African Journal of Cellular Pathology 1:23-29 (2013)
The Official Journal of the Society for Cellular Pathology Scientists of Nigeria
www.ajcpath.com

EFFECT OF ASCORBIC ACID ON MERCURIC CHLORIDE-INDUCED CHANGES ON THE CEREBRAL CORTEX OF WISTAR RATS

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ABSTRACT

Aim: The present work was aimed at evaluating the effect of ascorbic acid on mercuric chloride induced changes on the cerebrum of Wistar rats.
Methods: Thirty Wistar rats of average weight of 200g were divided into 6 groups of 5 rats each. In addition to normal diet, the animals in Group 1 were given distilled water, Groups 2 and 3 were administered 52mg/kg and 26.25mg/kg of mercuric chloride (HgCl) respectively while Groups 4 and 5 were administered 52mg/kg of HgCl and 5mg/kg of ascorbic acid and 26.25gm/kg of HgCl and 5mg/kg of ascorbic acid respectively. Group 6 was administered 5mg/kg of ascorbic acid through oral route, daily for 3 weeks.
Results: Oxidative stress assay showed a significant decrease ($P<0.05$) in the mean levels of catalase, superoxide dismutase and glutathione reductase in Groups 2, 3 and 4 when compared with the Control while lipid peroxidase showed a significant increase ($P<0.05$) in Groups 2 and 3. Histological observation of the cerebrum showed a normal architecture in Groups 1 and 6 while, Groups 2, 3, 4 and 5 showed degenerative changes, necrosis and clumping of cells.
Conclusion: Ascorbic acid administration has been shown to ameliorate induced degenerative changes in the cerebrum caused by mercuric chloride toxicity in Wistar rats.

Key words: Mercuric chloride, Cerebral cortex, Ascorbic acid, Oxidative stress.

INTRODUCTION

Human and animal populations interact with their environment on a daily basis and as such are exposed to a range of chemicals and heavy metals (Burger et al., 2011). These interactions with the environment occur through food, air and water (Burger et al., 2011). Mercury occurs in the environment owing to natural processes such as degassing from earth crust, emissions from volcanoes, evaporation from water bodies and anthropogenic processes from coal-fires, power stations, residential heating systems and waste incinerators (Cox, 1997; Burger and Gochfeld, 2011). Mercury can be present as a result of mining of mercury, gold, copper, zinc, lead and silver (ATSDAR, 1999). There is a growing appreciation of the effects that exposure to heavy metals such as mercury may have on the body and, in particular the brain and nervous system. This is because some of these metals can cross the blood brain barrier and accumulate in the brain and cause damage (Langford and Ferner, 1999; Valko et al., 2005). The ancient Greek used mercury in ointments

while the ancient Egyptians and Romans used it in cosmetics but in China, mercury was thought to prolong life, heal fractures and maintain general good health (Clarkson, 1989; Brian and Fred, 1995). There are many reported cases of mercury food poisoning in Sweden, Mexico, USA and the Minamata Bay incidence that led to the poisoning of over 800 people. 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 (De Bont et al., 1986; European Commission, 2005). Tilapia fishes from Lagos Lagoon are characterized with relatively high level of mercury concentration and this can be attributed to industrial effluents into the Lagoon (Fodeke, 1979). Kakulu and Osibanjo (1986), found the level of mercury in fishes from Niger Delta area of Nigeria to be less than 10mg/kg - 40g. In Nigeria, not much work has been done to investigate the relative bio-accumulation potential of mercury on local aquatic species (Oyewo, 1998). Some of the symptoms of mercury poisoning include irritability, excitability, restlessness, irrational outburst of temper, depression, headache and dizziness amongst others (Grant and Lipman, 2009). Other symptoms of mercury poisoning include itching, burning, pain, fingertips and toes swelling, and shedding of the skin. A person suffering from mercury poisoning may experience profuse sweating, tachycardia, increased salivation and hypertension (Lucky, 1987; Langford and Ferner, 1999). Affected children may show redness of cheeks, nose and lips, loss of hair, teeth, and nails, transient rashes, muscle weakness, kidney dysfunction, memory impairment and insomnia (Horowitz et al., 2002; Liuji et al., 2002). Ascorbic acid is an anti-oxidative substance that may protect the cells against the effects of free radicals. Free radicals are molecules produced when the body breaks down food or by exposure to tobacco smoke and radiation (Chihuailaf et al., 2002). Ascorbic acid is a natural antioxidant that prevents the production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues (Padayatty et al., 2003; WHO, 2003). Anti-oxides 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 regarded as a first-line protective agent that nullifies free radicals by donating a single electron to yield dehydro-ascorbic acid (Valko et al., 2005; UKFSA, 2007; Gemma et al., 2010). The aim of the present study was to evaluate the effect of ascorbic acid on mercuric chloride-induced changes on the cerebral cortex of Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Thirty adult Wistar rats of average weight of 200g 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 rats were divided into six groups of five rats per group.

Experimental Chemicals

Twenty grams of mercuric chloride manufactured by May and Bakers Chemical Laboratory Limited Dagenham England while Vitamin C tablets were manufactured by Jopan Pharmaceuticals Ltd.

Animal Experimentation

Thirty Wistar rats were divided into 6 Groups of 5 animals each. Group 1 (Control) was administered with distilled water, Group 2 was given 52.5mg/kg body weight of mercury chloride (Hg), Group 3 animals were administered with 26.25mg/kg body weight of HgCl. Animals in Group 4 were given 52.5mg/kg body weight of HgCl and 5mg/kg body weight of ascorbic acid, while Group 5 animals were administered with 26.25mg/kg body weight of HgCl and 5mg/kg body weight of ascorbic acid and Group 6 rats were administered with 5mg/kg body weight of ascorbic acid only. The animals were administered with mercuric chloride solution daily by oral route, for 3 weeks and were fed with animal feed and water was allowed ad bilitum at room temperature.

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 the mid

sagittal incision and the cerebrum was removed and fixed in Bouin's fluid. The tissues were routinely processed and stained using routine haematoxylin and eosin method and with crystal violet.

Estimation of Oxidative Parameters
Determination of Catalase Activity
Catalase activity was determined using the method described by Sinha, (1972) and the absorbance was read at 570 nm. Standard curve was made by plotting the absorbance obtained at various levels of the assay. Catalase activity was obtained from the graph of the standard curve.

Determination of Superoxide Dismutase Activity
Superoxide Dismutase (SOD) activity was determined by a method described by Fridovich, (1989). Absorbance was measured every 30 seconds up to 150 seconds at 480 nm from where the SOD activity was calculated.

Assessment of Lipid Peroxidation
Lipid peroxidation as evidenced by the formation of TBARS was measured by the method of Niehaus and Samuelson (1968). The absorbance of the pink supernatant was measured against a reference blank using a spectrophotometer at 535nm.

Assay of Reduced Glutathione Concentration
Reduced glutathione (GSH) concentration measurement was done according to the method of Ellman (1959) as described by Rajagopalan et al., (2004), and the absorbance was read at 412 nm.

Statistical Analysis
All data were presented as mean ± SD. For establishing significant differences, data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey post hoc test. Values were considered statistically significant when P value was less than or equal to 0.05 ($p \leq 0.05$).

RESULTS

Physical Observation of the Animals
The result of physical observation of the animals showed that rats in Group 1 were very active while Group 2 animals showed less activity, gnawing and restlessness with watery feces. The animals in Group 3 showed gnawing and restlessness characterized by watery feces while, Group 4 animals exhibited restlessness. The result of physical observation showed little gnawing in Group 5 animals with more activity when compared to Group 2 and Group 4 while Group 6 animals showed no changes in their physical activity as in Group 1.

Oxidative Parameters
The result of the analysis of oxidative stress markers namely catalase, SOD, glutathione reductase and lipid peroxidation following administration of mercury chloride and ascorbic acid showed increase and decrease in some parameters as shown in Table 1. The result showed a significant decrease ($P<0.05$) in catalase, SOD and glutathione reductase in Groups 2, 3 and 4 when compared with the Control, and a significant increase ($P<0.05$) in lipid peroxidase in Groups 2 and 3, and a significant decrease ($P<0.05$) in Groups 5 and 6 when compared with the Control as shown in table 1.

Histological Observations
The results of the histological observations showed the cerebrum of animals in Group 1 with normal structure of the cerebral cortical cells and layers as shown in plate 1 while the cerebral cortex of animals in Group 2 showed necrosis of cells and areas of degeneration of cells in the cerebral cortex as shown in plate 2. The results of the observations showed the cerebral cortex of animals in Group 3 with degenerated cells as shown in plate 3 while the results of the observations in animals in Group 4 showed reduced degeneration of cells with clumping and congestion of cells, necrosis of cells in the cerebral cortex as shown in plate 4. The observations of the cerebral cortex of animals in Group 5 showed clumping of cells as shown in plate 5 while the cerebral cortex of animals in Group 6 showed normal cells of the cerebral cortex as shown in plate 6.

DISCUSSION

The present study showed clumping of cerebral cortical cells and necrosis of cells in adult Wistar rats administered with different doses of mercuric chloride, while the layers and cells of cerebral cortex of the Control Group showed normal histology. The findings from the present study agree with other researchers who reported that many heavy metals such as mercury, lead,

cadmium and other organic compounds have the capacity to damage the nervous tissues (Farina et al., 2013). Permanent abnormalities are induced only by sustained use and exposure to these chemicals in large quantity. It has been reported that 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 (Farina et al., 2013). These toxins may adversely affect the cerebrum directly or as part of a more generalized encephalopathy (Jomova and Valko, 2011). It has been shown that oxidative stress particularly in the mitochondria, is a common feature of Fe, Mn and Hg toxicity. However, the primary molecular targets triggering oxidative stress are distinct. Free cationic ion is a potent pro-oxidant and can initiate a set of reactions that form extremely reactive products, such as hydroxyl ions. Manganese can oxidize dopamine, generating reactive species that can affect mitochondrial function, leading to accumulation of metabolites resulting in oxidative stress (Gemma et al., 2007; Farina et al., 2013). Studies have shown that metals like mercury undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radical and nitric oxide in biological systems (Gemma et al., 2007; Jomova and Valko, 2011; Farina et al., 2013). Disruption of metal ion homeostasis in the body system has been shown to lead to oxidative stress, a state where increased formation of reactive oxygen species overwhelms the body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects such as neurological disorders like Alzheimer's disease, Parkinson's disease and chronic inflammation. The mechanism of action for the metals involves formation of the superoxide radical, hydroxyl radical thus producing mutagenic and carcinogenic agents involved in the damage (Burger and Gochfeld, 2011; Burger et al., 2013). The present study has revealed the ameliorative effects of ascorbic acid on the cerebral cortex in the experimental animals induced with mercuric chloride toxicity. Administration of ascorbic acid has shown some improvement in the cerebral cortex when compared with rats exposed to mercuric chloride only. From the present observation, it could be deduced that there was a relationship between the amount of mercuric chloride an individual is exposed to, and the level of neurological damage in the cerebrum (Gemma et al., 2007; Burger et al., 2013). The present finding shows that the ascorbic acid has reduced the damage done to the cerebral cortex and this agrees to the fact that natural compounds that are rich in antioxidants help to reduce oxidative stress thus alleviating the effect of oxidative agents (Ahamed and Siddiqui, 2007; Burger et al., 2011). These antioxidants play significant roles in the reversion of the toxicity of mercury by forming inert complexes and inhibiting their toxicity on the dopaminergic neurons (Burger and Gochfeld, 2011). The present study revealed an increase in the mean SOD values in the experimental animals with a decrease in the mean LPO values. The respective increase and decrease in the mean SOD and LPO levels could be related to the ameliorative effects of the anti-oxidative action of the ascorbic acids on mercury-induced toxicity in the experimental animals. This is because heavy metals such as mercury, lead, thallium have been reported to reduce the anti-oxidative enzymes such as catalase, SOD, glutathione reductase and lipid peroxidase (Moreira et al., 2010). The present study agreed with the findings of Gutierrez *et al.*, (2006), who reported that oxidative stress is an important component of the mechanism of toxicity of metals. Acute exposure to mercury has been reported to increase LPO and decreases SOD (Jomova et al., 2010). Decrease in the activity levels of anti-oxidative enzymes such as superoxide dismutase and the elevation of lipid peroxidation suggests the formation of free radicals and the participation of free radical induced oxidative cell injury as in the toxic effects of mercury (Jomova and Valko, 2011). There is reduction in the antioxidant defense system such as SOD in mercury toxicity leading to the disruption of antioxidant balance in the body (Valko et al., 2005; Farina et al., 2013). The increase in lipid peroxidation might be due to peroxidation of unsaturated fatty acid. Thus, increased lipid peroxidation is suggestive of progressive increase in cellular deformity, increase in membrane permeability and disruption of structural and functional integrity of the cells.

Conclusion

It has been shown that exposure to mercury can induce degeneration in the cerebrum of adult Wistar rats. However, ascorbic acid has shown some level of protection against the

neurotoxicity induced by mercuric chloride. Consumers of mercury containing cosmetics, lipstick and ointments should always be conscious of the percentage of mercury in the products and pregnant women should avoid using such products since mercury has been reported to cross placental barrier. People in urban areas particularly those in industrial areas and those using mercury containing products should consume food and vegetables rich in ascorbic acid and other antioxidants to reduce the damage caused by mercuric chloride exposure.

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Table1: Effect of ascorbic acid on mercuric chloride-induced changes on the oxidative markers in experimental animals.

		CATmm/ml Mean ± SD	SOD(mUnits/L) Mean ± SD	GLU (mm/ml) Mean ± SD	LPO (µM/L) Mean ± SD
Group 1	(control)	23.25±1.19	604.65±60.32	14.29±1.19	57.46 ±3.35
Group 2	52.5mgHg Cl	12.52±5.30*	358.15±12.94*	10.04±1.16*	91.55±6.24*
Group 3	26.25mg HgCl	13.52±5.62*	382.75±24.39*	11.54±3.03*	70.39±6.59*
Group 4	52.5mg/kgHgCl +Vit. C	18.18±1.28*	472.75±77.15*	12.50±2.26*	52.82±14.79
Group 5	26.25mg/kg HgCl + Vit. C	20.29±3.57	526.50±50.21	13.57±0.81	47.75±3.25*
Group 6	Vit.C5mg/ kg	20.61±3.32	580.25±35.71	14.05±1.35	41.86±5.78*

*P<0.05,CAT=Catalase; SOD=Superoxide Dismutase; GLU=Glutathione Reductase; LPO=Lipid-peroxidation

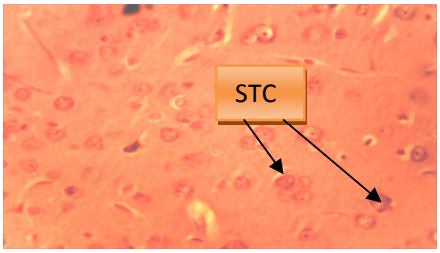

Plate 1: A transverse section of cerebral cortex of the Control (Group1), showing normal orientation of Stellate cells (STC). H&E, X 250

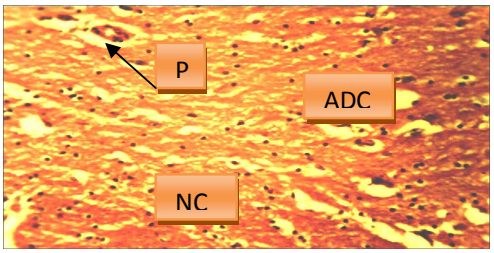

Plate 2: A transverse section of cerebral cortex of the Group 2, showing Pyramidal cell (P), Area of degeneration of cell (ADC) and Necrosis of cells (NC). H&E, X 250

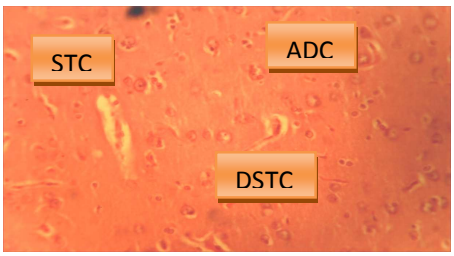

Plate 3: A transverse section of cerebral cortex of Group 3, showing Stellate cells (STC), Area of degeneration of cells (ADC) and Degenerated Stellate cells (DSTC). H&E, X 250

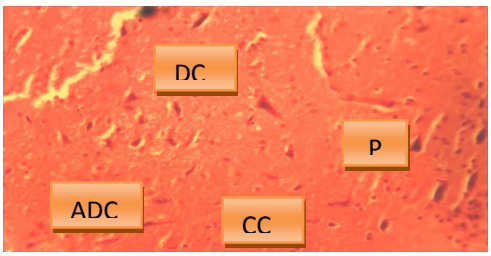

Plate 4: A transverse section of cerebral cortex of Group 4, showing Pyramidal cell (P), Area of degeneration of cells (ADC), Dead cell (DC) and Congestion cells (CC). H&E, X 250.

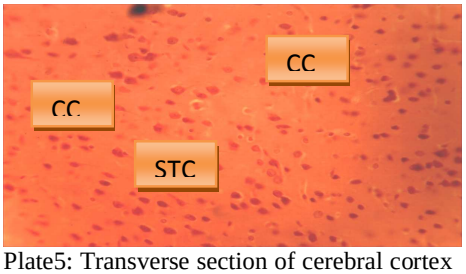

Plate5: Transverse section of cerebral cortex of Group 5, showing Stellate cells (STC) and Congestion of cell (CC). H&E, X 250

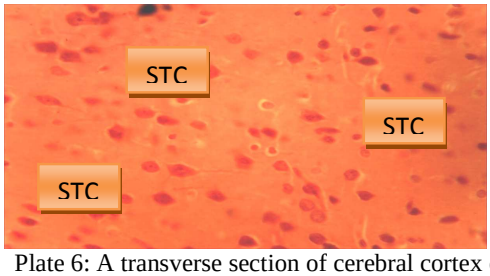

Plate 6: A transverse section of cerebral cortex of Group 6, showing normal orientation of Stellate cells (STC) resembling very closely that of Group 1. H&E, X 250.