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Comparative Neuroprotective Effect of *Celosia argentea Linn.* and Vitamin E on Mercury-induced Oxidative and Histological Parameters of Rat Brain

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Summary: Mercury contamination of our environment in Nigeria is increasing as mining activity increases. Its exposure causes human toxicological effects which include neurotoxicity through reactive oxygen species. This study investigated the ameliorative effects of the flavonoid-rich aqueous extract of *Celosia argentea* (AECA) and vitamin E (VitE) in the brain of rats treated with mercuric chloride ($HgCl_2$). Twenty-five adult male Wistar rats were randomized into five treatment groups (n=5). Group 1- control; Group 2- $HgCl_2$ (4 mg/kg); Group 3- AECA (400 mg/kg); Group 4- $HgCl_2$ (4 mg/kg) + AECA (400 mg/kg); Group 5- $HgCl_2$ (4 mg/kg) + VitE (500 mg/kg). All items were administered using an oral cannula daily for 14 days. Behavioural studies were carried out on the 16th day of experiment after which rats were euthanized. Thereafter, gross, haematological and biochemical parameters [malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT)] were assessed. Mercuric chloride significantly ($p<0.05$) reduced body weight of rats, SOD activity and GSH level but increased MDA level, CAT activity and the number of degenerated neurons in the cerebral cortex relative to control group. Microscopically, $HgCl_2$ induced degeneration of cerebral cortical neurons and Purkinje neurons of the cerebellum. Treatment of $HgCl_2$ and AECA and VitE caused a reversal of these $HgCl_2$-induced alterations. The behavioural and haematological parameters were not significantly affected through the groups. The results suggest *Celosia argentea Linn* and vitamin E protected against mercury-induced gross, oxidative, cerebral and cerebellar damage. Both AECA and Vitamin E demonstrated neuroprotection in this experiment.

Keywords*:* Neuroprotection, *Celosia argentea*, Oxidative stress, Mercuric chloride, Cerebrum, Purkinje neuron.

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INTRODUCTION

Mercury is a heavy metal that can be found in the environment in three forms namely: elemental mercury or metallic mercury (Hg_0), inorganic mercury (mercuric chloride, $HgCl_2$), and organic mercury (methyl mercury, MeHg), the latter being the most common form of intoxication in humans. However, MeHg is gradually metabolized to inorganic mercury by intestinal microflora at a low rate per day (Bernhoft, 2012). Inorganic mercury has been used for many years in medications, teething powders, skin creams and germicidal solutions thus exposing humans to its toxicological effects (Goldman *et al*., 2001). Despite its low liposolubility, inorganic mercury can be detected in the brain, disrupting neuronal homeostasis (Clarkson and Magos, 2006) which is an evidence of its ability to accumulate in the body causing CNS damage (Smith *et al*., 1994). In Nigeria, mercury exposure and toxicity has become important as gold mining activity increases in Zamfara state, Niger state and other mining areas. Mercury exposure has been shown to stimulate the rate of reactive oxygen species (ROS) production leading to oxidative stress (Abdel Moneim, 2015). The free radical stimulation mechanism of neurotoxicity of mercury suggests that natural products with antioxidant components and free radical scavenging capability can ameliorate or protect the brain from the effects of mercury, hence the consideration of a plant part from *Celosia argentea.*

Celosia argentea is an important tropical leafy vegetable crop of high nutritional value (Aladesanwa *et al*., 2001) belonging to the Amaranthacea family. It is popular vegetable in Nigeria, where it is known as "soko yokoto", (Yoruba) meaning *"make husbands fat and happy"*, "eriamionu" (Igbo) meaning "*eat and smack your lips*" and "alayyaho daji" (Hausa). There could also be a red "soko" because it has red pigment on the leaves, which differentiates it from the green "soko" (Malomo *et al*., 2011). The presence of phenols and flavonoids which suggests antioxidant properties in *Celosia argentea L.* has been reported (Malomo *et al*., 2011, Verma and Demla, 2012 and Ramesh *et al.*, 2013). Its antioxidant properties suggest a possible

role in the search for substances that can reduce mercury toxicity.

Alpha-tocopherol (vitamin E), a fat-soluble vitamin has been demonstrated as a potent antioxidant and radical scavenger in chemical and biological systems, and protects the cell membrane from injury through its ability to prevent oxidation of unsaturated fatty acid (Cerecetto and Lopez, 2007). A previous study showed the potency of vitamin E in reducing lipid peroxidation by about 80% compared with 65%, for methanolic extract of *Vernonia amygdalina* in brains of rats exposed to oxidative stress via gamma irradiation (Owoeye et al., 2010).

The cerebellum and cerebral cortex were reported to be the targets of mercury intoxication (Xu et al. (2012), extensive damage to the hippocampal formation of rats has also been reported (Owoeye and Farombi, 2015). The Purkinje cells provides the output of the cerebellar cortex to the cerebellar nuclei (Zeeuw and Hoogland, 2015), thus important in regulation of motor coordination, equilibrium, muscle tone while the cerebral cortex is responsible for regulating cognition and primary sensory functions among other functions (Snell, 2006). The abundant lipid content and relative deficit in antioxidant systems compared to other tissues and high oxygen demand makes the brain susceptible and particularly vulnerable to damage ROS than do most other organs (Ebokaiwe et al., 2013). This susceptibility of the brain to neurotoxicants may affect its microanatomy and physiology in the absence of ameliorating factors.

In view of the scanty literature on the effect of AECA on mercury toxicity in the brain of rat, this study was carried out to investigate the possible protective effect of aqueous extract of *Celosia argentea* (AECA) in mercury-induced oxidative stress in the brain of adult male rats and then compare such effects with those of a standard antioxidant vitamin E. This study will thus answer the research question of whether the aqueous extract of *Celosia argentea* (AECA) can modify the effect of mercuric chloride on the brain of rat.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Celosia argentea Linn* was purchased from Bodija Market, Ibadan, Nigeria in the month of June, 2015 and was authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria. A voucher specimen (FHI. 110229) was deposited at the herbarium of FRIN.

Extraction of the Aqueous Extract of *Celosia argentea L.*

The leaves were prepared using the method of Malomo *et al.* (2011). Briefly, the leaves were oven-dried at 40°C at a constant weight and the dried leaves were thereafter pulverized. Five hundred gramme of the powdered leaves was extracted in 5 L of distilled water at room temperature for 48 hours before been filtered. The filtrate was concentrated over a rotatory evaporator and dried to constant weight in an oven. This gave a yield 37.9% of aqueous extract of *Celosia argentea* and was given the acronym "AECA".

Preparation, dosage and administration of AECA

From a stock solution of 1 g AECA/ 10 mL of distilled water, the extract (AECA) was administered to the animals orally with the aid of an oral cannula at a dose of 400 mg/kg daily for fourteen (14) days. Dosage was based on the method of Malomo et al. (2011).

Preparation and administration of mercury chloride ($HgCl_2$)

Dry powder of Mercury Chloride ($HgCl_2$, 99% purity) manufactured by Loba Cheme PVT Ltd, Mumbia, 40005, India. From a stock solution of 500 mg of $HgCl_2$ to 20 mL of distilled water, $HgCl_2$ was administered to the animal at a dose of 4 mg/kg/day with the aid of an oral gavage for 14 days. Dosage was based on published method (Sheikh et al., 2013).

Preparation, dosage and administration of vitamin E

Each soft gelatin capsule containing 100 mg of DL-α-tocopheryl acetate as 100 mg vitamin E acetate (Sinopharm Xingsha Pharmaceuticals Co. LtD, China). Each 100 mg capsule was punctured with a new size 21G needle (Hypojet, Spain) attached to a new 1 mL hypothermic syringe, completely aspirated and then attached to a clean cannula through which each rat was administered orally the measured dose of 500 mg/kg body weight/daily (Owoeye et al., 2010).

Ethical approval

The research protocol was approved by the Animal Care and Use Research Ethics Committee (UI-ACUREC) of the University of Ibadan, Nigeria, with reference number UI-ACUREC/App/2014/003. The experiments were carried out at the Anatomy Department, while biochemical assays were carried out at the Drug Metabolism and Toxicology Unit, Department of Biochemistry and haematological studies were done at the Veterinary Pathology Laboratory of University of Ibadan. The animals received humane care in accordance with the principle of humane care and use of laboratory animals.

Experimental Animals

Adult male Wistar rats, weighing 150–240 g, aged about 3 months, were obtained from the breeding colony of the Department of Veterinary Physiology University of Ibadan, Nigeria. Five animals were kept in plastic cages having dimensions of 39 × 29 × 27 cm and soft wood shavings employed as bedding in the cages. They were housed in the College of Medicine Central Animal House in a light/dark cycle and had access to rat pellets (Ladokun Feeds, Mokola, Ibadan)

and water *ad libitum* where they were acclimatized for two weeks before randomization into experimental and control groups.

Experimental Design

Twenty five adult rats were after acclimatization divided into five groups of five animals each as detailed below:

Group 1: served as control, received distilled water only
Group 2: received $HgCl_2$ (4 mg/kg of $HgCl_2$)
Group 3: received 400 mg/kg of AECA extract
Group 4: received $HgCl_2$ (4 mg/kg of $HgCl_2$) + 400 mg/kg of AECA extract
Group 5: received $HgCl_2$ (4 mg/kg of $HgCl_2$) + 500 mg/kg of VitE.

AECA = Aqueous extract of *Celosia argentea L.*, $HgCl_2$ = Mercury chloride, VitE = Vitamin E.
All treatments were administered to the rats for 14 days orally by gavage.

Behavioural Tests

On experimental day 16, rats in each group were weighed and then subjected to behavioural studies namely **o**pen field test and forelimb grip strength test.

Open Field Test. The animals were placed for 5 min in an open-field arena. The apparatus, made of wood covered with impermeable formica, had a white floor of 100 × 100 cm (divided by black lines into 25 squares of 20 × 20 cm) and 40-cm high white walls. Each rat was placed at the center of the open field and was free to explore the unfamiliar arena; the total number of squares crossed and rearing was measured (Olopade *et al.*, 2012). The quadrant was considered crossed when the animal has four paws in the adjacent square. The test was carried out on day 15 of experiment.

Forelimb Grip Strength Test: This test involved the forepaws of the rats being placed on a horizontally suspended metal wire (measuring 2mm in diameter and 1m in length), placed one meter 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 time of 2 minutes was given to each rat after which it was removed. The test reflects muscular strength in the animals (Olopade *et al.*, 2012).

Haematological Studies

On completion of behavioural studies on the 16th day of the experiment, blood for haematological parameters was obtained from the retro-ocular plexus of the animals using heparinized capillary tubes into Lithium heparin treated sample bottles for the determination of blood parameters namely: red blood cell count (RBCC), white blood cell count (WBCC), haemoglobin count (HB), packed cell volume count (PCV) and white cell differential cell count. These procedures were performed at the Veterinary Pathology Laboratory of the University of Ibadan, Nigeria.

Assessment of Oxidative Stress Parameters

The left hemisphere of the brain was used for biochemical assays. Malondialdehyde (MDA) level was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Varshney and Kale (1990). The activity of Superoxide dismutase (SOD) was determined according to the method of Misra and Fridovich (1972) and SOD activity was expressed as µ/mg protein. Catalase (CAT) was determined according to the method of Clairborne (1985) and enzyme activity was expressed as U/mg protein. The method of Beutler *et al.* (1963) was followed in estimating the level of reduced Glutathione (GSH) and was expressed in µmole/mg protein.

Histology

The animals were sacrificed by cervical dislocation on the 16th day of the experiment after final body weight measurement and blood samples were obtained. The whole brain was dissected and brain weights recorded. The full brain was divided into two halves using the method of Owoeye and Onwuka (2016); the right hemisphere was fixed in 10% neutral buffered formalin (10% NBF), dehydrated using a grade ethanol series and embedded in paraffin and then sectioned at 6 - 7µm thickness. Sections were stained with haematoxylin and eosin. The tissue sections were evaluated under light microscope (Olympus BX51, Tokyo, Japan) and photographed with a digital camera (100 Olympus, Olympus Optical Co. Ltd., Japan). The left hemisphere of the brain preserved for biochemical assays 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.
Histomorphometric analysis was done using computerized image analyzer (TSView CX image software file version 6.2.4.3 and Image motic 2000 (China). On the cerebral cortex sections, the number of non-viable pyramidal cells in the external pyramidal cell layer was counted per 5 high-power fields (x400).

Statistical Analysis

Data were analysed using one way ANOVA (Analysis of Variance) test, followed by Bonferroni's post-test analysis using the statistical software package Graphic Prism Version 5.04 (2010). The statistical significance was set at $p < 0.05$, for the null hypothesis being true by chance and the confidence interval at 95% level.

RESULTS

Body and brain weight changes

Mercuric chloride significantly ($p<0.05$) reduced the body of rats compared with control at the end of this

Table 1: Effect of mercuric chloride and *Celosia argentea* on body and brain weight of rats.

Groups	Initial BW (g)	Final BW (g)	Difference (g)	Brain Weight (g)	Relative brain weight (%)
CTRL	177.50±15.00	215.00±12.90	37.5±3.60	1.580±0.05	0.73±0.05
$HgCl_2$	220.00±21.60	212.50±17.07	-7.50±0.18♯	1.787±0.07	0.84±0.08
AECA	171.25±6.29	192.50±17.07	20.75±1.40*	1.632±0.04	0.85±0.06
$HgCl_2$+AECA	183.00±8.24	192.50±16.58	9.5±1.25⁎	1.672±0.15	0.87±0.11
$HgCl_2$+VitE	195.00±8.40	181.25±13.14	-13.75±0.05⁎	1.715±0.09	0.95±0.03

Values are expressed as mean ± SD of 5 rats. CTRL=Control, AECA=*Celosia argentea*, $HgCl_2$=Mercuric chloride, VitE = Vitamin E. BW=Brain weight. ♯P<0.05 compared to control group, *P<0.05 compared to $HgCl_2$ group.

Table 2: Effect of mercuric chloride and *Celosia argentea* on behavioural parameters and forelimb grip of male Wistar rats.

Groups	Fore limb grip (s)	No. of grooms	No. of squares crossed	No. of rearing
CTRL	7.87±4.93	45.52±2.18	29.50±15.15	8.25±06.39
$HgCl_2$	8.25±2.59	42.75±2.59	30.75±24.91	13.25±09.17♯
AECA	5.75±1.19	44.75±1.19	32.75±17.65	6.50±00.57
$HgCl_2$+AECA	6.25±1.70	38.75±1.70	36.50±9.32	13.50±05.19
$HgCl_2$+VitE	4.62±0.47	75.25±0.47*	26.00±24.49	13.75±08.73

Values are expressed as mean ± SD of 5 rats. CTRL = Control, AECA = *Celosia argentea*, $HgCl_2$ = Mercuric chloride, VitE = Vitamin E. ♯P<0.05 compared to control group, *P<0.05 compared to $HgCl_2$ group.

Table 3: Effect of mercuric chloride and *Celosia argentea* on red blood parameters.

Groups	PCV%	RBC (10_6/µL)	HB (g%)
CTRL	52.25±0.96	8.44±0.05	17.33±0.28
$HgCl_2$	54.75±1.71	8.56±0.06	18.28±0.40
AECA	50.00±2.94	8.29±0.48	16.60±0.83
$HgCl_2$+AECA	47.00±2.71	7.93±0.47	15.50±0.74
$HgCl_2$+VitE	50.50±0.58	8.46±0.07	16.65±0.13

Values are expressed as mean ± SD of 5 rats. CTRL = Control, AECA = *Celosia argentea*, $HgCl_2$ = Mercuric Chloride, VitE = Vitamin E.

Table 4: Effect of *Celosia argentea* and vitamin E on mercuric chloride-induced antioxidant status of rat brain.

Groups	MDA (µmol/mg.pr-)	SOD (Units/ mg.pr-)	CAT(U/mg.pr-)	GSH (µmole/ mg.pr-)
CTRL	5.002±0.60	0.799±0.29	19.09±0.91	1.037±0.09
$HgCl_2$	22.56±0.70♯	0.570±0.21	23.16±0.40♯	1.011±0.08
AECA	6.365±2.38	1.028±0.06♯	15.13±1.35♯	1.322±0.02♯
$HgCl_2$+AECA	7.900±1.70⁎	1.314±0.15*	25.23±1.18	1.352±0.05⁎
$HgCl_2$+VitE	8.111±0.56⁎	0.933±0.37*	23.21±0.98	1.183±0.04*

Values are expressed as mean ± SD of 5 rats. CTRL=Control, AECA=*Celosia argentea*, $HgCl_2$=Mercuric chloride, VitE = Vitamin E. ♯P<0.05 compared to control group, *P<0.05 compared to $HgCl_2$ group.

experiment (Table 1) but co-treatment of $HgCl_2$ with AECA reversed this alteration relative to $HgCl_2$ group whereas co-treatment with Vitamin E did not. There were, however, no significant differences in the relative weight of rat's brain across the experimental groups as shown in Table 1.

Behavioural test assessments

Table 2 shows there was no significant difference between the control and other experimental groups in the number of squares crossed and forelimb grip strength. Rearing number was significantly ($p<0.05$) increased in the $HgCl_2$ alone group compared with the control. Only the $HgCl_2$ +VitE treatment elevated the number of grooms significantly ($p<0.05$) when compared with the control group**.**

Haematological parameters

Generally, there were no significant differences between the control and experimental groups as regards the red blood cell parameters (Tables 3) and the leukocytes (not displayed).

Biochemical analysis of antioxidant parameters

As shown in Table 4, $HgCl_2$ increased the MDA level significantly ($p<0.05$) when compared with control which co-treatment of $HgCl_2$ with AECA or VitE reduced relative to the $HgCl_2$ group. Additionally, $HgCl_2$ caused a reduction in the activity of SOD and level of GSH whereas it significantly elevated the activity of CAT compared with control. However, co-treatment of $HgCl_2$ with AECA or VitE reversed theses alterations relative to $HgCl_2$ group.

Table 5: Effect of mercuric chloride and *Celosia argentea* on viability of pyramidal cells of the external pyramidal layer of the cerebral cortex.

Groups	Mean number of non-viable pyramidal cells **/hpf**
CTRL	4.01±0.01
$HgCl_2$	185.00±7.70♯
AECA	5.01±0.30*
$HgCl_2$+AECA	39.00±0.60*
$HgCl_2$+VitE	25.00±0.56*

Values are expressed as mean ± SD of 5 rats. CTRL= Control, AECA = *Celosia argentea*, $HgCl_2$ = Mercuric chloride, VitE = Vitamin E, hpf = high power field. ♯P<0.05 compared to control group, *P<0.05 compared to $HgCl_2$ group.

Histological examination of cerebral and cerebellar cortices

Figure 1C demonstrated the varying degrees of degeneration observed in cortical neurons elicited by $HgCl_2$ treatment when compared with control. The ameliorative effect of AECA and VitE relative to $HgCl_2$ group was demonstrated in Figures 1D and E. In Figure 2B, most of the Purkinje neurons of the cerebellum were devoid of nuclei material (karyorhexis) after treatment with $HgCl_2$ when compared with the control group. However, co-treatment with AECA and VitE demonstrated some protection relative to the $HgCl_2$ group (Fig. 2D and E).

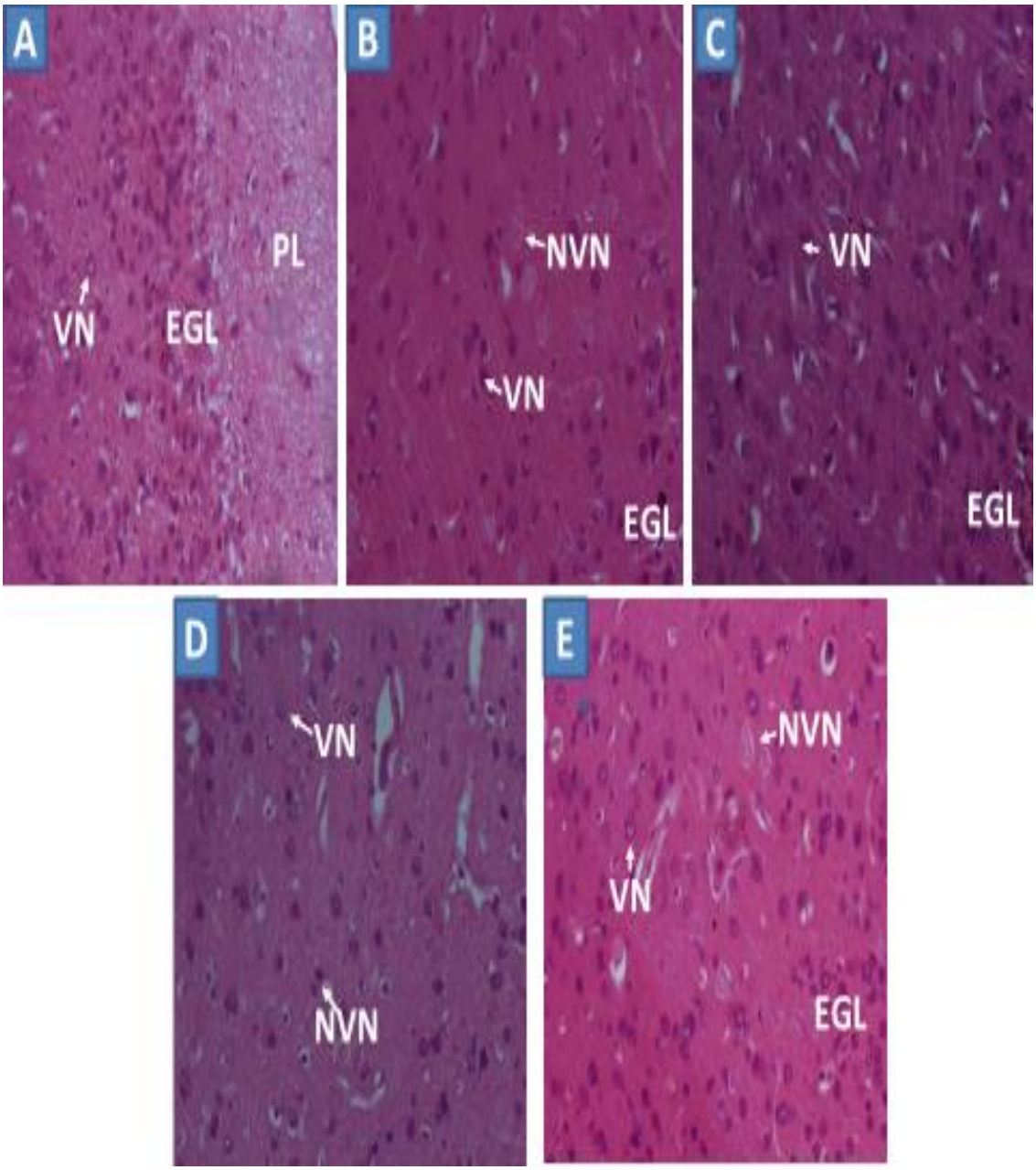

Fig. 1: **Representative photomicrograph of cerebral cortex.** (A) Control (B) $HgCl_2$ (C) AECA (D) $HgCl_2$+AECA (E) $HgCl_2$+VitE. PL = Plexiform layer, EGL = External granular layer, VN = Viable cortical neuron, NVN = non-viable cortical neuron. AECA = *Celosia argentea*, $HgCl_2$ = Mercuric chloride, VitE = Vitamin E. H&E x 400.

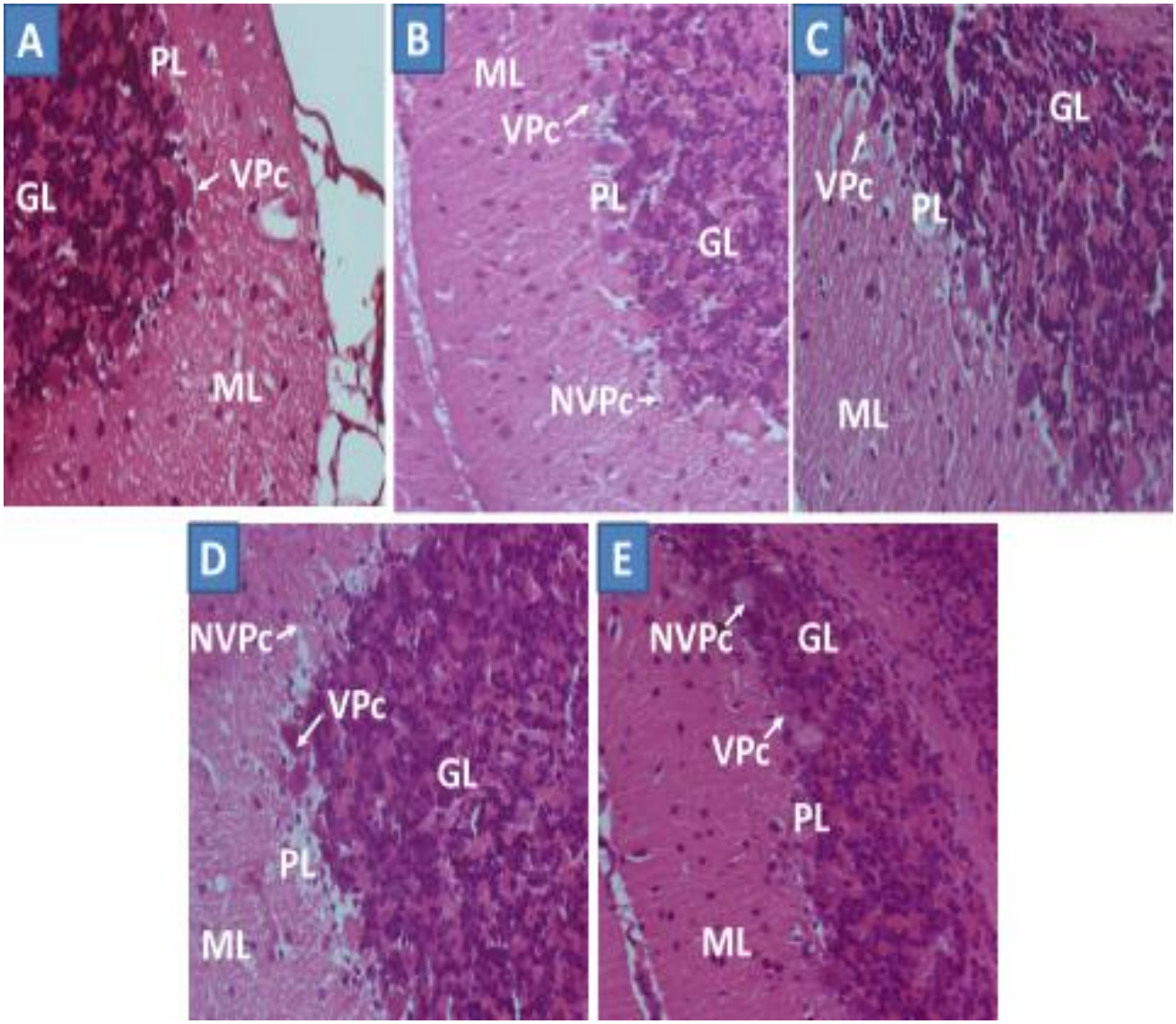

Fig. 2: **Representative photomicrograph of cerebellar cortex.** (A) Control (B) $HgCl_2$ (C) AECA (D) $HgCl_2$+AECA (E) $HgCl_2$+VitE. ML=Molecular layer, GL=Granular layer, PL= Purkinje layer, VPc=Viable Purkinje cell, NVPc=Non-Viable Purkinje cell. AECA = *Celosia argentea*, $HgCl_2$ = Mercuric chloride, Vit E = VitE. H&E x 400.

Histomorphometry evaluation of pyramidal neurons

Mercuric chloride increased the number of degenerated pyramidal neurons in the cerebral cortex significantly ($p<0.05$) when compared with the control (Table 5). This was reduced in the $HgCl_2$+AECA and $HgCl_2$+VitE treatment groups compared with the $HgCl_2$ thus demonstrating the ameliorative effect of AECA and VitE cotreatment with the toxicant as shown in Table 5.

DISCUSSION

In this present study, our data demonstrated the capability of the aqueous extract of *Celosia argentia* (AECA) and vitamin E to ameliorate the oxidative and histological alterations induced by mercuric chloride ($HgCl_2$) treatment of male rats.

Although body weight change serves as a sensitive indication of the general health status of an animal, gross changes in organ weight and weight coefficients (organ–body weight ratio) induced by chemical substances are a reliable marker of toxicity (Elias and Nelson, 2012). Therefore, the significant reduction of body weight in the $HgCl_2$-treated rats signifies its toxicity on the general wellbeing of the rats, whereas the lack of a significant increase in the absolute brain weight brain of rats across the groups might have indicated the absence of inflammation in the brain (Rossi et al, 2003). There was no significant alterations in the haematological parameters in agreement with previous studies (Owoeye and Farombi, 2015; Owoeye and Arinola, 2017).

Behavioural changes showed little effect of the different treatments on the motor abilities of the various groups although the results suggest some anxiety in the $HgCl_2$ +VitE treatment group as demonstrated in the increased number of grooming.

Our observation of increased lipid peroxidation demonstrated by increased thiobarbituric acid reactive

substances (TBARS) formation in rat brain treated with mercury alone $HgCl_2$ is supported by previous reports (Hussain *et al*., 1997; Ibegbu *et al.,* 2014). This was an indication of free radical generation induced by mercury toxicity as one of its mechanisms is overproduction of ROS (Abdel Moneim, 2015). The brain contains large amounts of polyunsaturated fatty acids and is particularly susceptible to free radical attack and, therefore, lipid peroxidation (Ebokaiwe, 2013).

The reduction of MDA in the $HgCl_2$+AECA group when compared with $HgCl_2$ group demonstrated the antioxidant capacity of *Celosia argentea Linn* extract which is in agreement with the report of Rukhsana *et al.* (2013). So also did the reduction of lipid peroxidation in the $HgCl_2$+VitE as indicated by lower levels of MDA when compared with $HgCl_2$ demonstrated the potency of the antioxidant capacity of vitamin E. While the decline in GSH levels in the $HgCl_2$ group is a reflection of the oxidative stress it induced (Vekaria, 2012), its elevation in the $HgCl_2$+AECA and $HgCl_2$+VitE groups relative to the $HgCl_2$ group demonstrated the potency of AECA and VitE to protect the brain against oxidative damage.

Superoxide dismutase is an important endogenous antioxidant that deals with superoxide radicals converting them to H_2O_2 (Chaudhary *et al*., 2003). Reports have shown that antioxidant enzyme activities might be reduced after Hg exposure *in vivo* (Vijayaprakash *et al*., 2013). However, the elevation of SOD in the $HgCl_2$+AECA and $HgCl_2$+VitE groups indicated the enhancement of the depressed activity of the enzyme by the antioxidant property of both AECA (Malomo et al., 2011) and VitE (Ulatowski et al., 2014). The elevation of the activity of CAT by $HgCl_2$ has the potential of increasing its ability to decompose H_2O_2 and convert it to water and diatomic oxygen. In AECA-treated rats there was an increase in the activity of the antioxidant enzyme SOD, and the level of GSH both of which play important roles in the detoxification of many environmental chemicals (Malomo et al., 2011). The decrease in the activity of SOD in $HgCl_2$-treated rats may be due to the enhanced lipid peroxidation or inactivation of the antioxidant enzymes (Ansar, 2015).

With the exception of the vertical movement (rearing), mercuric chloride did not alter behavioural results suggesting that AECA and VitE did not significantly influence the behavioural parameters studied in rats with induced mercury-toxicity in this study.

Histology results clearly demonstrated the neurotoxicity of $HgCl_2$ in both cerebral and cerebellar cortices by the degenerative features observed in the cerebral cortical neurons and the karyolitic and karyorhexic Purkinje neurons of cerebellum, which agrees with previous findings (Ferraro et al., 2009; Uma et al., 2012; Owoeye and Farombi, 2015; Owoeye and Arinola, 2017). The implication of damage to frontal cerebral cortical neurons is the attendant alteration of cortical functions like decision making, control of movement, cognition among others (Tranel, 2005). On the other hand, damage to Purkinje neurons has the consequence of reducing the cerebellar output to the deep cerebellar nuclear from where the major cerebellar output emerges to target organs like the spinal cord, vestibular nuclei, brainstem and the nucleus ventrolateralis of thalamus (Afifi and Bergman, 2005). Being the focal neuron of the cerebellar cortex on which all afferent fibres ultimately converges; such a damage done by $HgCl_2$ might affect the cerebellar coordinating activity of voluntary movement, posture and balance, as well as the coordination of the saccadic and slow eye movements with neck movements (Affi and Bergman, 2005). The recovery of the neurons of the $HgCl_2$+AECA and $HgCl_2$+VitE groups when compared with the $HgCl_2$ group suggested that antioxidant capacity of both AECA and VitE ameliorated the toxicity of effects of $HgCl_2$ qualitatively and quantitatively as shown in the histomorphometry results. This implies the possible sparing of these animals from the aforementioned consequences of mercury damage (Owoeye and Farombi, 2015) in both the frontal cortex and the cerebellum. Vitamin E appears to be more potent than AECA in their ameliorative and antioxidant activity as the latter preserved more viable cells compared with the latter. The protective effects of AECA have been attributed to the presence of compounds like flavonoids and phenolics (Malomo *et al*., 2011).

From our data, mercuric chloride demonstrated neurotoxicity in this experiment as shown by gross, biochemical and histological alterations observed. The antioxidant activity of AECA and VitE demonstrated protection against the gross, oxidative stress and neuronal damage induced by mercury toxicity in the rats.

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