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Afr. J. Biomed. Res. Vol.19 (September, 2016); 241- 247

Full Length Research Paper

Protective Effects of Aqueous Extract of *Telfairia occidentalis* on Mercury-Induced Histological and Oxidative Changes in the Rat Hippocampus and Cerebellum

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ABSTRACT

Mercury intoxication in rodents causes damage to various organs including the brain via oxidative stress. Aqueous extract of *Telfairia occidentalis* (TOAE) may be a preventive agent by virtue of its reported antioxidant property. The present study was carried out to investigate the possible protective role of TOAE against mercuric chloride (HgCl₂)-induced changes in rat brain. Twenty-four adult female rats (150g-200g) were randomized into four groups of six rats each after acclimatization viz: I: Control, tap water; II: TOAE (400 mg/kg body wt.); III: HgCl₂ (4 mg/kg body wt.); IV: TOAE (400 mg/kg body wt.) + HgCl₂ (4 mg/kg body wt.). All treatments were oral by gastric gavage and lasted 14 days. Behavioural tests were conducted on the 15th day after which rats were euthanized with i.p Ketamine (100mg/kg) same day. Brain weight, antioxidant parameters [malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and super oxide dismutase (SOD)], behavioural studies, and histology of rat hippocampus and cerebellum with regard to micro-anatomical parameters were examined in all groups. Mercuric chloride (HgCl₂) induced significant ($p < 0.05$) elevation of the MDA level, activities of CAT and SOD, grooming and locomotion frequency but reduced GSH level relative to control. Also, it induced the death of granule cells, pyramidal cells and Purkinje neurons thus altering the microanatomy of these brain structures. However, concomitant administration of TOAE with HgCl₂ caused a reversal of these parameters relative to HgCl₂-treated rats. In conclusion, aqueous extract of *Telfairia occidentalis* demonstrated protective effects against HgCl₂-induced oxidative and histological changes of the microanatomy of rat hippocampus and cerebellum.

Keywords: Mercuric chloride, *Telfairia occidentalis*, hippocampus, cerebellum, antioxidant

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Received: March 2016; Accepted: August, 2016

Abstracted by:

Bioline International, African Journals Online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Mercury contamination of the environment continues to be a concern and a major source of this contamination is from human activities such as mining, smelting, extensive industrial and agricultural usage, combustion of fossil fuels and other industrial waste which release mercury into the environment (Rao and Purohit, 2011). Much of these wastes find their way into oceans, lagoons and rivers where fishes consume them and then contaminate humans via diets comprising of fish from polluted water.

Upon ingestion, tissue damage from mercuric chloride (HgCl₂) has been linked to oxidative damage resulting from the formation of highly reactive hydroxyl radical (OH) and other intermediate products which stimulate lipid peroxidation. This may lead to damage to and alteration of the

function of biomembranes, ultimately resulting in the development of different pathological processes (Bharathi *et al.*, 2012). Additionally, free radicals from the mercuric ion can also inactivate a number of enzymes by blocking the functional sites through binding to sulfhydryl groups, which are part of catalytic or binding domains (Sanders *et al.* 1996; Rao and Purohit, 2011). Mercury manifests its central nervous system toxicity by generating high levels of reactive oxygen species (ROS) and oxidative stress (Xu *et al.*, 2012; Vanithasri and Jagadeesan, 2013), thus altering the functions and structure of cerebrum, cerebellum and medulla oblongata (Rao and Purohit, 2011).

The ability of plant based products in mitigating the neurotoxicity of mercury by protecting neurons against oxidative stress and restoring oxidative balance was reported by Mottay and Neergheen-Bhujun (2015). *Telfairia*

occidentalis Hook f. (fluted pumpkin) is plant that belongs to the family Cucurbitaceae and is a popular and widely grown vegetable crop in different parts of Nigeria whose common names include fluted gourd, fluted pumpkin, and “*ugwu*”. It is a tropical vine grown as a leaf vegetable, has edible seeds and contains nutrients such as proteins, carbohydrates, vitamins and minerals (Fasuyi, 2006). Bioactive compounds found in it include oxalates, saponins, glycosides, flavonoids, alkaloids and resins (Akubue *et al.*, 1980). The aqueous extract of *Telfairia occidentalis* has been reported to demonstrate hepatoprotective property and increase haematological parameters (Alada, 2000), while the antioxidant and free radical scavenging property have been reported (Nwanna & Oboh, 2007; Kayode *et al.*, 2010). Folklore medicine has employed it for the treatment of convulsion (Gbile, 1986), liver and high blood sugar problems (Eseyin *et al.*, 2005; Adaramoye *et al.*, 2007) and reproductive and fertility issues (Nwangwa *et al.*, 2007).

While the mammalian cerebellum regulates motor coordination, equilibrium, both saccadic and smooth eye movements and maintains muscle tone, the hippocampal formation is involved in long term spatial and episodic memory storage (Albertini and Kandel, 2015). The anatomy and physiology of these vital parts of the central nervous system may be adversely affected when exposed to mercury. Although literature abounds with reports of the antioxidant activity of *Telfairia occidentalis* on various organs of rodents e.g. liver, testis etc; information is scanty concerning the central nervous system, hence the need for this study.

This study is aimed at determining the possible protective effect of aqueous extract of *Telfairia occidentalis* (TOAE) on mercury chloride-induced brain injury so our research question is: “What modulatory role would TOAE have on the neurotoxicity induced by HgCl₂ in rat brain?”

MATERIALS AND METHODS

Plant material and extraction method: Fresh *Telfairia occidentalis* Hook f., (Cucurbitaceae), plants were purchased from Ojoo market, Ibadan, Oyo State, Nigeria, in the month of July 2015. The leaves were identified at the Botany Department, University of Ibadan, Nigeria. An aqueous extract of the leaves was prepared according to the method of Bolaji and Olabode (2011) with slight modification. Briefly, after washing off sand and dirt with water, the fresh vegetable was blended in warring blender and the paste sieved through prewashed white cloth in distilled water. The sieved liquid was then filtered using a Whatman No. 2 filter paper to obtain a clear aqueous extract of the vegetable. The obtained filtrate was then lyophilized to obtain a solid extract by Mr A. Attah of Department of Pharmacognosy of the University of Ibadan, Nigeria which was then preserved in clean bottles at room temperature until required for used.

Preparation, dosage and administration of extract: For each fresh preparation, one gramme of the extract was dissolved in 10 mL of distilled water to make the stock solution termed *Telfairia occidentalis* aqueous extract (TOAE). Based on the method of Ajao and Akindele (2013),

400 mg/kg body weight was chosen as dosage for administration in this experiment.

Animals: Twenty-four male Wistar rats initial weight between 150 g - 200 g were acclimatized for two weeks on rat chow and water ad libitum and thereafter randomized into experimental and control groups. The rat chows used were the product of Ladokun Feeds, Ibadan, Nigeria. The animals were housed in the Animal House of the College of Medicine, University of Ibadan in well-ventilated cages. All procedures on animal handling were in accordance with guidelines of the University of Ibadan Ethical Committee which conformed to the ethical use of animals in research (PHS, 1996).

Chemicals: Mercuric chloride powder (Loba Cheme PVT Ltd, Mumbai, 40005, India) was purchased from Julimark Enterprises, Yemetu, Ibadan, Nigeria. Ketamine was manufactured by Rotex Medica, Trittau, Germany. All other chemicals and reagents were purchased from British Drug Houses Poole, Dorset, UK. Phosphate Buffer Saline (PBS) at pH = 4.0 was prepared and stored in the refrigerator at 4°C.

Preparation, dosage and administration of mercuric chloride (HgCl₂): A stock solution of 100 mg of HgCl₂ to 20 mL of distilled water was prepared. From the stock solution, HgCl₂ was administered to the animals at a dose of 4 mg/kg/daily for 14 days based on the method of Sheikh *et al.* (2013) orally using a syringe.

Research design: Twenty-four adult female rats (150g-200g) were used for the experiment. Rats were randomly assigned into four groups of five rats each after acclimatization to animal house condition for one week with free access to feed and water. The four grouping are shown in Table 1.

Table 1:
Animal grouping and Experimental schedule

Groups	No. of animals	Treatment	Duration	Sacrificed
Control	6	Tap water	14 days	15th day
TOAE	6	400 mg/kg of TOAE daily	14 days	15th day
HgCl ₂	6	4 mg/kg daily	14 days	15th day
TOAE+ HgCl ₂	6	400 mg/kg of TOAE+ 4 mg/kg of HgCl ₂	14 days	15th day

TOAE – *Telfairia occidentalis* aqueous extract;
HgCl₂ –Mercuric chloride.

Neurobehavioural tests: On the 15th day of the experiment, the open field test and forelimb grip strength test were carried out.

The open field test: This test was performed according to the method of Olopade *et al.* (2012) to assess the locomotor capacity of rodents. Briefly, each rat was placed in an open field, a 72 by 72 cm square box with lines on the floor dividing it into 18 by 18 cm square that allowed the definition of central and peripheral parts. Each animal was placed in the centre of the arena and the total locomotion (number of floor units entered with all four paws), rearing frequency (number of

times the animal stood up on its hind limb or with the fore limbs against the wall of the observation box or free in the air) and grooming frequency (number of body cleaning with paws or picking of the body and pubis with mouth and face washing actions) within each 5 min interval were video-recorded. These parameters were assessed by the same set of observers who ensured the arena was cleaned with 70% ethanol to eliminate olfactory bias and allowed to dry before introducing a fresh animal.

The forelimb grip strength test: This is a test of muscular strength in the forelimbs (Tamashiro *et al.*, 2000). In this test, the forepaws were placed on a horizontally suspended metal wire 2 mm in diameter, 1 m in length and placed 1 m above a landing area filled with soft bedding. The length of time each rat was able to stay suspended before falling off the wire was recorded; a maximum of 2 minutes was given to each rat. Each animal was given two trials with a period of rest interval. This test assessed muscle strength and balance.

Sample collection and histological preparation : On the completion of the behavioural tests on the 15th day of the experiment, animals were euthanized under ketamine anesthesia (100 mg/kg) i.p., followed by cervical dislocation after which the skulls were opened and the brains quickly dissected out and divided into two sagittal sections. Based on the method of Igado *et al.* (2012), the right hemisphere of the each brain was preserved for histology by fixing it in 10% buffered formalin. The formalin-fixed tissue was serially dehydrated and embedded in paraffin, and using a Rotary Microtome (Leica RM2125 RTS), 5-6 µm thick coronal sections of the brain and cerebellum were processed and slide sections stained with Haematoxylin and Eosin according to the method of Bancroft and Gamble (2008). After staining, the slides were viewed with an Olympus CH (Japan) light microscope and images captured with a Sony DSC-W 30 digital camera (Japan) and photomicrograph calibration done with 'Image J' (Abramoff *et al.*, 2004). The slides were then assessed for histologic alterations like form and shape changes, loss of cellular integrity and other micro-anatomical alterations of brain sections. The left half of the brain reserved for biochemical tests was rapidly rinsed, mopped with filter paper, weighed and kept in freshly prepared cold phosphate buffered solution (PBS) at pH = 4 in the freezer till processed.

Biochemical Assays: The left hemisphere of the brain samples was homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride, and the homogenate

centrifuged at 10,000 g for 15 minutes at 4 °C. The supernatant was collected for the estimation of the various biochemical bioassays. Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi *et al.* (2000) and expressed as micromoles of MDA per milligram protein. Protein concentration was determined by the method of Lowry *et al.* (1951). Reduced glutathione (GSH) was determined at 412 nm using the method described by Jollow *et al.* (1974). Catalase (CAT) activity was determined using hydrogen peroxide as substrate according to the method of Clairborne (1995). Superoxide dismutase (SOD) was assayed by the published method of Misra and Fridovich (1972). Biochemical assays were conducted at the Drug Metabolism & Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria.

Statistical analysis: Data will be presented as the Means ± Standard Error of Mean and analysed using one-way analysis of variance (ANOVA) followed by LSD post-test using computer based fitting program (IBM SPSS) version 20. Statistical significance was set at $p < 0.05$

RESULTS

General: There was no mortality throughout the duration of the experiment.

Brain weight changes: Brain weight changes and the relative weights of the brains were not affected in a significant manner as shown in Table 2.

Biochemical parameters: Data presented in Table 3 shows that HgCl₂ elevated MDA and reduced GSH levels significantly ($p < 0.05$) relative to control. Similarly, it increased the activity of the enzymes SOD and CAT relative to control. However, in rats co-treated with TOAE, all the induced changes were significantly ($p < 0.05$) reversed to near control values when compared with HgCl₂ treated animals.

Behavioural tests: As displayed in Table 4, HgCl₂ administration increased the grooming frequency and locomotion frequency but reduced the rearing frequency of the rats significantly ($p < 0.05$) relative to control, while the increase in forelimb grip was not significant. However, concomitant administration of TOAE with HgCl₂ significantly (< 0.05) reduced the locomotion frequency but increased the rearing frequency relative to HgCl₂.

Table 2:
Effect of TOAE and HgCl₂ on brain changes in rats

Groups	Initial average weight(g)	Final average weight(g)	Body weight difference(g)	Brain weight(g)	Relative brain weight (%)
Control	176.00±2.92	184.00±5.79	16.67±8.82	1.60±0.06	0.88±0.05
TOAE	156.00±6.21	173.00±5.39	17.00±1.23	1.56±0.08	0.90±0.04
HgCl ₂	160.00±3.16	174.00±3.67	14.00±2.92	1.54±0.05	0.87±0.04
TOAE+HgCl ₂	167.00±9.57	183.00±5.39	16.00±4.85	1.50±0.07	0.82±0.04

Data is presented as Mean ± S.E of 6 animals each per group.

TOAE – *Telfairia occidentalis* aqueous extract, HgCl₂ – Mercuric chloride.

Table 3:

Effects of TOAE and HgCl₂ treatment on biochemical parameters of rat brain.

Groups	LPO (μmol MDA/mg protein)	SOD (units/mg protein)	CAT (Units/mL)	GSH (ug/ml/mg protein)
Control	2.828±0.40	0.705±0.08	1.438±0.14	4.615±0.55
TOAE	3.142±0.47	0.876±0.08	2.001±0.28	3.704±0.98
HgCl ₂	4.538±0.13*	1.162±0.06	2.639±0.31*	1.033±0.05*
TOAE+HgCl ₂	2.674±0.09**	0.781±0.06	1.429±0.14**	4.537±0.56**

Data presented as Mean ± SEM of 6 animals each per group. LPO- Lipid peroxidation, SOD- Superoxide dismutase, CAT- Catalase, GSH- reduced Glutathione, TOAE- *Telfairia occidentalis* aqueous extract, HgCl₂ –Mercury chloride. *P< 0.05 versus Control, **P< 0.05 versus HgCl₂.

Table 4:

Effects of TOAE and HgCl₂ treatment on animal behaviour.

Groups	Grooming frequency	Forelimb grip (s)	Locomotion frequency	Rearing frequency
Control	49.33±7.33	8.75±1.18	28.60±1.29 b	16.50±2.22
TOAE	51.75±9.15	8.50±0.96	32.25±6.07 b	14.33±0.67
HgCl ₂	69.67±6.74*	10.25±0.48	45.50±3.40*	4.00±0.55*
TOAE+HgCl ₂	69.33±6.89	10.00±1.05	28.67±3.18**	10.00±1.23**

Data is presented as Mean ± S.E of 6 animals each per group. *P< 0.05 versus Control, **P< 0.05 versus HgCl₂. TOAE – *Telfairia occidentalis* aqueous extract, HgCl₂ –Mercury chloride

Histological parameters

Dentate gyrus: The layers of the gyrus are well displayed in Figure 1 showing the molecular, granular and polymorphic. In Figures 1A and 1B, the granule neurons show dispersed chromatin. In the HgCl₂ treated Figure 1C, the granule neurons are observed to be dark and pyknotic when compared with control. In Figure 1D, the effect of co-treatment of TOAE with HgCl₂, are observed with the granule neurons comparable with the control

Cornu ammonis3: All the groups showed normal histological features of CA3 viz: the alveus, stratum oriens, stratum pyramidalis, stratum radiatum, and stratum lacunosum moleculare. In Figure 2C, mercuric chloride toxicity on the pyramidal neurons was demonstrated by neuronal degeneration (orange arrowheads), while black arrowheads indicate normal pyramidal neurons. Plate 2D shows the effect of co-treatment of TOAE with HgCl₂, the pyramidal neurons are relatively healthy with nucleolus prominent in many as compared with the HgCl₂ in Plate 2C.

Cerebellum: The histological layers of adult rat cerebellum are shown in all the figures viz: outer molecular inner granular and middle Purkinje as observed in all the groups. The Purkinje cells are noted to be the largest cell with prominent basophilic staining. The effect of HgCl₂ is observed in Plate 3C with loss of basophilia and complete eosinophilic staining of the Purkinje cell bodies when compared with the control and TOAE group. However, Figure 3D shows the effect of co-treatment with TOAE shown by a normal basophilic staining of the Purkinje cells when compared with the HgCl₂ group of Plate 3C.

DISCUSSION

In this study, we presented data showing that administration of mercuric chloride (HgCl₂, 4 mg/kg) to rats for 14 days induced changes in oxidative, behavioural and histological parameters observed. The concomitant treatment of these rats

with 400 mg/kg aqueous extract of *Telfairia occidentalis* (TOAE) with HgCl₂ ameliorated these changes.

The elevated level of MDA (a biomarker of lipid peroxidation, LPO) in the brains of HgCl₂-treated rats was an indication of increased generation of free radicals it induced which could damage and disrupt the lipid bilayers of membrane leading to cellular dysfunction (Abolaji *et al.*, 2016). The combination of raised LPO and reduction of GSH support the presence of oxidative stress which agrees with previous report (Abdel, 2015). The elevation in the activities of SOD and CAT may be an adaptive process to counter the increased free radicals generated by HgCl₂ exposure in the brain which agrees with reports (Ebokaiwe *et al.*, 2013). However, we observed the significant lowering of the MDA level and elevation of the GSH by TOAE. The antioxidant activity of this plant is attributable to the presence of flavonoids as bioactive compounds in this extract. Particularly, the aqueous extract (that we used in this study) was reported to possess a higher total phenol, reducing power and free radical scavenging ability than the ethanolic extract (Obboh *et al.*, 2010). Javanmardi *et al.* (2003) asserted that the antioxidant activity of phenolic compounds is mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides The property may also account for the significant reduction of SOD and CAT activities having been augmented by the antioxidant content of TOAE. Activities of antioxidant enzymes were also restored concomitantly when compared to the HgCl₂ control rats after TOAE administration.

The neurotoxicity of HgCl₂ in the rat hippocampus was well demonstrated by the neuronal degeneration noted by the pyknotic neurons in the granule layer of the dentate gyrus (DG) of the rats which is in agreement with previous report (Owoeye and Farombi, 2015). Similarly observed, was the HgCl₂-induced death of pyramidal neurons of the cornu ammonis3 (CA3) with distortion of the layered microanatomy of the neurons with some neurons showing features compatible with pyknosis and some with karyolysis (Stevens and Lowe, 2000).

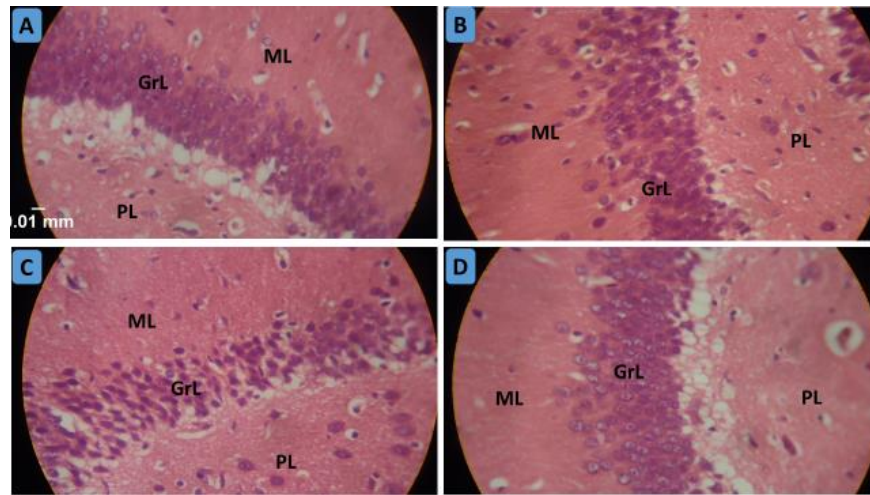


Plate 1:

Representative stained coronal sections of gyrus dentatus of hippocampal formation of rats: (A) Control rats (B) TOAE-treated (C) HgCl₂-treated (D) HgCl₂+ TOAE group. HgCl₂-treated show granule neurons with condensed nuclei in the GrL. TOAE, *Telfairia occidentalis* aqueous extract; HgCl₂, Mercuric chloride. ML, molecular layer; PCL, Purkinje cell layer; GrL, granular layer. H&E stain. Scale bar is 0.01mm (10 µm) for all figures.

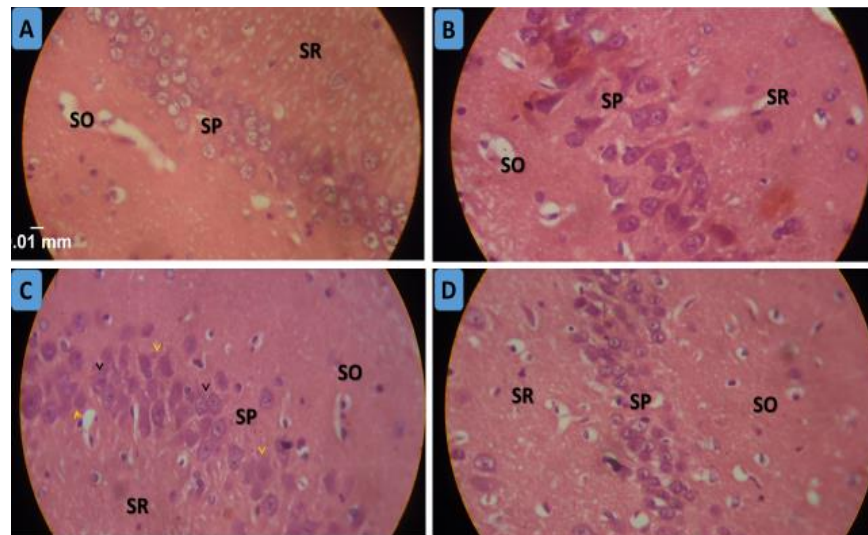


Plate 2:

Representative stained coronal sections of Cornu Ammonis3 of rats: (A) Control rats (B) TOAE-treated (C) HgCl₂-treated (D) HgCl₂ + TOAE group. HgCl₂-treated show degenerated neurons (orange arrowheads) and a distortion of the layered structure observed in the control group. Normal pyramidal neurons in this group show dispersed chromatin (black arrowheads). TOAE, *Telfairia occidentalis* aqueous extract; HgCl₂, Mercuric chloride. SO, stratum oriens layer; SP, stratum pyramidalis; SR, stratum radiatum. H&E stain. Scale bar is 0.01mm (10 µm) for all figures.

These findings agreed with published findings (Owoeye and Farombi, 2015). The degeneration of Purkinje cells observed in HgCl₂-treated cerebellum especially the complete loss of nuclear materials shows clearly the damage to DNA by its toxicity (Uma *et al.*, 2012; Bernhoft, 2012). The eosinophilia, lack of nuclear material, death of the Purkinje cells, and loss of basophilia are in agreement with previous reports (Ibegbu *et al.*, 2014; Owoeye and Farombi, 2015).

The implication of HgCl₂-induced granule cell neuronal death is the possible reduction in effectiveness of impulse transmission from those neurons via the mossy fibres to the CA3 pyramidal neurons as part of the trisynaptic pathway. Excitatory synaptic input from layer II of entorhinal cortex project on apical dendrites of granule cells, which give rise to mossy fibers. Mossy fibres project in turn, synapse on CA3

pyramidal neurons which via its glutamatergic Schaffer collaterals then project onto ipsilateral CA1 pyramidal neurons, thereby completing the hippocampal trisynaptic circuit (Stepan *et al.*, 2015). The combination of granule neuronal death and CA3 pyramidal neuronal death will inevitably affect the trisynaptic pathway associated with the flow of hippocampal neural flow. The resultant effect of this is reduction in the acquisition and recall of episodic and spatial memories in such affected animal (Scharfman, 2007; Alberini and Kandel, 2016). Similarly, since is the focal neuron of the cerebellum, Purkinje cell death will inevitably affect the coordinating role of the cerebellum as regards movement of voluntary skeletal muscle, posture and gait leading to such features as muscular hypotonia, intentional tremor, nystagmus (Snell, 2006).

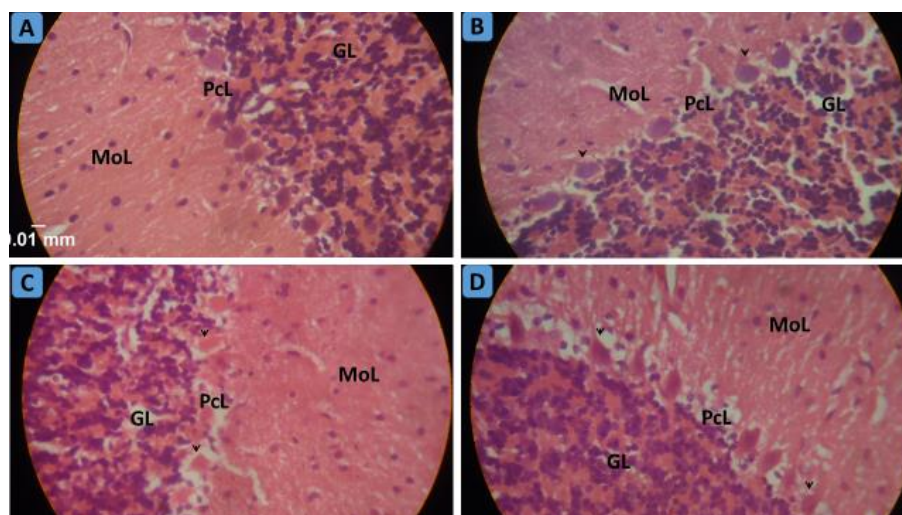


Plate 3:

Representative stained sections of cerebellum of rats: (A) Control rats (B) TOAE-treated (C) HgCl₂-treated (D) HgCl₂ + TOAE group. HgCl₂-treated show degenerated Purkinje neurons (arrowheads) that have undergone karyolysis. TOAE, *Telfairia occidentalis* aqueous extract; HgCl₂, Mercuric chloride. ML, molecular layer; PcL, Purkinje cell layer; GL, granular layer. H&E stain. Scale bar is 0.01mm (10 µm) for all figures.

The increase in line crossing and forelimb strength of HgCl₂-treated rats was in contrast to the report of (Owoeye and Farombi (2015) which reported a diminution of these parameters. However, Masur *et al.* (1971) and Morais *et al.* (1998) explained that mobile and rearing activities of rodents are functions of CNS excitability which may support the excitability of the HgCl₂ treated rats.

In summary, the finding of ameliorative changes observed biochemically and histologically in all the groups concomitantly treated with TOAE and HgCl₂ suggests the potency of the antioxidant potential of TOAE to abolish or reduce most of the above implications in the rats affected. This supports previous reports by Rao and Purohit (2011) and Uma *et al.* (2012) that HgCl₂-induced toxicity via oxidative damage can be mitigated by antioxidants which in this experiment have been demonstrated by TOAE.

In conclusion, our findings suggest that TOAE has the capacity to protect rat brain from HgCl₂-induced oxidative stress and degeneration of hippocampal and cerebellar neurons possibly via its antioxidant capacity.

Acknowledgement

We appreciate the kindness of Prof. E. O. Farombi, who permitted the biochemical assays to be done in his Drug Metabolism & Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria and Dr. I.A. Adedara and Mr. D. Iramofu of the same laboratory who helped with biochemical data acquisition and interpretation. We equally thank Mr A. Attah of Department of Pharmacognosy of the University of Ibadan, Nigeria who lyophilized the filtrate.

Conflict of interest

The authors declare no area of conflict as they are wholly responsible for the funding of this research and the writing of the manuscript

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