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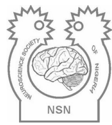

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Ameliorative Effect of Ascorbic Acid (Vitamin C) on Mercury Induced Temporal Lobe Damage in Rats

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ABSTRACT

Mercury is a heavy metal of known toxicity, noted for inducing public health disasters in Minamata Bay, Japan and in Iraq. The present study investigated the effect of ascorbic acid on the histology of mercury induced temporal lobe damage in Wistar rats. Twenty five adult Wistar rats (average weight 185 g) were randomly divided into five groups (each n = 5); a control group administered normal saline, mercuric chloride ($HgCl_2$; 49.8 mg/kg), $HgCl_2$ with distilled water, $HgCl_2$ with low dose ascorbic acid (595 mg/kg) and $HgCl_2$ with high dose ascorbic acid (1,190 mg/kg), groups, orally administered daily for three weeks. Results revealed alteration of temporal lobe histoarchitecture; neuronal degeneration, such as necrosis, clumping of cells, neuronal vacuolation, cytoplasmic shrinkage and reduction in the number of cells ($p \leq 0.05$) in $HgCl_2$ intoxicated group. The administration of ascorbic acid remarkably ameliorated $HgCl_2$ induced temporal lobe damage, notably with ascorbic acid 1,190 mg/kg treatment suggesting that ascorbic acid has neuroprotective potentials against $HgCl_2$ induced temporal lobe damage in Wistar rats.

Key words: *Temporal lobe, Histology, Mercuric Chloride, Ascorbic acid, Wistar rats*

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 air, water and food chain as a result of anthropogenic activities, mining of mercury, gold and metals including copper, zinc, lead and silver (Ghosh and Sil 2008; Burger et al. 2011). Many populations worldwide have been exposed to doses of mercury through the consumption of fishes and sea foods, and some have experienced neurotoxic effects (Valey et al. 1980; WHO 2003). Some population have experienced subsequent neurotoxic effects, and since the epidemic of mercury poisoning from contaminated fish consumption in Minamata, Japan in the late 1950s, mercury has been one of the most documented examples of bio-accumulation of toxins in the environment, particularly in the aquatic food chain (ATSDR 2011). The toxicity of mercury can also result from vapour inhalation and ingestion or absorption through the skin. However, diagnosis of mercury toxicity can be challenging because the commonly used modalities (blood, urine and hair levels) do not always correlate with total body burden and offer little diagnostically useful information. There are currently no consensus criteria for the diagnosis of mercury overload, nor for overload of other toxic metals (Bernhoft 2012). There is a growing appreciation of the effects that exposure to mercury has on the nervous system, because mercury crosses the

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blood brain barrier and accumulates in brain tissues to induce pathologies (Farina et al. 2011). Animal studies linking mercury toxicity to neurodegenerative diseases (Fujimura et al. 2009) raise clinical concern, as do a series of associations between mercury and neurodegenerative diseases in humans (Johnson and Atchison 2009; Mutter et al. 2010). Mercury occurs in several chemical forms, with complex pharmacokinetics; being capable of inducing a wide range of clinical presentations with nervous, digestive and renal systems being most affected (Burger et al. 2011; Bernhoft 2012). A 1997 population study conducted in Faroe Islands demonstrated that children born to mothers who consumed mercury-contaminated whale meat during pregnancy, exhibited cognitive delays and irregular cardiovascular development (Grandjean et. al. 1997; Booth and Zella 2005). Hence, children and pregnant women are vulnerable to mercury exposure (WHO 2003; EC 2005). In Nigeria, tilapia fish from Lagos lagoon and the use of "Kohl" a traditional cosmetic had been reported to predispose people to mercury toxicity (Fodeke 1979; Onyeike et al. 2002). Symptoms of mercury poisoning involves depression, headache, dizziness, itching, burning, shedding of the skin, irritability, excitability, restlessness, irrational outburst of temper, profuse sweating, tachycardia, frequent urination, increased salivation, and hypertension (Grant and Lipman 2009; ATDRS 2011).

Ascorbic acid is an essential nutrient for humans and some other animal species. In living organisms ascorbate acts as an antioxidant by protecting the body against oxidative stress (Padayatty et al. 2003; Ibegbu et al. 2014). It is also a cofactor in at least eight enzymatic reactions including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy (Jacob 1999). Antioxidants have been shown to react with superoxide (Nishikimi 1975; WHO 2005), hydroxyl radicals (McGregor and Biesalski 2006) and singlet oxygen (Moreira et al. 2010).

Antioxidants are substances that nullify the effect of free radical by either inhibiting the initial production or inhibiting the preparative phase of free radicals (Sujatha et al. 2011). Examples of antioxidants are ascorbic acid (vitamin C), vitamin E and vitamin A (Vasudevan and Sreekumari 2007). These anti-oxides are generally regarded as primary first-line protective agent that nullifies free radicals by donating a single electron to yield dehydro-ascorbic acid (UKFSA 2007; Gemma et al. 2010). Vitamin C is an antioxidant that scavenges free radicals (Padayatty et al. 2003), prevents scurvy (WHO 2001), pneumonia (Hemila and Louhiala 2007) and maybe useful in lowering the incidence of gout (Choi et al. 2009). The study seeks to evaluate the ameliorative effects of ascorbic acid on mercury induced temporal lobe damage in rats.

MATERIALS AND METHODS

Twenty five adult Wistar rats of average weight 185 g were used for this study. After acclimatization in the Animal House of the Department of Human Anatomy, Ahmadu Bello University, Zaria, the animals were grouped into five groups of five animals each (n = 5). Mercuric chloride (X-N202, May and Bakers, England) was utilized at LD_{50} of 166 mg/kg body weight as adopted from ATSDR (2011). While the LD_{50} of ascorbic acid (S42238, Sam Pharmaceuticals, Nigeria) was adopted from MSDS (2008) as 11,900 mg/kg body weight. The mercury chloride was the approved laboratory grade chemical by Standard Organization of Nigeria, marketed and sold in Nigeria, while the ascorbic acid tablets was approved by National Agency for Food and Drug Administration and Control to be marketed and used in Nigeria. Before the commencement of the study, ethical approval was sort and obtained from the Ahmadu Bello University Zaria Ethical and Animal Use Committee, Faculty of Veterinary Medicine with reference Number ABU/FVM/EAUC/2015/12.

The animals were dosed as follows: control group was administered with normal saline, group 2 with 30% mercuric chloride ($HgCl_2$, 49.8 mg/kg) only, group 3 received $HgCl_2$ with distilled water only, group 4 received $HgCl_2$ with 5% low dose ascorbic acid (595 mg/kg), while group 5 received $HgCl_2$ with 10% high dose ascorbic acid (1,190 mg/kg). However, administrations of distilled water and ascorbic acid from weeks 3-6 were done in order to observe for any possible natural recovery and possible ameliorative potentials of ascorbic acid respectively (Table 1). The administration was by oral route daily and lasted for 3-6 weeks, while animal feed and water were allowed *ad libitum*.

Table 1: Animal grouping and duration of administration of mercuric chloride and ascorbic acid

Groups (n=5)	Dosage/kg body weight	Treatment Duration (Weeks)
1	Distilled water (Control)	1 – 3
2	49.8mg/kg of mercuric chloride	1 – 3
3	49.8mg/kg of mercuric chloride	1 – 3
	Distilled water	3 – 6
4	49.8mg/kg of mercuric chloride	1– 3
	595mg/kg of ascorbic acid	3 – 6
5	49.8mg/kg of mercuric chloride	1– 3
	1,190mg/kg of ascorbic acid	3 – 6

After the administration, the animals were anaesthetized by inhalation of chloroform in the sacrificing chamber. Incision was made through the skin and muscle of the skull. The skull was opened through a mid sagittal incision with the aid of a brain

opener, while brain tissue was removed and fixed in Bouin's fluid. The tissues were routinely processed for paraffin embedded histology and stained using Cresyl violet staining method.

Tissue Processing Procedure

The fixed tissues were removed from the Bouin's fluid and dehydrated using ascending grades of alcohol. This method involved dehydration of tissues in two (2) changes of 70% alcohol and two (2) changes of 90% alcohol, three (3) changes of 95% alcohol and three (3) changes of absolute alcohol, each of which lasted for 30 minutes. The dehydrated tissues were further cleared in two (2) changes of chloroform for two (2) hours each. The cleared tissues were infiltrated by immersion into molten paraffin wax. The embedded tissues were blocked in rectangular blocks, while tissues were sectioned coronally using the rotary microtome at 5 μm per section. The tissue sections were allowed to float in water bath at 30 °C to help the spreading of the paraffin ribbons. The clean slides were used to pick the tissues from the warm water bath. The slides were left to dry and later stained using Cresyl violet solution.

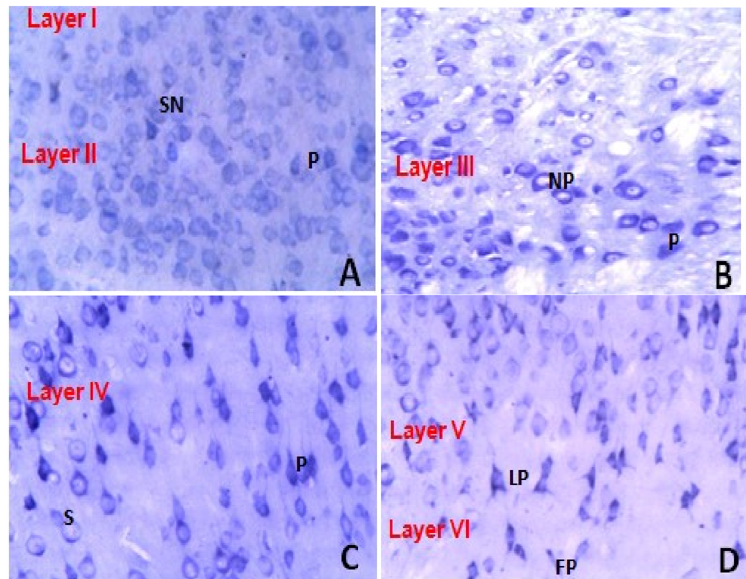

Figure 1: Photomicrographs showing the cellular layers of the temporal cortex (Cresyl Violet × 250)
A: Layer I contains few scattered neurons (SN) and extensions of pyramidal neurons (P), and Layer II with small pyramidal neurons (P). B: Layer III contains small and medium size pyramidal neurons (P) as well as non-pyramidal neurons (NP). C: Layer IV contains different types of stellate (S), and pyramidal neurons (P). D: Layer V contains large pyramidal neurons (LP), and Layer VI contains few but large pyramidal neurons (FP).

Cresyl violet Staining Method

The tissue sections were deparaffinized and hydrated to distilled water and then stained for 5 minutes in Cresyl violet solution. The stained sections were rinsed in two changes of distilled water and placed in 95% alcohol for 30 seconds. Sections were transferred to absolute alcohol for 30 seconds and then placed in xylene for 1 minute and 2 minutes sequentially. Differentiations were made in absolute alcohol, two changes for 10 and 30 seconds each. The sections were then taken through several changes of xylene and mounted with synthetic resin. Digital photomicrographs were made from all the experimental groups with the aid of MD900 Amscope microscope digital camera.

Cell Count Analysis

Pyramidal cells involving the temporal lobe was counted using Digimizer image analysis software. Photomicrographs involving the

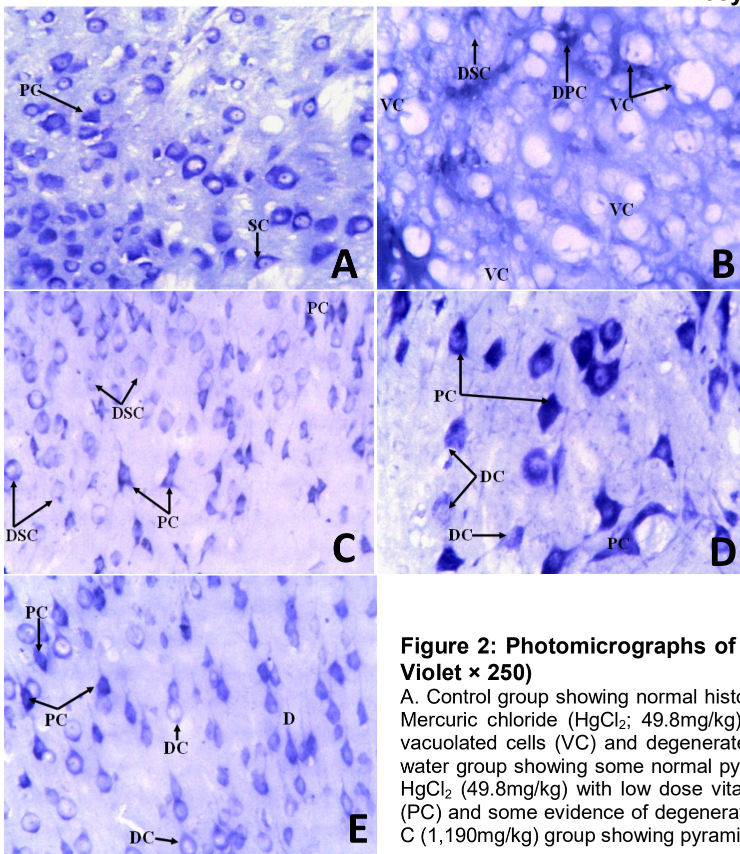

Figure 2: Photomicrographs of the cellular layers of the temporal cortex (Cresyl Violet × 250)
A. Control group showing normal histoarchitecture with pyramidal (PC) and stellate cells (SC). B. Mercuric chloride ($HgCl_2$; 49.8mg/kg) only group showing degenerated pyramidal cells (DPC), vacuolated cells (VC) and degenerated stellate cells (DSC). C. $HgCl_2$ (49.8mg/kg) with distilled water group showing some normal pyramidal cells (PC) and degenerated stellate cells (DSC). D. $HgCl_2$ (49.8mg/kg) with low dose vitamin C (595 mg/kg) group showing normal pyramidal cells (PC) and some evidence of degenerated cells (DC). E. $HgCl_2$ (49.8mg/kg) with high dose vitamin C (1,190mg/kg) group showing pyramidal cells (PC) and some evidence of degenerating cells.

cortical cell layers and hippocampal cells of the temporal lobe region were uploaded into the image area interphase of the software. This was followed by the utilization of marker tools to mark and count cells in the aforementioned regions. The numbers of the counted cells were automatically indicated on the statistics area of the software, while results obtained were further subjected to statistical analysis.

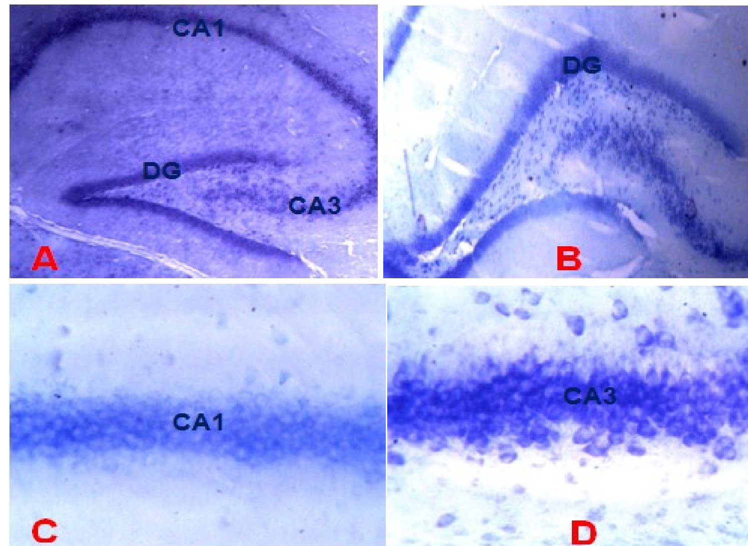

Figure 3: Photomicrographs of the hippocampus of the control group
Architecture of: A. hippocampus (×40); B. Dentate gyrus; DG (×100); C. CA1 region (×250); and D. CA3 region (×250). Cresyl violet, (×250).

Statistical Analysis

All the results were analyzed using the Statistical Package for Social Scientist (SPSS version 20) and the results were expressed as Mean ± Standard Error of Mean (SEM). The statistical significance between means were analyzed using one-way analysis of variance followed by post-hoc test; Tukey's multiple comparison test was utilized to test for significant difference between control and experimental groups. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Physical Observation of the Animals

On physical observation of the animals, the control group animals were very active and behaviourally stable while mercury treated animals were observed to be ataxic, apathetic, agitated, distressed, with diarrhea for the first 3 weeks of administration. However, there were improvements in activity, agility, and behavioural stability as observed in animals treated with ascorbic acid in the last 3 weeks of administration. .

Histological Observations of Temporal Cortex

The results revealed normal histoarchitecture and cellular layers of the temporal cortex in the control group (Fig. 1 and 2A), while animals in the $HgCl_2$ (49.8 mg/kg) only and $HgCl_2$ with distilled water groups revealed necrosis of cells, area of cellular degeneration characterized by vacuolation, congestion, clumping of cells (Fig. 2B and C). However, $HgCl_2$ with low dose vitamin C (595 mg/kg) and $HgCl_2$ with high dose vitamin C (1,190 mg/kg) groups showed minimal cellular degeneration with some normal cerebral cortical cells (Fig. 2D and E).

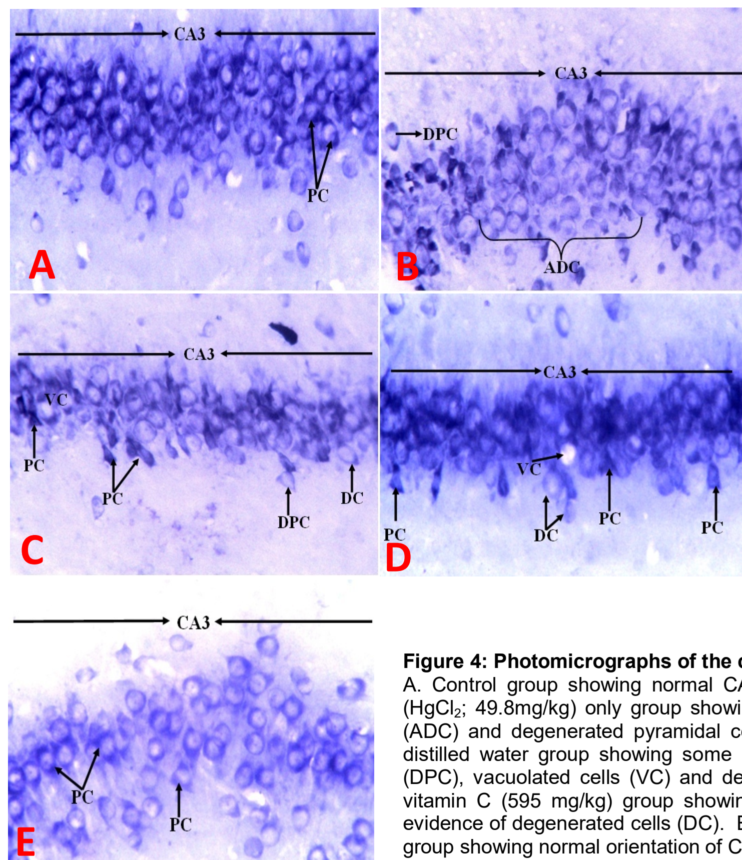

Figure 4: Photomicrographs of the cellular layers of the hippocampus (Cresyl Violet × 250)
A. Control group showing normal CA3 region with pyramidal cells (PC). B. Mercuric chloride ($HgCl_2$; 49.8mg/kg) only group showing disorientation of CA3 region, area of degenerating cell (ADC) and degenerated pyramidal cells (DPC) with loss of nuclei. C. $HgCl_2$ (49.8mg/kg) with distilled water group showing some normal pyramidal cells (PC), degenerated pyramidal cells (DPC), vacuolated cells (VC) and degenerated cell (DC). D. $HgCl_2$ (49.8mg/kg) with low dose vitamin C (595 mg/kg) group showing normal pyramidal cells (PC), vacuolated cell (VC) and evidence of degenerated cells (DC). E. $HgCl_2$ (49.8mg/kg) with high dose vitamin C (1,190mg/kg) group showing normal orientation of CA3 region, pyramidal cell (PC) with intact nuclei

Histological Observations of the Hippocampus
The results revealed normal histoarchitecture of the hippocampus in the control group (Fig. 3 and 4A), while animals in the $HgCl_2$ (49.8 mg/kg) only and $HgCl_2$ with distilled water groups revealed area of cellular degeneration characterized by loss of nuclei, vacuolation of cells and degenerated pyramidal cells (Fig. 4B and C). However, $HgCl_2$ with low dose vitamin C (595 mg/kg) and $HgCl_2$ with high dose vitamin C (1,190 mg/kg) groups showed minimal cellular degeneration with some normal pyramidal cells (Fig. 4D and E).

Cell Count Analysis
Number of Pyramidal cells in the Temporal cortex
The number of pyramidal cells was significantly ($p \le 0.05$) decreased in the temporal cortex of the $HgCl_2$ (49.8mg/kg) only group compared to the control, $HgCl_2$ (49.8 mg/kg) with low dose vitamin C (595 mg/kg) and $HgCl_2$ (49.8 mg/kg) with high dose vitamin C (1,190 mg/kg) groups. This significant decrease ($p \le 0.05$) in the pyramidal cell was also observed in $HgCl_2$ (49.8mg/kg) with distilled water group compared to the control and the $HgCl_2$ (49.8mg/kg) with high dose vitamin C (1,190 mg/kg) groups. However, significant decrease ($p \le 0.05$) in the number of pyramidal cells involving the temporal cortex was observed in $HgCl_2$ (49.8 mg/kg) with low dose vitamin C (595 mg/kg) compared to the control group (Table 2).

Table 2: Number of pyramidal cells counted from temporal lobe sections

Groups	Administration	Hippocampus (Pyramidal cell)	Temporal Cortex (Pyramidal cell)
		Mean ± SEM (n)	Mean ± SEM (n)
GI	Control	30.33 ± 0.88	30.00 ± 3.05
GII	($HgCl_2$ 1st-3rd Weeks)	7.33 ± 0.33*	4.00 ± 1.15*
GIII	($HgCl_2$ and Distilled H_2O)	10.33 ± 1.45*	12.33 ± 1.45*
GIV	($HgCl_2$ and Vit.$C_{595mg/kg}$)	20.67 ± 1.20*[cd]	18.33 ± 2.03*[c]
GV	($HgCl_2$ and Vit.$C_{1,190mg/kg}$)	26.00 ± 2.08*[ab]	26.67 ± 2.73*[ab]

*$p \le 0.05$, significantly different compared to Control; a, significantly different between mercuric chloride ($HgCl_2$) with high dose Vit. C (Vitamin C) group and $HgCl_2$ only group; b significantly different between $HgCl_2$ with high dose Vitamin C group and $HgCl_2$ with distilled water group. c significantly different between $HgCl_2$ with low dose; Vit. C group and $HgCl_2$ only group; d significantly different between $HgCl_2$ with low dose. Vit. C group and $HgCl_2$ with distilled water group; n= number of cells counted. SEM: Standard Error of Mean.

Number of Pyramidal cells in the Hippocampus
The pyramidal cell number was significantly ($p \le 0.05$) decreased in the CA3 region of the hippocampus of the $HgCl_2$ (49.8 mg/kg) only and $HgCl_2$ (49.8 mg/kg) with distilled groups compared to the control, $HgCl_2$ (49.8 mg/kg) with low dose vitamin C (595 mg/kg) and $HgCl_2$ (49.8 mg/kg) with high dose vitamin C (1,190 mg/kg) groups. This decrease ($p \le 0.05$) in the pyramidal cell involving the CA3 region of the hippocampus was also observed in the $HgCl_2$ (49.8mg/kg) with low dose vitamin C (595 mg/kg) group compared to the control group (Table 2).

DISCUSSION

Mercury is a toxic heavy metal which is widely dispersed in nature and it is known to have direct and indirect effects on biological systems and cells (Bjornberg et al. 2011). The present study revealed degeneration and necrosis of cells in the temporal lobe in adult Wistar rats administered with $HgCl_2$ with or without ascorbic acid, while the control group showed normal histology. These neurodegenerative changes could invariably affect learning, memory and hearing associated with the hippocampus and temporal lobe (Wolf et al. 2009). In addition, degeneration and decrease in the number of pyramidal cells which result from exposure to $HgCl_2$ implies the activity of hippocampus in memory formation and learning will be impaired and the role of hippocampus that involved storage and retrieval of information could also be lost. These results are in agreement with the study of Wolf et al. (2009), Ibegbu et al. (2013) and Mahmoud et al. (2017), who reported clumping of cerebral cortical cells, necrosis of cells in animals following administration of different doses of mercury for a period of three weeks. The findings from the present study also agree with other researchers who reported that heavy metals such as mercury, lead, cadmium and other organic compounds have the capacity to impair the nervous system (Ibegbu et al. 2014; Animoku et al. 2015; 2016) because these metals can cross the blood-brain barrier to accumulate in brain tissues (Farina et al. 2011).

The brain uptake of mercury in rats is enhanced from the blood to the central nervous system across the blood-brain barrier by the L-type neutral amino acid carrier transport system (Aschner and Clarkson 1987). Glutamate dyshomeostasis in the central nervous system represents another critical target in mercury induced neurotoxicity (Aschner et al. 2007). Cerebral dysfunction may occur in association with exposure to a wide variety of toxins including heavy metals such as mercury, lead, thallium, manganese, drugs and solvents, while permanent abnormalities are induced only by sustained use and exposure to these chemicals in large quantity (Jomova et al. 2010; Farina et al. 2011). Since, it has been shown that heavy metals such as mercury, lead and thallium have the potential to induce oxidative

stress via reduction of antioxidative enzymes such as superoxide dismutase, catalase, glutathione and proliferation of lipid peroxidation levels. These decrease in the activity of antioxidative enzymes such as superoxide dismutase level and the elevation of lipid peroxidation, suggest the formation of free radicals and the participation of free radical induced oxidative cell injury in mediating the toxic effect of mercury (Jomova et al. 2010). Neurodegenerative disorders in cerebral cortex can affect the functional areas of the cerebral cortex which deal with skill movement, ability to speak, appreciate pains and temperature (Mahmoud et al 2017). However, the present study revealed that animals administered with ascorbic acid both at low and high doses showed significant improvements ($p<0.05$) when compared with animals intoxicated with mercury alone and this agrees to the fact that ascorbic acid can improve the reduced superoxide dismutase, catalase, glutathione and increased Lipid peroxidase levels (Farina et al., 2013) caused by mercury exposure to the brain (Ibegbu et al. 2014; Animoku et al. 2016). Ascorbic acid administration has ameliorated to certain degree the induced impairments to the temporal lobe as a result of exposure to $HgCl_2$ and this agrees to the fact that natural compounds rich in antioxidants can reduce oxidative stress thus alleviating the effect of oxidative agents (Burger et al. 2011; Ibegbu et al. 2013). Hence, ascorbic acid as an antioxidant plays significant ameliorative role ($p \leq 0.05$) in the reversion (to certain level) of mercury induced temporal lobe impairment possibly by forming inert complexes and inhibiting their toxicity (Burger et al. 2011; Ibegbu et al. 2014).

CONCLUSION

The findings from the present study justify the ameliorative effect of ascorbic acid against mercury induced temporal lobe neurotoxicity and hence populations exposed to mercury poison should consume foods rich in ascorbic acid (vitamin C) along with other antioxidants.

Conflict of Interest
None declared.

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