativum* extract, p.o); Group 4 (administered 3.75mg/kg $Hgcl_2$ +50mg/kg, *Allium saivum* p.o) and group 5 rats were given 100mg/kg of Vitamin C (Okey and Ayo, 2015) and 3.75 mg/kg, $Hgcl_2$, p.o. *Allium sativum* extract and vitamin C was administered one hour before the administration of $Hgcl_2$ for 28 days.

Sacrifice of the animals and harvest of brain and cerebellum: At the end of the interventions the rats were weighed, neurobehavioural tests conducted and animals were sacrificed by quick cervical dislocation. The brains and cerebella dissected out, and some cerebella fixed in 10% formol-saline for histological and histomorphometric studies, while others, preserved in phosphate buffered saline at $4^{\circ}C$ and pH 7.2 for oxidative stress evaluation.

Neurobehavioural Test:

Two neurobehavioural tests were conducted to access the cerebellar function of motor coordination in both the control and treated groups.

Forelimb Grip Strength Test: This test involved placing the fore paws of the rat on a horizontally suspended metal wire (measuring about 2 mm in diameter and 1m in length), placed one meter above a landing surface filled with soft bedding. The length of time that each rat was able to stay suspended by

its forelimb alone, before falling off the wire was recorded. A maximum time of 2 minutes was allotted to each rat for this test (Tamashiro *et al.*, 2000). This test will be used to measure muscle strength.

Negative Geotaxis: In this test, the rats were placed facing downwards and against gravity on a wooden plane, inclined at an angle of 45 degrees to the horizontal. The rats were left to re-orient themselves properly to a position where their tail would point in the direction of gravity, and the time taken for each rat to successfully re-orient itself properly were recorded. For every rat that failed to re-orient itself, the time was recorded as 60s (Motz and Alberts, 2005). This test was used to determine the unlearned response to gravitational cues as well as balance/equilibrium.

Oxidative stress markers

Cerebellar tissue was homogenized in ice cold phosphate buffer at pH 7.4. The resulting homogenate was centrifuged at 4 °C at 1500 rmp for 10 minutes. The supernatant collected from the centrifugation was used for all oxidative stress and antioxidant markers using spectrophotometer. The following biochemical markers were assayed for; lipid peroxidation (LPO) by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Varshney and Kale (1990), reduced glutathione (GSH) by the method of Beutler *et al.*, (1963), Catalase (CAT) activity was determined according to the method of Claiborne (1985), activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich (1972) and glutathione peroxidase (GPx) activity by the method of Rotruck *et al.* (1973).

Tissue processing for histological and immunohistological studies

Histological preparations: Cerebellar tissues from the pups of all groups were fixed in 10% formo-saline, processed employing routine paraffin embedding and stained with Haematoxylin and Eosin for histomorphological evaluation. The slides were examined and evaluated under a 500-pixel Leica digital binocular microscope and the following were evaluated in the cerebellar cortex; Molecular layer (ML), and Purkinje cell density and diameter, and astrocyte population using the computer software, image-j.

Immunohistochemistry: Cerebellar tissues were immunostained with Glial fibrillary acidic protein (GFAP) for astrocyte population (neuroglia) using the Avidin biotin immunoperoxidase method. Briefly, cut formalin-fixed paraffin sections were treated with 3% hydrogen peroxide (H_2O_2) for 15min, to block endogenous peroxidase. Then, washed in phosphate buffered saline (PBS) and treated with GFAP primary antibody (GFAP, mouse monoclonal antibody 1:100 dilution, Leica Biosystems Inc. Ilinois, USA) at room temperature for 60min. The sections were washed in 3 changes of PBS for 5min each, incubated with horseradish peroxidase (HRP) secondary biotinlated anti-mouse antibodies and washed in 3 changes of PBS for 5mins. The sections were then incubated with diaminobenzidine (DAB) for 3 to 5min and counterstained with Haematoxylin solution for 2 mins and blued briefly. Sections were dehydrated in alcohol, cleared in xylene and mounted in DPX. Images were captured from the cerebellar cortex with a 500-pixel Leica binocular microscope. Astrocyte population was counted using the software, image-j.

Statistical analysis

Data collected was further analysed as mean±SD employing one-way analysis of variance (ANOVA) followed by Tukey Posthoc for multiple comparison using the GraphPad prism 6.0 at $p<0.05$**.**

RESULTS

General observation: In the course of the study, the control, *Allium sativum*, $Hgcl_2$ + *Allium sativum*, and $Hgcl_2$ + vitamin C groups were active throughout the experimental period while the group administered with $Hgcl_2$ only shows general body weakness, decline in food and water intake and reduction in physical activities. No gross morphological defect was observed between the control and the treated groups.

Body weight: Decreased percentage weight gain was seen in the $Hgcl_2$- and $Hgcl_2$+*A sativum*-treated groups compared with the control and other treated groups, at $p<0.05$ (Figure 1).

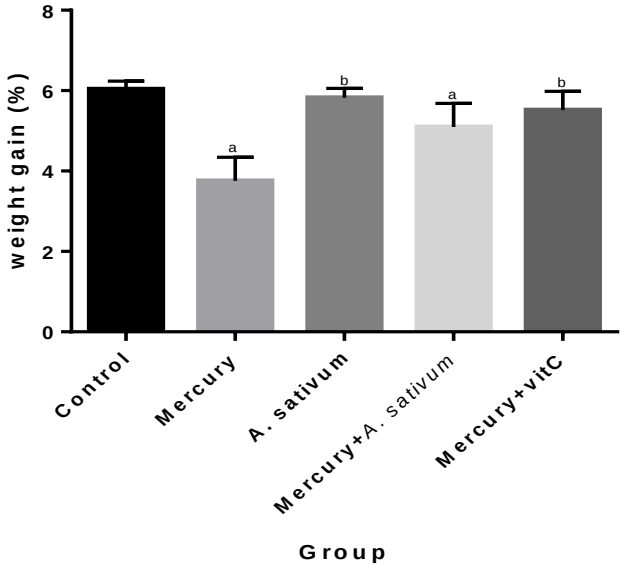


Figure 1:
Mean percentage body weight gain (%) of the control and treated animals. Values (n=5) are expressed as mean±SD. *A. sativum- Allum sativum*, vit C- Vitamin C. [a]$p<0.05$ compared with control, [b]$p<0.05$ compared with mercury group.

Neurobehavioural assessment

Forelimb grip test and Negative geotaxis: A shorter drop-off time was observed in the $Hgcl_2$-treated group compared with the control and *A. sativum*-treated groups at $p<0.05$ (Figure 2a). There was no statistical difference in the time (seconds) taken to turn against gravity on an inclined plane (45^o) flat surface in all the groups at $p>0.05$ (Figure 2b).

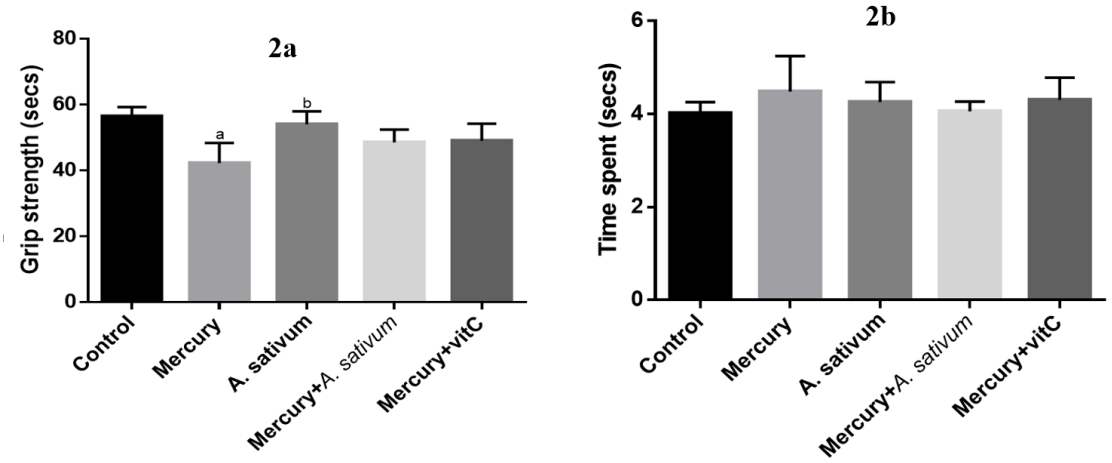


Figure 2:
Neurobehavioural assessment
2a. Time spent on forelimb grip (secs) between control and treated groups. Values (n=5) are expressed as mean±SD. *A. sativum- Allum sativum*, vit C- Vitamin C. [a]p<0.05 compared with control, [b]p<0.05 compared with mercury group.
2b. Time spent on Negative geotaxis (secs) between the control. and treated groups. Values (n= 5) are presented as mean±SD. *A. sativum- Allum sativum*, vit C- Vitamin C. p>0.05.

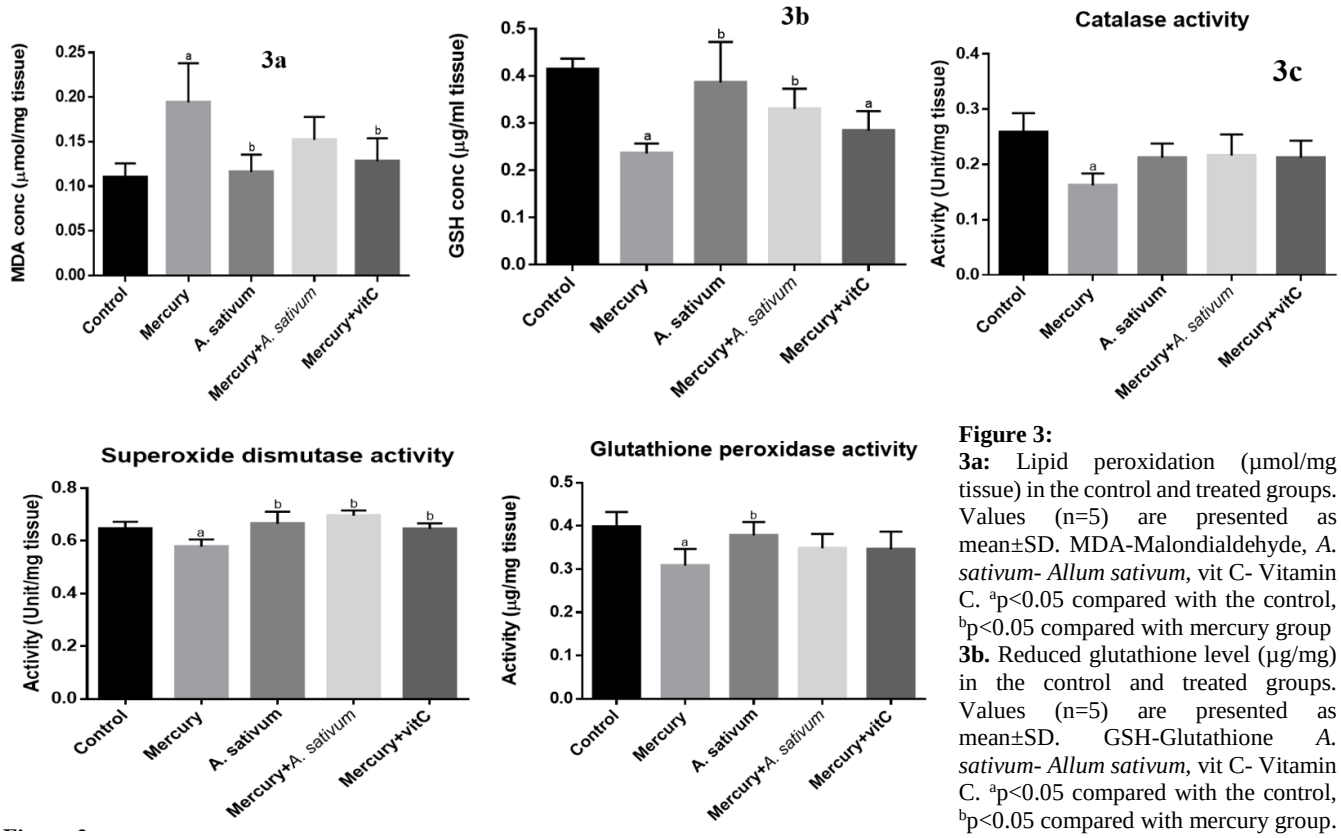


Figure 3:
3a: Lipid peroxidation (µmol/mg tissue) in the control and treated groups. Values (n=5) are presented as mean±SD. MDA-Malondialdehyde, *A. sativum- Allum sativum*, vit C- Vitamin C. [a]p<0.05 compared with the control, [b]p<0.05 compared with mercury group
3b. Reduced glutathione level (µg/mg) in the control and treated groups. Values (n=5) are presented as mean±SD. GSH-Glutathione *A. sativum- Allum sativum*, vit C- Vitamin C. [a]p<0.05 compared with the control, [b]p<0.05 compared with mercury group.

3c. Catalase (CAT) activity (U/mg) in the control and treated groups. Values (n=5) are presented as mean±SD. *A. sativum- Allum sativum*, vit C- Vitamin C. [a]p<0.05 compared with the control group
3d: Superoxide dismutase (SOD) activity (U/mg) in the control and treated groups. Values (n=5) are presented as mean±SD. *A. sativum- Allum sativum*, vit C- Vitamin C. [a]p<0.05 compared with the control, [b]p<0.05 compared with mercury group
3e: Glutathione peroxidase (GPx) activity (µg/mg) in the control and treated groups. Values (n=5) are presented as mean±SD. *A. sativum- Allum sativum*, vit C- Vitamin C. [a]p<0.05 compared with the control, [b]p<0.05 compared with mercury group

Oxidative stress markers: Increased MDA concentration, a bye-product of lipid peroxidation was observed in the $Hgcl_2$-treated group compared with the *A. sativum* amd $Hgcl_2$+*A. sativum* groups at $p<0.05$. (Figure 3a). Decreased GSH levels was seen in the $Hgcl_2$- and $Hgcl_2$+vit C-treated groups compared with the control and other treated groups at $p<0.05$ (Figure 3b). Decreased catalase activity was observed in the $Hgcl_2$-treated group compared with the control group at $p<0.05$ (Figure 3c). Decreased SOD activity was seen in the $Hgcl_2$-treated groups compared with the control and other treated groups at $p<0.05$ (Figure 3d). Decreased activity of GPx was observed in the $Hgcl_2$-treated group compared with the control and *A. sativum* groups at $p<0.05$ (Figure 3e).

Histomorphological assessment of the cerebellar cortex

Thickness of molecular layer of cerebellar cortex: There was reduction in the thickness of the molecular layer of the cerebellar cortex in the $Hgcl_2$-treated group but not statistically significant compared with the control and other treated groups at $p>0.05$ (Plate 1 and Figure 4).

Cerebellar Purkinje cell count and diameter: Decreased number of Purkinje cells was seen in the $Hgcl_2$-treated group compared with the control and *A.sativum*-treated groups at $p<0.05$. A non-significant reduction in the Purkinje cell diameter was observed in the $Hgcl_2$-treated group compared with the control group at $p>0.05$ (Plate 2 and Figures 5a, 5ab).

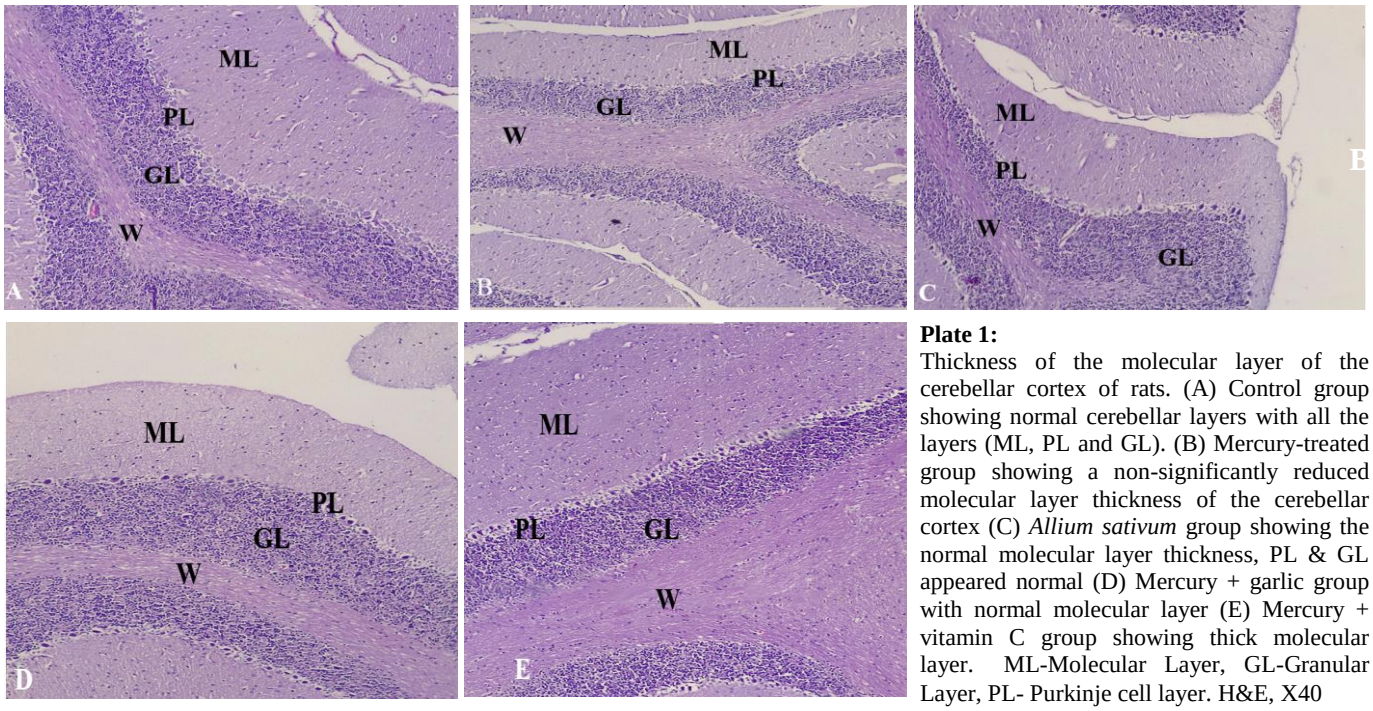


Plate 1:
Thickness of the molecular layer of the cerebellar cortex of rats. (A) Control group showing normal cerebellar layers with all the layers (ML, PL and GL). (B) Mercury-treated group showing a non-significantly reduced molecular layer thickness of the cerebellar cortex (C) *Allium sativum* group showing the normal molecular layer thickness, PL & GL appeared normal (D) Mercury + garlic group with normal molecular layer (E) Mercury + vitamin C group showing thick molecular layer. ML-Molecular Layer, GL-Granular Layer, PL- Purkinje cell layer. H&E, X40

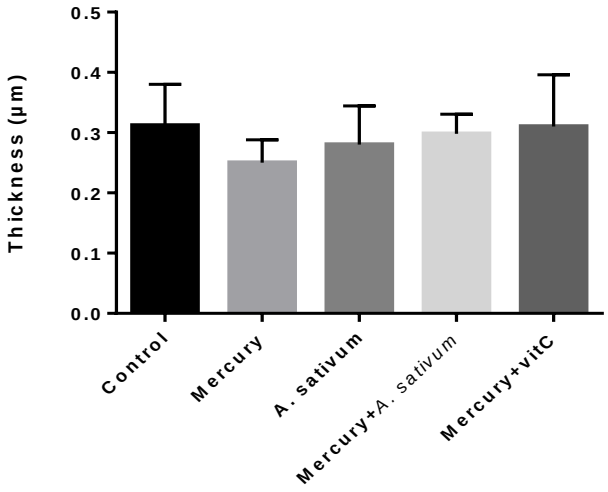


Figure 4:
Mean thickness of the molecular layer (ML) of cerebellar cortex (µm) of the control and treated rats. Values (n=5) are presented as mean±SD. *A. sativum- Allum sativum*, vit C- Vitamin C. $p>0.05$

Cerebellar astrocyte count: Increased astrocyte population was seen in the $Hgcl_2$-treated group compared with the control group at $p<0.05$ (Plate 3 and Fig.6).

DISCUSSION

Mercury is one of the most toxic heavy metals existing in the environment in three forms, namely, elemental, inorganic, and organic, causing human health problems (Takahashi and Shimohata, 2019). The principal sources of exposure to mercury compound in the general population are ingestion and inhalation of mercury from dental amalgams, and ingestion of fish (fresh water and marine) and seafood (Gandhi *et al.*, 2014). The protective effect of *Allium sativum* on $Hgcl_2$-induced oxidative stress on the cerebellum of adult Wistar rat was studied. In the study, there was a significant reduction in percentage weight gain in the $Hgcl_2$-treated group when compared to the control and other treated groups.

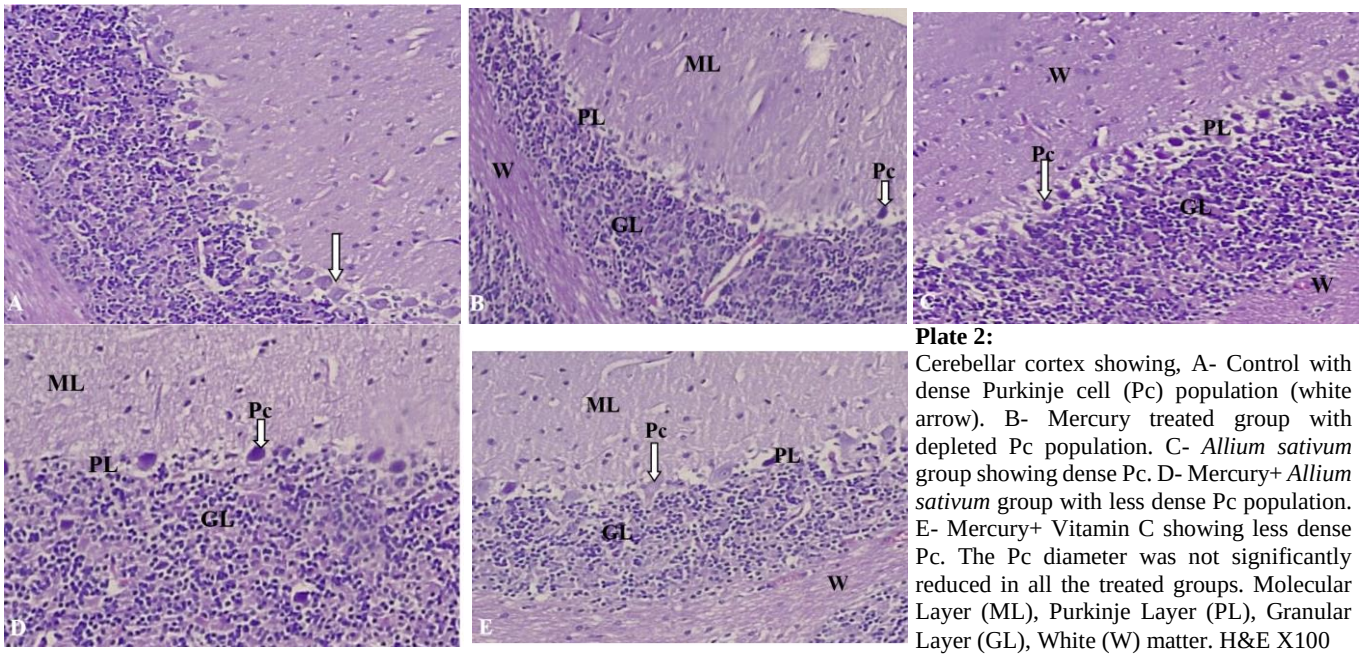
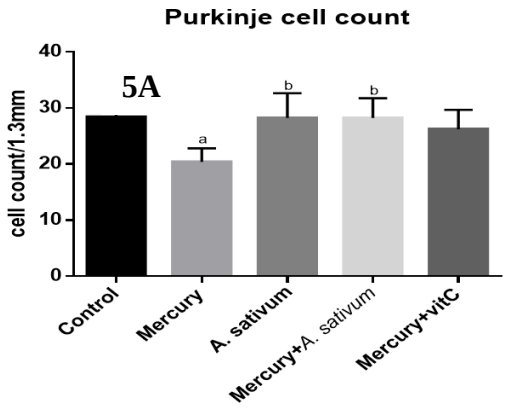


Plate 2:
Cerebellar cortex showing, A- Control with dense Purkinje cell (Pc) population (white arrow). B- Mercury treated group with depleted Pc population. C- *Allium sativum* group showing dense Pc. D- Mercury+ *Allium sativum* group with less dense Pc population. E- Mercury+ Vitamin C showing less dense Pc. The Pc diameter was not significantly reduced in all the treated groups. Molecular Layer (ML), Purkinje Layer (PL), Granular Layer (GL), White (W) matter. H&E X100



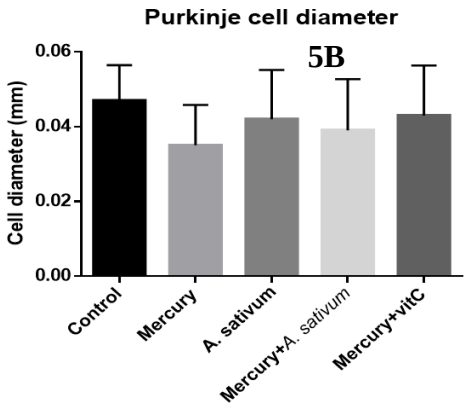


Figure 5
Cerebellar Purkinje cell count/1.3mm (**5A)** and Cerebellar Purkinje cell diameter (mm)/1.3mm (**5B**) in control and treated rats. Values (n=5) are presented as mean±SD. *A. sativum*- *Allum sativum*, vit C- Vitamin C. [a]p<0.05 compared with the control, [b]p<0.05 compared with mercury group.

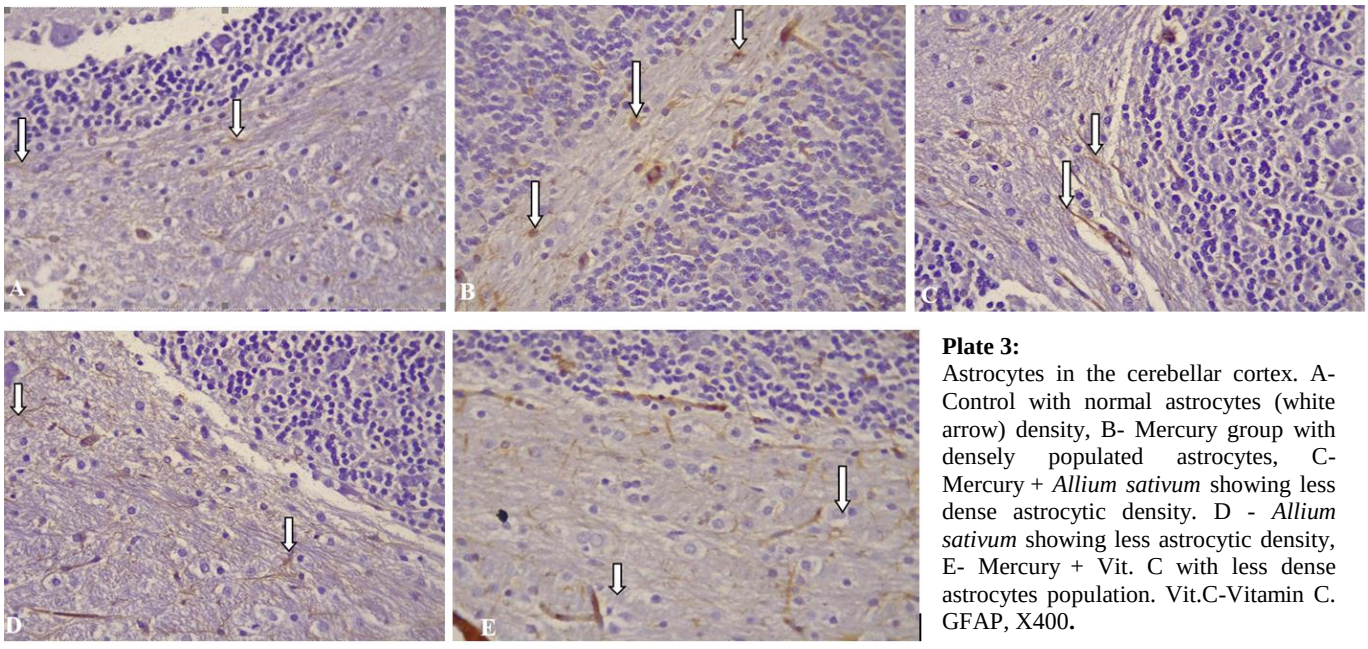


Plate 3:
Astrocytes in the cerebellar cortex. A- Control with normal astrocytes (white arrow) density, B- Mercury group with densely populated astrocytes, C- Mercury + *Allium sativum* showing less dense astrocytic density. D - *Allium sativum* showing less astrocytic density, E- Mercury + Vit. C with less dense astrocytes population. Vit.C-Vitamin C. GFAP, X400**.**

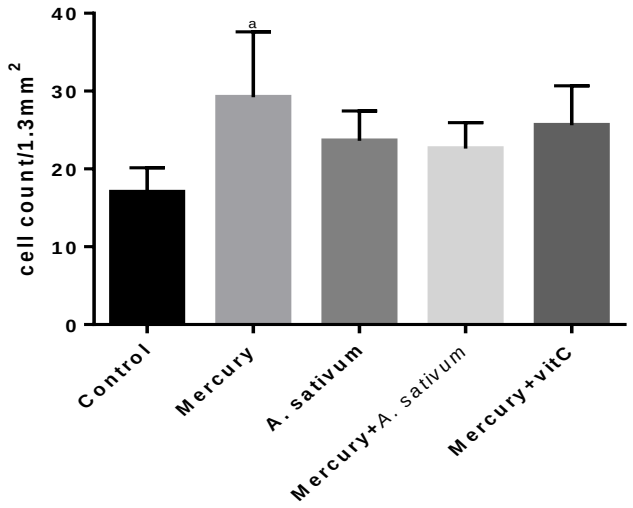


Figure 6:
Cerebellar astrocyte population/1.3mm^2 . Values (n=5) are presented as mean±SD. *A. sativum- Allum sativum*, vit C- Vitamin C. $^a p<0.05$ compared with the control.

The decreased body weight may be due to reduction in food and water intake observed during the periods of administration in the mercury treated group. This finding agrees the report of Ibegbu *et al*. (2014) that oxidative effect of mercury caused a reduction in the body weight of the animals. This however, is in contrast with the report of Teixeira *et al*. (2018) that chronic exposure to $Hgcl_2$ did not alter the body weight of the animals. *Allum sativum* administered to rats exposed to $Hgcl_2$ increased the body weight but not significantly. The insignificant increase in body weight recorded may be due to increased appetite stimulation by *Allium sativum* as it contains several enzymes, minerals, vitamins, protein, carbohydrate, fibres and amino acids (Josling, 2005).

The cerebellar cortex is also important for learning of movements (for example, in learning to write) and through its vestibular and spinal connections, the cerebellum is responsible for maintaining the equilibrium of the body (Singh *et al*., 2014). Previous studies have shown that mercury has the ability to reduce the number of neuron and alters the cytoarchitecture in individuals with prenatal exposure to mercury (Grandjean *et al*., 2006). Although alterations in balance and fine motor coordination are usually associated with cerebellar damage (Karl *et al*., 2003), the motor cortex plays an important role in fine motor control and fractionation of movement, sensorimotor integration and higher order cognitive–motor movement (Sanes and Donoghue, 2000). Chronic exposure of animals to mercury results in accumulation of inorganic mercury in cerebellum and temporal and frontal lobes, causing neurobehavioural deficits (Ostertag *et al*., 2013). The forelimb grip strength test is used to measure muscle strength and balance while negative geotaxis is used to determine the unlearned response to gravitational cues (Motz, and Alberts, 2005). Our study revealed that mercury-treated rats had decreased muscular strength with a shorter drop-off time compared with the control and *A. sativum* rats. This however, may be due to the depletion in the cerebellar neurons as seen in the histological evaluation as a result of oxidative stress induced by $Hgcl_2$. Our results also showed a non-significantly increased time to complete negative geotaxis response in the $Hgcl_2$ exposed groups. Axevedo *et al.* (2012) reported that high exposure to mercury induces changes in the central nervous system, potentially resulting in irritability, fatigue, behavioral changes, tremors, headaches, hearing and cognitive loss, dysarthria, incoordination, hallucinations, and death. The high amount of minerals, vitamins, protein, carbohydrate, fibres and amino acids present in *A. sativum* has been reported to increase its biological activities (Josling, 2005).

The brain is particularly vulnerable to oxidative stress because of its high oxygen consumption; in addition, it contains unsaturated fatty acids that are targets of lipid peroxidation (Dringen, 2000; Perry *et al*, 2002). In the present study, mercuric chloride induced oxidative stress as indicated by increased lipid peroxidation (LPO), and decreased glutathione (GSH) levels, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in the cerebellum. *Allum sativum* and vitamin C reduced oxidative stress by decreasing the rate of lipid peroxidation, and increasing the GSH levels and SOD activity, thereby boosting the antioxidant capacity of the cerebellum. Superoxide dismutase is considered to be one of the most active enzymes involved in the dismutation of superoxide anions produced during oxidative stress in cells (Halliwel, 1999). Increased SOD activity may suggest increased survival mechanism for the rats exposed to mercury to cope with stress so as to reduce possible neurobehavioral effects such as aggression and body tremor. The antioxidant system has been reported to modify the highly reactive oxygen species to form less reactive intermediate which helps in reducing stress and pose no threat to the cell (Al-Omar *et al*. 2004).

The cerebellum plays an important role in the control of movement, ensuring smooth movement taking place in the right direction and to the right extent. Cerebellar stimulation modifies movements produced by stimulation of motor areas of the cerebral cortex. The Molecular layer (ML) is the outermost layer of the adult cerebellar cortex, consisting mainly of cell processes, dendrites and axons of neurons, and sparsely populated with two types of nerve cells namely; outer stellate cells basket cells. The ML also contains the dendritic arbors of Purkinje neurons and parallel fiber tracts from the granule cells (Greenstein *et al.*, 2000). The thickness of the ML has been shown to depend on the neurons and granule cell fibres (parallel fibres) present (Rakic and Sidman, 1970). Histomorphologically, there was a non-significant reduction in the molecular layer thickness of the $Hgcl_2$-treated group compared with the control and other treated groups. The mechanism for the insignificant reduction in not completely clear, however, the oxidative toxicity of mercury has been documented (Ibegbu *et al*., 2019). The Purkinje cell layer is the middle layer containing large flask-shaped cells arranged in a single row called Purkinje cells, which has a cytoplasm consisting of large number of Nissl granules and prominent

nucleus (Greenstein *et al.*, 2000). The present study demonstrates a significant depletion of and a non-significant reduction in the diameter of the Purkinje cell in the $Hgcl_2$-treated rats compared with the control and *A.sativum*-treated groups. The mechanism for the depletion of the Purkinje cells is not completely clear however, oxidative stress caused by mercury resulting in complex sequence of cascading molecular events, such as mitochondrial dysfunction, excitotoxicity, intracellular calcium dyshomeostasis and decreased antioxidant capacity may have been implicated (Farina *et al*., 2011). In humans, the Purkinje cell can be damaged by autoimmune diseases, genetic mutation and exposure to a variety of toxic substances such as alcohol or lithium, causing spinocerebellar ataxias and neurodegenerative diseases (Mitoma *et al*., 2016). *Allum sativum* restored the depleted Purkinje cell probably by mopping up the free radical generated by mercury as it contain high amount of phenolic compounds and flavonoids capable of scavenging free radicals and increasing the antioxidant capacity.

Astrocytes, the largest and most prevalent type of glial cell in the central nervous system consist of two main subclasses: protoplasmic and fibrous (Pekny and Pekna, 2014). Activation of astrocytes can occur in response to a variety of injuries to the brain as well as in inflammation or pathological neurodegeneration (Pekny and Pekna, 2014). Immunohistochemically, using the glial fibrillary acidic protein (GFAP) antibody, there was increase astrocytes density in the cerebellar cortex in the treated rats but significantly in the $Hgcl_2$-treated group, an indication of astrogliosis. The increased astrocytes density may be due to the oxidative stress induced by mercury toxicity. Astrocytes have high antioxidant capacity and protect neurons from oxidative stress. However, astrocytes are vulnerable to oxidative stress when cultured with neurons (Takahashi and Shimohata, 2019). *Allum sativum* and vitamin C non-significantly decreased the astrocyte population by increasing the antioxidant capacity of the cerebellum and decreasing the rate of oxidative stress.

In conclusion, the results of the study showed that mercuric chloride induced oxidative stress by disruption of the antioxidant defense system and generation of free radical in the cerebellum of rats, causing neurobehavioural and morphological alterations. Aqueous extracts of *Allium sativum* and vitamin C decreased the rate at which mercuric chloride induced oxidative damage in the cerebellum of adult female Wistar rats.

Acknowledgement: *We appreciate Professor E. O. Farombi for the use of his Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria for the biochemical assays.*

Conflicts of Interests: *The authors have declared that there is no conflict of interest in this study.*

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