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Research article

## Bromocriptine and Vitamin E were Protective Against Mercury-Induced Purkinje Neuron Injury in Male Wistar Rats

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### ABSTRACT

Mercuric chloride ( $HgCl_2$ ) is poisonous to both human and animals often causing systemic and nervous damage. The possible neuroprotective activity of bromocriptine (BRC), a dopamine agonist and alpha-tocopherol (TOCO) against mercuric chloride intoxication was investigated. Forty-two rats were randomized into six groups for this study. Group 1 rats served as control; Group 2, TOCO (500 mg/kg); Group 3, BRC (10mg/kg); Group 4,  $HgCl_2$  (4mg/kg); Group 5, BRC (10mg/kg) +  $HgCl_2$  (4mg/kg); Group 6, TOCO (500 mg/kg) +  $HgCl_2$  (4mg/kg).  $HgCl_2$  and TOCO were administered with oral cannula for 14 days while BRC was given i.p. On day 15, behavioural studies were conducted and rats were euthanized by cervical dislocation the next day. Skulls were carefully dissected open and brains carefully extracted, rinsed, blotted with filter paper, weighed and fixed in 10% formalin. The cerebellum was separated from the brain and processed for paraffin wax embedment and slides stained with Haematoxylin and Eosin. There was significant difference in body weight of rats of TOCO group when compared with control. Behavioural results showed that  $HgCl_2$  treatment significantly ( $p<0.05$ ) reduced the number of lines crossed, vertical movement (rearing) and forelimb grip when compared with the control. These parameters were significantly ( $p<0.05$ ) elevated by BRC+ $HgCl_2$  treatment demonstrating the ameliorative effect of BRC co-treatment with  $HgCl_2$ . The forelimb grip strength was elevated by BRC treatment while  $HgCl_2$  treatment elevated the duration recorded for negative geotaxis. Histological studies demonstrated degenerated Purkinje neurons in the cerebellum of  $HgCl_2$  -treated rats confirming neural damage. Mercuric chloride caused behavioural alterations in rats and was also toxic to rat Purkinje neurons. Both effects were mitigated by bromocriptine and alpha-tocopherol.

**Keywords:** Bromocriptine, alpha-tocopherol, mercuric chloride, Purkinje neurons, neuroprotection

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### INTRODUCTION

Mercury in elemental form is a silver-colored metal that exists as a thick liquid at room temperature, familiar to most people as the silvery liquid inside mercury thermometers. Mercury is a naturally-occurring metal that is used in man-made products and processes, and is also emitted into air from industrial sources. Human exposure to mercury occurs from a variety of sources, e.g., breathing mercury-containing air, using commercial products that contain mercury, and consumption of fish and seafood containing methyl mercury (Wong and Lye, 2008). Three types of mercury exists namely: elemental mercury, found in thermometers, fluorescent bulbs, dental amalgam fillings, and other sources; organic mercury, predominantly methylmercury, found in foods such as fish, and ethyl mercury found in some vaccine preservatives and

some antiseptics; non-elemental forms of inorganic mercury, found primarily in batteries, some disinfectants, and some health remedies and creams (Chang *et al.*, 2015). Mercury is a ubiquitous contaminant generated by human activity and natural environmental change. Mercuric chloride is a white powder that is soluble in water (Otto *et al.*, 1994) whose human exposure may occur following ingestion of items containing inorganic mercury, such as some batteries; or use of some homeopathic remedies or skin bleaching creams (Litovitz *et al.*, 2002). Mercuric chloride may exhibit chemical toxicity since it can damage a tissue, organ or organism (Matsumara and Ananthaswamy, 2004). Its exposure can be toxic to the kidney, stomach, and intestines and also may be embryo-toxic increasing rates of miscarriage and stillbirths, and still may increase blood pressure and is neurotoxic sometimes presenting neuropsychiatric symptoms (Gale, 1981; Khan *et*

al., 2004; Chang *et al.*, 2015). There has been an increasing occurrence of mercury poisoning in Nigeria due to gold mining activities (Idowu *et al.*, 2013).

The toxicity of mercuric chloride in animals has been attributed (among other causes) to oxidative stress due to generation of excess of free radicals or reactive oxygen species (ROS) in the living system. Although ROS can be beneficial when used by the immune system as a tool to attack and kill pathogens (Segal, 2005) and short term oxidative stress may be important in prevention of aging by induction of hormesis (Gems and Patridge, 2008), oxidative stress will occur when oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them as this may lead to lipid peroxidation, oxidative DNA damage, production of peroxides and free radicals that can damage cell components including proteins, lipids and disruptions in normal mechanism of cellular signaling (Yoshikawa, 1998). However, antioxidants e.g. natural products or synthetic compounds like bromocriptine or vitamin E, have been reported to be useful in mitigating the oxidative damaged induced by mercuric chloride intoxication.

Bromocriptine (trade names Parlodel, Bromergan), an ergot derivative of ergoline and also an amide derivative of the d isomer of lysergic acid is a white crystalline almost odorless powder (Gilman *et al.*, 2001). Bromocriptine is a dopamine agonist that exerts its actions at striatal D1 and D2 adenylyl cyclase-linked dopamine receptors (Lieberman, 1985). It inhibits prolactin secretion and inhibits glutamate release by reversing the glutamate GLT1 transporter (Missale *et al.*, 1998; Mah *et al.*, 2002). It is used in the treatment of Parkinson's disease and has also been found valuable in the treatment of a number of endocrinology and gynecologic disorders (Sherwal *et al.*, 2010). It induces behavioral changes like motor hyperactivity in animals and hormonal changes that could last several hours following a single systemic dose (Johnson *et al.*, 1974). Bromocriptine's antioxidant properties demonstrated neuroprotective effects in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced toxicity and reversed glutathione and dopamine depletion (Mulakrishnan and Mohanakumar, 1998).

Vitamin E is a fat-soluble vitamin and one of the most effective liposoluble antioxidant which protects against lipid peroxidation (Waghmode, 2012) *in vivo*. Known also as alpha-tocopherol, vitamin E, is found in almonds, oils including wheat germ, safflower, corn, soybean oils, mangoes, nuts, broccoli, and other foods (Herrera, 2001).

The cerebellum is the largest part of the hindbrain and overlies a substantial portion of the posterior surface of the pons and medulla oblongata. The important motor functions of the cerebellum include: the control of posture and balance while standing and walking, coordination of eye movements with movements of the head and coordination of skilled movements (Glickstein *et al.*, 2005 Morton *et al.*, 2007; Goodlett, 2008). However, all of these important cerebellar functions can be deranged by the chemical toxicity induced by mercuric chloride, making it imperative to continue the search for substances that could prevent or mitigate this potential situation.

Considering these important functions of the cerebellum and our earlier studies that have shown that mercury damage

of cerebellum was mitigated by antioxidant-containing plant products, this study was designed to investigate the possible neuroprotective effect of bromocriptine on mercuric chloride toxicity employing vitamin E as a standard antioxidant. Based on the above, we here asked whether bromocriptine can modulate mercuric chloride toxicity in Purkinje cells of rat cerebellum.

## MATERIALS AND METHODS

**Experimental animals:** A total of 42 male Wistar rats weighing between 120 to 140 g were purchased from Olu Research animals farm Ibadan and acclimatized for 2 weeks in the Animal House of the College of Health Sciences, Bowen University, Iwo, Nigeria in a 12 hour light/dark cycle. They were thereafter randomly divided into 6 groups and kept in netted wooden cages having dimensions 43cm × 40cm × 29cm and soft wood shavings employed as bedding and were given standard rat chow and were watered *ad libitum*. Animal experiments were carried out according to acceptable guidelines on the ethical use of animals in research (Public Health Service, 1996).

**Preparation and administration of mercuric chloride solution:** Dry powder of Mercury Chloride ( $HgCl_2$ , 99% purity) manufactured by Loba Chemie PVT Ltd, Mumbai, 40005, India, was purchased from Julimark Enterprises, Yemetu, Ibadan, Nigeria from which 100 mg was measured. To this, 20 mL of distilled water was added and the mixture stirred thoroughly with a glass rod and then kept in a specimen bottle for administration. From the stock solution  $HgCl_2$  was administered to the each experimental animal as 4 mg/kg orally body weight based on the method of Sheikh *et al.* (2013).

**Preparation and administration of Bromocriptine:** Bromocriptine 2.5mg tablets (30 in a bottle) with batch number EP9471 and expiry date of 08/2017 were purchased from Twin's venture Pharmaceutical Store, Iwo, Nigeria which was manufactured by Lek pharmaceutical and chemical company in Slovenia. The tablets were crushed to very fine powder after which the powder was carefully poured into a 100 mL beaker. Distilled water (50 mL) was added to it after being diluted in 8% methanol, the mixture was mixed thoroughly with a stirring rod and then kept in a specimen bottle for administration to rats. Bromocriptine was given as 10 mg/kg intraperitoneally based on the method of Mulakrishnan and Mohanakumar (1998).

**Preparation and administration of alpha-tocopherol (vitamin E):** Alpha-tocopherol capsules 100 mg were purchased from Twin's Venture Pharmaceutical store in Iwo, Nigeria. The batch number was GI50466 with an expiry date was 06/2018. Each soft gelatin capsule containing 100 mg of DL- $\alpha$ -tocopherol acetate as 100 mg vitamin E acetate (Gujarat liqui Pharmacaps Pvt. Ltd country India) was punctured with a new size 21G needle (Hypojet, Spain) attached to a new 1 ml hypothermic syringe (Becton Dickinson, La Portde Clair, France). The oily formulation of vitamin E was then neatly

and completely aspirated out with the syringe. Each aspirate measured approximately 0.2 ml containing 100 mg of DL- $\alpha$ -tocopherol. The insulin syringe was thereafter attached to a clean intra-gastric gavage through which each rat was administered orally the measured dose of 500 mg/kg/daily for 14 days based on the report of Viana *et al.* (2003).

**Research Design:** Treatment grouping was as follows:

Group 1 (n = 6): Control group given rat chow and water

Group 2 (n = 6): TOCO = Alpha-tocopherol alone for 14 days

Group 3 (n = 6): BRC = Bromocriptine alone for 14 days

Group 4 (n = 8): HgCl<sub>2</sub> = Mercuric chloride alone for 14 days

Group 5 (n = 8): BRC (10mg/kg) + HgCl<sub>2</sub> (4mg/kg)

Group 6 (n = 8): TOCO (500 mg/kg) + HgCl<sub>2</sub> (4mg/kg)

All treatments lasted 14 days..

**Behavioural tests:** On the day 15, the animals were subjected to three forms of behavioural tests: Open field test, geotaxis test and forelimb grip test according to the methods described by Adebiyi *et al.* (2016).

**Open field test:** A wide box approximately 120 cm by 120 cm with an open roof was used. The box had lines drawn horizontally and vertically from end forming square grids. The animal was placed in the centre square quadrant and then left free to move around. The parameters checked for included frequency of grooming, rearing, line crosses, and droppings of faeces. The animal was subject to this test for a period of 5 minutes, after which the box was cleaned with 70% alcohol and dried before introduction of the next animal.

**Negative Geotaxis test:** A wooden slab was inclined at about 45° to a hard vertical surface and then the animal was placed on the slab in a head down position and its movement down the slab monitored, and latency to turn 180° to a head up position observed and recorded. This duration for each animal was 2 minutes. The mean of three (3) trials was recorded.

**Forelimb grip test:** The ends of a metal wire about 1 metre long was placed on two stools and heavy weights were placed in turn on the ends the space between the stools on the floor had a soft cushioning surface should the animal lose its grip and fall down. The animal was then gently placed on the metal wire in such a way as to grasp it with on the forelimbs supporting the body weight. It suspended itself in that position until it either fell down or used its hind limbs to support the weight of its body. Each animal was given three trials with a 30 min inter-trial rest interval (Shabani *et al.*, 2012).

**Sacrifice, tissue harvesting and histological procedures:**

On day 16 of the experiment, animals in all the groups were weighed and euthanized by cervical dislocation. Each animal was then decapitated and the skull opened and the brain carefully removed, rinsed in normal saline, mopped with a filter paper and then weighed. The cerebellum from each rat was careful dissected out and homologous sampling was assured by obtaining transverse sections of the right cerebellum from each specimen from the lateral zone portions

of the cerebellar hemisphere for uniformity. The tissues were sectioned at 5-6  $\mu$ m thickness and then stained with haematoxylin and eosin according to the method of Bancroft and Gamble (2008) to assess Purkinje cell damage in the cerebellum. Slides were viewed with an Olympus CH (Japan) light microscope while images were captured with a Sony DSC-W 3 digital camera (Japan).

**Histomorphometry:** Quantification of density of Purkinje neurons using a microscope with a graticule at different magnifications was done according to reported methods (Ebokaiwe *et al.*, 2013). Briefly, the micrometer was calibrated using a stage micrometer slide with a customized 2 mm ruler engraved on the cover slip (Leitz, Wetzlar, Germany). This was done by using the eyepiece of an Olympus CH (Japan) binocular microscope at  $\times 40$  magnification. The radius of the eye piece at  $\times 40$  was calibrated with the graticule to be 0.19 mm, and the area of the view at  $\times 40$  magnifications was thus estimated as 0.11 mm<sup>2</sup>. The densities of the cells on the histological slides were determined by counting the number of viable neurons (defined as those with blue hue, visible nucleus and excluded pyknotic neurons) observed within a given square area in a section (Zhen and Dore, 2007). The densities were also quantified using measured squares of the OpenOffice.org Draw (Apache Open OfficeTM3) and values compared with those from graticule measurements. The widest diameter of Purkinje neurons were made using the "dimension line" component of the Open Office TM3 software. Measurements were made on each slide section from all experimental and control groups at high-power fields. The means of each of the densities and diameters were calculated and compared by two investigators who independently quantified using the graticule and OpenOffice software methods.

**Statistical analysis**

Data were expressed as means  $\pm$  standard deviation. Data were analyzed using one way analysis of variance (ANOVA). Student's t test using Microsoft Excel application of the Microsoft office package 2011 and GraphPad Prism 5, were used to carry out the analyses. Statistical significance was set at P<0.05.

## RESULTS

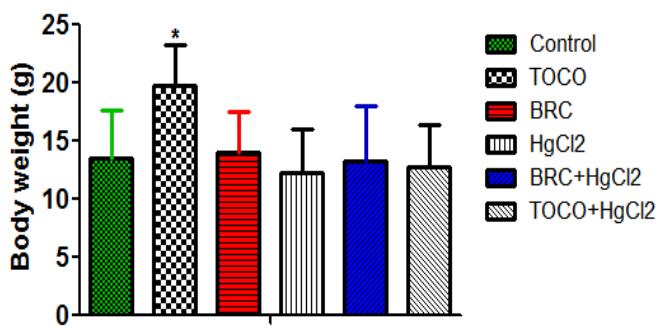
**Body and brain weight changes:** The body weight of rats in TOCO group was significantly (p<0.05) higher than that of control, whereas other alterations were insignificant as shown in Figure 1. The relative brain weight changes were insignificant as reflected in Figure 2.

**Behavioural test evaluation:** The alterations in the behavioural parameters are shown in Table 1. Mercuric chloride treatment significantly (p<0.05) reduced the number of lines crossed, vertical movement (rearing) and forelimb grip when compared with control. All of these parameters excluding grooming were significantly (p<0.05) elevated by BRC+HgCl<sub>2</sub> demonstrating the ameliorative effect of BRC co-treatment with HgCl<sub>2</sub>. However, co-treatment of TOCO with HgCl<sub>2</sub> was unable to reverse the toxicity of HgCl<sub>2</sub> on these

same parameters as shown in Table 1. The forelimb grip was elevated by BRC treatment while HgCl<sub>2</sub> treatment significantly ( $p<0.05$ ) increased the duration recorded for negative geotaxis.

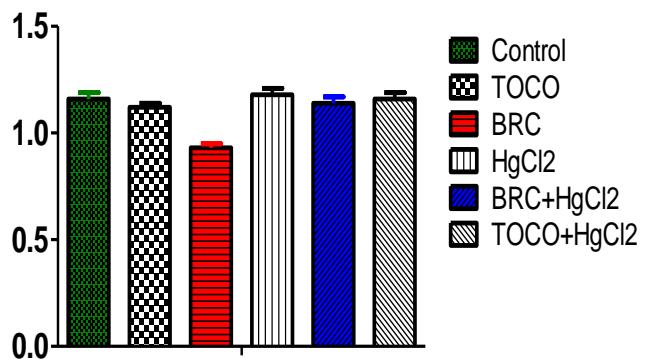
**Histological examination of cerebellar tissue:** Figure 3 demonstrates the normal layers of the cerebellar cortex of these groups of animals and the alterations observed in the Purkinje neurons. In the control, the molecular layer (M) and granular layer (G) show normal cytoarchitecture. Purkinje neurons (blue arrows) are noted to be healthy with plumb soma, nucleic material and even some exhibiting nucleoli as in Figs. 3A-3C. Note that Purkinje neurons show evidence of HgCl<sub>2</sub> toxicity in Fig. 3D, few are healthy (blue arrow) while majority were devoid of nuclei material and appeared shrunken (black arrowhead) when compared with the control and other HgCl<sub>2</sub>-cotreated groups.

**Morphological evaluation of cerebellar tissue:** The widest diameter and density of Purkinje neurons were significantly ( $p<0.05$ ) reduced by HgCl<sub>2</sub> when compared with the control. However, BRC and TOCO co-treatment mitigated this effect significantly by elevating these values as shown in Table 2.



**Fig. 1:**

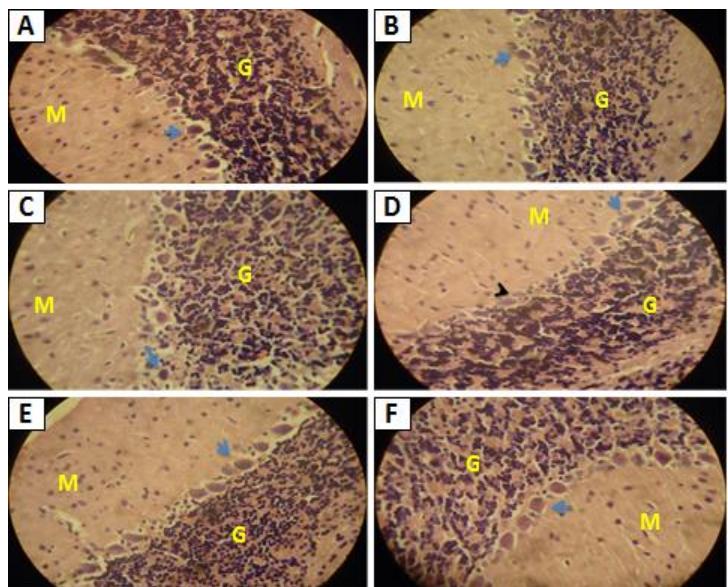
**Mean of body weight changes of rats at end of experiment.**  
Values are presented as means  $\pm$  standard deviation of 5 rats. TOCO-Alpha-tocopherol, BRC-Bromocriptine, HgCl<sub>2</sub>-Mercuric chloride.  
\*P<0.05 against Control.



**Fig. 2:**

**Brain/body weight ratio of rats.**

Values are presented as means  $\pm$  standard deviation of 5 rats.  
TOCO-Alpha-tocopherol, BRC-Bromocriptine, HgCl<sub>2</sub>-Mercuric chloride.



**Fig. 3:**

**Representative photomicrograph of cerebellar cortex.**

(A) Control; (B) TOCO; (C) BRC; (D) HgCl<sub>2</sub>; (E) BRC+HgCl<sub>2</sub>; (F) TOCO+HgCl<sub>2</sub>. M-Molecular layer, G-Granular layer, Blue arrow-Viable Purkinje neuron, Black arrowhead-degenerated Purkinje neuron, BRC-Bromocriptine, TOCO-Alpha tocopherol, HgCl<sub>2</sub>-Mercuric chloride. H&E-stained sections. Final magnifications: 768x.

**Table 1:**

Effect of treatments on behavioural studies

Groups	Lines crossed	Rearing	Grooming	Forelimb grip (s)	Negative geotaxis (s)
Control	30.83 $\pm$ 10.06	9.50 $\pm$ 5.43	2.0 $\pm$ 2.61	16.50 $\pm$ 5.79	2.72 $\pm$ 1.26
TOCO	17.17 $\pm$ 10.84*	5.83 $\pm$ 5.23	1.0 $\pm$ 1.55	10.35 $\pm$ 4.17	3.98 $\pm$ 1.83
BRC	19.80 $\pm$ 5.54*	4.50 $\pm$ 2.89	2.33 $\pm$ 2.94	19.20 $\pm$ 12.29	3.24 $\pm$ 1.96
HgCl <sub>2</sub>	19.0 $\pm$ 13.08*	6.83 $\pm$ 5.46*	2.0 $\pm$ 1.27	9.80 $\pm$ 5.20*	6.92 $\pm$ 2.96*
BRC+HgCl <sub>2</sub>	29.33 $\pm$ 9.64**	8.33 $\pm$ 2.46**	3.5 $\pm$ 1.64	12.80 $\pm$ 9.78**	2.01 $\pm$ 1.11**
TOCO+HgCl <sub>2</sub>	11.83 $\pm$ 7.31**	2.2 $\pm$ 1.30	1.33 $\pm$ 1.41	15.03 $\pm$ 8.3**	1.95 $\pm$ 1.44**

Values are presented as means  $\pm$  standard deviation mean of 5 rats. TOCO-Alpha-tocopherol, BRC-Bromocriptine, HgCl<sub>2</sub>-Mercuric chloride.  
\*P<0.05 against Control; \*\*P<0.05 against HgCl<sub>2</sub>

**Table 2:**

Effect of treatments on parameters of Purkinje neurons in cerebellar cortices.

Groups	Widest diameter Purkinje neurons ( $\mu\text{m}$ )	Density of viable Purkinje neurons (/ $0.11\text{mm}^2$ )
Control	9.39 $\pm$ 0.78	13.8 $\pm$ 0.75
TOCO	8.62 $\pm$ 0.52	12.8 $\pm$ 0.75
BCR	8.9 $\pm$ 0.83	12.8 $\pm$ 1.12
HgCl <sub>2</sub>	4.91 $\pm$ 1.41*	6.0 $\pm$ 1.41*
BCR+HgCl <sub>2</sub>	9.07 $\pm$ 0.56**	13.4 $\pm$ 0.49**
TOCO+HgCl <sub>2</sub>	9.04 $\pm$ 0.86**	13.2 $\pm$ 0.75**

Values are presented as means  $\pm$  standard deviation mean of 5 rats per group. TOCO-Alpha-tocopherol, BRC-Bromocriptine, HgCl<sub>2</sub>-Mercuric chloride. \*P<0.05 against Control; \*\*P<0.05 against HgCl<sub>2</sub>.

## DISCUSSION

In the present study, we presented data demonstrating the ability of bromocriptine (BCR) and alpha-tocopherol (vitamin E) in mitigating some of the behavioural and micro-anatomical alterations induced by mercuric chloride (HgCl<sub>2</sub>) treatment in male rats.

Body weight change serves as a sensitive indication of the general health status of an animal (Adebiyi *et al.*, 2016), however, weight loss can occur because of inadequacy of nutritious diet or presence of disease processes, changes in metabolism, hormonal changes, medications or other treatments. Changes in organ weight and weight coefficients (organ-body weight ratio) induced by chemical substances have been shown to be a reliable marker of toxicity (Elias & Nelson, 2012). There was no noticeable alteration observed in the feeding pattern of rats in the toxicant (HgCl<sub>2</sub>) group or of other rats yet the group treated with alpha-tocopherol (TOCO) alone showed a significant increase in body weight as shown in Figure 1 although the alterations in the relative brain weight were insignificant as Figure 2 shows, which at best suggest a state of wellbeing.

Behavioural studies are used to evaluate the emotional state and locomotor activity of an animal (Adebiyi *et al.*, 2016). The open field model examines anxiety related behaviour characterized by the normal aversion of the animal to an open area. Thus animals removed from their acclimatized cage and placed in a new environment express anxiety and fear by showing alteration in all or some parameters (Adebiyi *et al.*, 2016). While the frequency of lines crossed is an indication of the central nervous system stimulation or depression and the rearing (vertical movement) is an index of locomotor activity, the length of time a rat was able to hold on to the hanging wire is an indirect assessment of muscle coordination and strength. From the results, the significant reduction of the number of lines crossed, vertical movement (rearing) and forelimb grip by HgCl<sub>2</sub> treatment significantly demonstrated its toxicity previously reported (Owoeye and Farombi, 2015). This reduction implied HgCl<sub>2</sub>

treatment caused a reduction in the locomotor activity as well as the coordination and strength of muscles in these rats suggesting early fatigue of their forearm muscles. This indicated the possibility of problems with motor coordination which is chiefly controlled by the cortical motor association areas and the cerebellum. The significant elevation of number of lines crossed, vertical movement (rearing) and non-significant increase in grooming observed in the BRC+HgCl<sub>2</sub> group demonstrated the ameliorative effect of BRC co-treatment with HgCl<sub>2</sub> agrees with the report of Onaolapo and Onaolapo (2014) who reported similar findings. Additionally, we recorded a significant increase in the forelimb grip strength in the same group. Both findings suggested that BRC demonstrated the capability to improve the locomotor and exploratory activities as well as the coordination/ muscles strength, thus preventing early muscle fatigue in the affected rats. TOCO was more potent in reversing the effect of mercury on the forelimb grip test and also recorded a shorter time for the rats to re-orientate themselves in the negative geotaxis test as shown in Table 1. Hence TOCO was more effective in restoring the muscle strength and coordination and reorientation of the affected rats. Negative geotaxis refers to an orienting response and movement expressed in opposition to cues of a gravitational vector hence negative geotaxis is considered diagnostic of vestibular and proprioceptive function (Motz *et al.*, 2005). The toxicant group (HgCl<sub>2</sub>) took the longest duration to reorient after being set free on the inclined plane which may suggest loss of proprioceptive and vestibular function (Motz *et al.*, 2005).

The observed damage of the Purkinje neurons in the cerebellum of rats in the (HgCl<sub>2</sub>) treated rats shown in Figure 3 and Table 2 demonstrated its neurotoxicity which is in agreement with previous findings (Ibegbu *et al.*, 2014; Owoeye and Farombi, 2015). The damage to these neurons may be due to the fact that mercury attacks nuclear DNA (Uma *et al.*, 2012) leading to inability to synthesize new neuronal proteins for transport to axon terminals. Thus there may be reduction in the effectiveness of the Purkinje cells who are the focal neurons of the cerebellum and upon which the excitatory (glutamatergic) parallel fibres which are axons of granule cells, the climbing fibres and the glutamatergic inputs from the mossy fibre pathway (Acsadyet *et al.*, 1998) ultimately converge and from which inhibitory efferent cerebellar (GABAergic) inputs proceed to the deep cerebellar nuclei namely: the dentate, the interpositus and the fastigial. It is from these deep nuclei that most of the output fibres of cerebellum originate (Snell, 2006). Damage of Purkinje cells evidenced by reduction in its density and diameter as in Table 2 might consequently affect movement coordination, sensing, learning and other cerebellum-dependent cognitive function (Bastian, 2011). The cerebellum is functionally divided into parts that define its motor functions, the vestibulo-cerebellum portion which controls posture and balance while standing and walking (Morton *et al.*, 2007) as well as coordinating eye movements with movements of the head via the vestibular nuclei that control extra ocular muscles. The neo-cerebellum portion is concerned with planning, programming and

coordination of skilled movements (Glickstein *et al.*, 2005), thus facilitating the ability to progress smoothly from one movement to the next in orderly succession, while the spinocerebellum/vestibulo-cerebellum portion is responsible for maintaining balance (Goodlett, 2008). The chemical injury from mercuric toxicity to the cerebellum in this experiment would therefore affect any or all of these important functions. As presented in Figure 3, BRC and TOCO either alone or as co-treatment with HgCl<sub>2</sub> demonstrated the ability to maintain the cellular integrity of the Purkinje neurons. Alpha-tocopherol is particularly required to maintain the integrity of Purkinje neurons (Ulatowski *et al.*, 2014).

All of the alterations observed in this experiment might have been due to the oxidative damage induced by the toxicity of HgCl<sub>2</sub> since it has been specifically established that HgCl<sub>2</sub> induced the elevation of lipid peroxidation and reduced glutathione levels in rat brain thus establishing a state of oxidative stress (Vanithasri and Jagadeesan, 2013; Ibegbu *et al.*, 2014; Ansar, 2015). It has however been reported that oxidative stress can be ameliorated by an effective liposoluble antioxidant like alpha-tocopherol (Herrera, 2001; Owoeye *et al.*, 2011; Waghmode, 2012) or bromocriptine (Mulakrishnan and Mohanakumar, 1998) which were demonstrated by the findings of this experiment. Additionally, bromocriptine's enhancement of the locomotor activity was probably due to a complex involvement of both noradrenaline and dopamine pre-and postsynaptic neurons, possibly due to its partial agonist action or as a result of its metabolite according to the report of Onaolapo and Onaolapo (2014).

In conclusion, mercuric chloride caused significant behavioural alteration in this study which was also histologically demonstrated as mercuric chloride-induced Purkinje neuron lesion in the cerebellum. These adverse effects were mitigated by treatment with bromocriptine and alpha-tocopherol whose antioxidant effects on the parameters studied supported their antioxidant roles in neuroprotection thus reducing the toxic effects of mercury on the cerebellum and its movement-coordinating function. A further study applying immunohistochemical analysis of cerebellar Purkinje neurons using a specific biomarker e.g. Calbindin D28k might yield further information on the reaction of these neurons to both HgCl<sub>2</sub> and BRC.

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