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HISTOMORPHOLOGICAL EFFECT OF ASCORBIC ACID ON MERCURY EXPOSURE ON TEMPORAL LOBE OF THE CEREBRAL CORTEX OF ADULT WISTAR RATS

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ABSTRACT

Aim: Histomorphological effect of ascorbic acid on mercury exposure on temporal lobe of the cerebral cortex of Adult Wistar rats was investigated.

Methods: Twenty five adult Wistar rats of average weight of 185 grams were randomly divided into five groups of five rats per group namely; a control group administered with normal saline, a mercuric chloride only group ($HgCl_2$; 49.8mg/kg), $HgCl_2$ with distilled water group, $HgCl_2$ with low dose Vitamin C group (595mg/kg) and, $HgCl_2$ with high dose Vitamin C (1,190mg/kg) group. The animals were orally administered with concentrations of mercuric chloride and ascorbic acid daily for three weeks each.

Results: Histological result showed normal architecture in the control Group while histological changes involving necrosis, clumping of cells, reduction in the number of cells and vacuolation of cells were observed in experimental Groups.

Conclusion: Ascorbic acid administration has been shown to ameliorate induced degenerative changes in the temporal lobe of the cerebral cortex caused by mercury exposure in Adult Wistar rats.

Key words: Mercuric Chloride, Temporal lobe, Ascorbic acid

INTRODUCTION

Mercury is one of the most hazardous environmental contaminants to living organisms and the central nervous system has been shown to be the main target (WHO, 2005). Human and animal populations are exposed to this environmental contaminant via daily interaction with their environment. These interactions with the environment occur through food, air and water (Burger et al., 2011). Mercury is present in the environment owing to natural and anthropogenic processes such as degassing from earth crust, emissions from volcanoes, evaporation from water bodies, residential heating systems and waste incinerators (WHO, 2005). Mercury can also be present as a result of mining of mercury, gold and other metals such as Copper, Zinc, Lead and Silver (Cox, 1997; Burger et al., 2011). There is a growing appreciation of the effects that exposure to heavy metals such as mercury may have on the nervous system, this is because some of these metals can cross the blood brain barrier and accumulate in brain tissues thus causing damage to these tissues (Volko et al., 2005; Farina et al., 2011). Toxicity of mercury can result from vapor inhalation and ingestion or absorption through the skin. Nervous, digestive and renal systems are most commonly affected in mercury exposure while children and pregnant women are most vulnerable to mercury exposure (WHO, 2003; EC, 2005). In Nigeria, Sates like Katsina, Sokoto, Gombe were characterized with exposure to mercury due to increase use of Kohl; a traditional cosmetic which had been reported to predispose people to mercury toxicity (Onyeike, 2002). Some of the symptoms of mercury poisoning include depression, headache and dizziness, itching, burning, pain, fingertips and toes swelling, 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). Vitamin C or 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 (Sies et al., 1992; 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, 2004), hydroxyl radicals (McGregor and Biesalski, 2006) and singlet oxygen (Moreira et al., 2010). 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).

MATERIALS AND METHODS

Experimental Animals

Twenty five (25) Adult Wistar rats of average weight 185g were used for this study. They were acclimatized for three weeks and kept in the Animal house of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria. After acclimatization, the animals were divided into five groups of five animals each.

Experimental Chemicals

Mercuric chloride manufactured by May and Bakers limited, Dagenham England with batch number X-N202 was used for this study. The LD_{50} of Mercuric Chloride was adopted from ATSDR, (2011) as 166mg/kg body weight. 30% (49.8mg/kg) of the LD_{50} per kg body weight of mercuric chloride was used in this study. Ascorbic acid manufactured by Sam Pharmaceuticals Limited, Ilorin, Kwara State, Nigeria with batch number S42238 was used for this study. The LD_{50} of Ascorbic acid was adopted from MSDS, (2008) as 11,900mg/kg body weight. 5% (595mg/kg) and 10% (1,190mg/kg) of the LD_{50} per kg body weight of ascorbic acid was used in this study.

Experimental Procedure

Twenty five rats were grouped into five groups of five animals each as GI-GV. Group I (Control) received normal saline, Groups II and III were administered with 49.8mg/kg body weight of mercuric chloride, 49.8mg/kg body weight of mercuric chloride and distilled water respectively. While Groups IV and V received 49.8mg/kg of mercuric chloride and 5% of the LD_{50} of ascorbic acid (595mg/kg) and 49.8mg/kg of mercuric chloride and 10% of the LD_{50} of ascorbic acid (1,190mg/kg) respectively (Table 1). The administration was by oral route daily and lasted for 3-6 weeks while animal feed and drinking water were allowed ad libitum.

Table 1: Animal grouping, number of rats, treatment and duration of administration of mercuric chloride and ascorbic acid

Groups	Treatment	Duration (Dosage/kg body weight)
GI	Distilled water (Control)	1st – 42nd day
GII	49.8mg/kg of mercuric chloride	1st – 21st day
GIII	49.8mg/kg of mercuric chloride	1st – 21st day
	and	
	Distilled water	22nd – 42nd day
GIV	49.8mg/kg of mercuric chloride	1st – 21st day
	and	
	595mg/kg of ascorbic acid	22nd – 42nd day
GV	49.8mg/kg of mercuric chloride	1st – 21st day
	and	
	1,190mg/kg of ascorbic acid	22nd – 42nd day

Animal Sacrifice

After the administration, the animals were weighed and 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 and brain tissue was removed and fixed in Bouin's fluid. The tissues were routinely processed and stained using haematoxylin and eosin (H&E) method.

Cell Count Analysis

Pyramidal cells of cerebral cortex involving the temporal lobe region was counted using Digimizer image analysis software. Photomicrographs were uploaded into the image area of the software, while marker tool was used to mark and count the cells.

Statistical Analysis

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

RESULTS

Histological Observations

The results of histological observation showed normal architecture of the temporal lobe region with cerebral cortical cells and layers in Control Group as shown in Plate I while Groups II and III showed necrosis of cells, area of degeneration of cells characterized by vacuolation and clumping of cells as shown in Plates II and III respectively. Groups IV and V showed evidence of degenerated cells with some normal cerebral cortical cells as shown in Plates IV and V respectively.

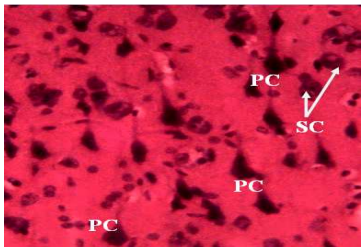

Plate I: Temporal lobe of Group I (Control). Normal cytoarchitecture with Pyramidal cells (PC) and Stellate cells (SC) (H&E × 250).

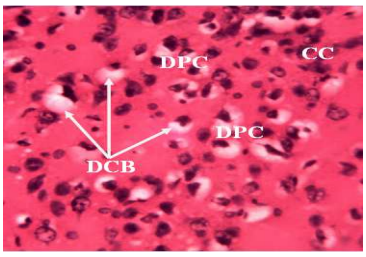

Plate II: Temporal lobe of Group II. Degenerated Pyramidal cells (DPC) with vacuolated degenerated cell bodies (DCB) and Clumping of cells (CC) (H&E × 250).

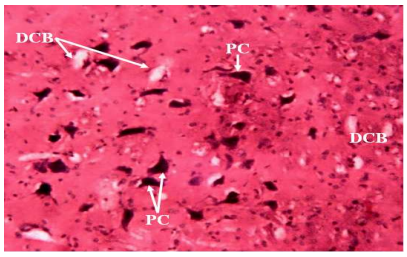

Plate III: Temporal lobe of Group III. Degenerated Pyramidal cells with vacuolated cell bodies (DCB) and some normal Pyramidal cells (PC) (H&E × 250).

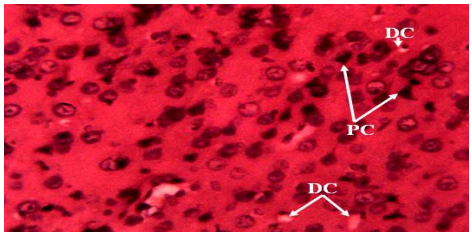

Plate IV: Temporal lobe of Group IV. Normal Pyramidal cells (PC) and some evidence of degenerated cells (DC) (H&E × 250).

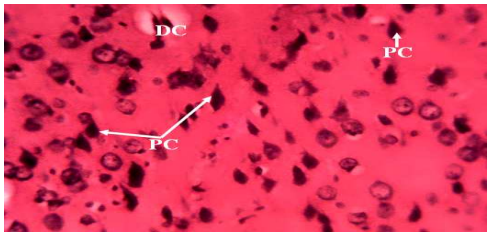

Plate V: Temporal lobe of Group V. Normal Pyramidal cells (PC) and some evidence of degenerating cells (DC) (H&E × 250).

Table: 2: Number of pyramidal cells counted from temporal lobe sections of the cerebral cortex of Adult Wistar rats.

Groups	Administration	Temporal lobe (Pyramidal cell)
		Mean ± SEM (n)
GI	Control	30.00 ± 3.05
GII	($HgCl_2$ 1st-3rd Weeks)	4.00 ± 1.15*
GIII	($HgCl_2$ and Distilled H_2O)	12.33 ± 1.45*
GIV	($HgCl_2$ and Vit.$C_{595mg/kg}$)	18.33 ± 2.03*^{c}
GV	($HgCl_2$ and Vit.$C_{1,190mg/kg}$)	26.67 ± 2.73*^{ab}

*p<0.05 indicates significant difference compared to Group I (Control). *^{a} indicates significant difference between Group V and Group II. *^{b} indicates significant difference between Group V and Group III. *^{c} indicates significant difference between Group IV and Group II. n= number of cells counted. SEM: Standard Error of Mean. $HgCl_2$: Mercuric Chloride. Vit. C: Vitamin C.

CELL COUNT ANALYSIS

The result of cells counted showed significant decreased (p<0.05) in the number of pyramidal cells counted in the temporal lobe of the cerebral cortex in Groups II when compared to Groups I, IV and V. This significant decrease (p<0.05) in the pyramidal cell was also observed in Group III when compared to Groups I and V. However, significant decrease (p<0.05) in the number of Pyramidal cells of the temporal lobe was observed in Groups IV when compared to Group I (Control) as shown in Table 2.

DISCUSSION

Mercury is a common environmental and occupational toxic heavy metal, which is known to have direct and indirect effects on biological systems and cells (Bjomberg et al., 2011). Results from the present study revealed neuronal changes involving clumping of cerebral cortical cells, vacuolation of cells, necrosis of cells, loss of cerebral cells and reduction in number of Pyramidal cells counted in adult Wistar rats exposed to mercury. These neurodegenerative changes could invariably affect learning, memory and hearing abilities associated with the temporal lobe of the cerebral cortex. This study agrees with the study of Ibegbu et al., (2013) 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 damage the nervous system (Ibegbu et al., 2014; Animoku et al., 2015). Since 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 (LAT) 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 and Valko, 2011; 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 SOD, CAT, GLU 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). However, the present study revealed that administration of ascorbic acid both at low and high dose showed significant improvement ($p<0.05$) in the temporal lobe of the cerebral cortex of animals when compared with animals exposed to mercury alone and this agrees to the fact that ascorbic acid can improve the reduced SOD, CAT, GLU and the increased Lipid peroxidase (LPO) levels (Farina et al 2013) caused by mercury exposure to the brain (Ibegbu et al., 2014; Animoku et al., 2015). Ascorbic acid administration has reduced the damage done to the temporal lobe of the cerebral cortex and this agrees to the fact that natural compounds that are rich in antioxidants can help to 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 role in the reversion of the toxicity of mercury by forming inert complexes and inhibiting their toxicity (Burger et al., 2011; Ibegbu et al., 2014).

CONCLUSION

It has been shown that exposure to mercury can induce degeneration in the temporal lobe of the cerebral cortex of adult Wistar rats. However, ascorbic acid has shown some level of protection against the neurotoxicity induced by mercuric chloride. Hence, people exposed to mercury poison should consume food rich in ascorbic acid along with other antioxidants.

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