

**The Role of Ascorbic acid on Mercury Induced Hippocampal (CA1 and CA3 region)
Damage and Memory impairments in rats.**



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ABSTRACT

Introduction: *Mercury is well known hazardous environmental contaminants with potential for global mobilization following its give off through air, soil, water and food while central nervous system has been shown to be the main target. Aim: The present study emphasis the effects of ascorbic acid administration against hippocampal damage and spatial learning impairments induced by mercury exposure in Wistar rats. Methods: Twenty five wistar rats (average weight 185 g) were randomly divided into five groups of five rats per group. Earlier the rats were trained for spatial navigation task in Morris water maze for 7 days and were simultaneously treated with mercuric chloride (49.8mg/kg) orally for 21 days, the animals were further subjected to ascorbic acid (595 mg/kg and 1,190 mg/kg) treatment orally for 21 days. After each administration the rats were tested for retention of spatial learning and memory in Morris water maze and latency were determined. The normal pyramidal cell density in the CA1 and CA3 region of hippocampus was also counted. Results: Results revealed histological alterations in hippocampal regions; (CA1 and CA3) involving necrosis, neuronal vacuolation, neuronal degeneration, pyknosis, cytoplasmic shrinkage and reduction in pyramidal cell density ($p<0.05$) in HgCl₂ intoxicated groups. The results from Morris water maze navigation test showed significant increase ($p<0.05$) in mean latency time taken by the animals to locate the hidden platform in mercury treated groups when compared to animals in the control and ascorbic acid treated groups, suggestive of neurological toxicity of mercury to learning and memory loss and ameliorative potential of ascorbic acid. Conclusion: The outcome of this study suggests that ascorbic acid could ameliorate pyramidal cell damage of CA1 and CA3 region of hippocampus and retention of hippocampal associated spatial learning and memory in rats treated with ascorbic acid, this finding confirms the ameliorative role of ascorbic acid against mercury induced neurotoxicity.*

Key words: Mercury, Hippocampal region (CA1and CA3), cognition, memory, Ascorbic acid.

INTRODUCTION

Heavy metals and organic compounds have the capacity to damage the nervous system. These compounds include mercury, arsenic, lead, manganese, thallium, cadmium, dichlorodiphenyl-trichloroethane (DDT) etc. The toxic effects of these compounds are variable and diffuse, involving different parts of nervous system as well as other organ systems (Maurice and James, 1972; Volko et al., 2005).

Mercury is highly toxic heavy which has been a major nervous system problem over decades (Brian and Fred, 1995), it is a potential factor in brain damage (Ibegbu et al., 2013; 2014), mental impairment and behavioral anomalies (Sadeeq et al., 2013).

Mercury is highly toxic heavy metal present in the environment via air, food and water (Wade et al., 2002; Burger et al., 2011). Most of the mercury in the environment results from human activity, particularly from coal-fired power stations, residential heating systems and waste (WHO, 2005). Mercury also enters the environment from fertilizers, fungicides and from solid wastes; thermometers or electrical switches (Department for Environment Food and Rural Affairs, 2002; Zhang 2002). Mercury exists in three (3) forms namely; elemental, organic and inorganic mercury (WHO, 2005; Burger et al., 2011).

Many populations Worldwide have been exposed to mercury through the consumption of fishes and sea foods (European Commission, 2005), dental amalgam and mining of gold, silver in industries (WHO, 2007). 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 (WHO, 2005). In Nigeria, Tilapia fishes from Lagos Lagoon and the use of “Kohl” a traditional cosmetic had been reported as an agent of mercury toxicity (Onyeike et al., 2002).

Some of the symptoms reported from mercury poisoning include, excitability, restlessness, irritability, irrational outburst of temper, depression, headache, dizziness, itching and pain (Grant and Lipman, 2009; WHO, 2005) while, tachycardia, frequent urination, salivation, hypertension, inactivity, memory impairment and insomnia were also reported in mercury exposed individuals (WHO, 2005; ATDRS, 2011). The excretory pathways of mercury compounds are urine, feces, expired air, sweat and saliva (Booth and Zeller, 2005; WHO, 2007). Ascorbic acid is well known for its antioxidant activity acting as a reducing agent to reverse oxidation in liquids and lipoproteins in various cellular compartments and tissues (Padayatty et al., 2003; Ibegbu et al., 2014). It is primary 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). Ascorbic acids can scavenge free radicals (Padayatty et al., 2003), prevent scurvy (WHO, 2001), pneumonia (Hemila and Louhiala, 2007) and are useful in lowering the incidence of gout (Choi, et al., 2009). Ascorbic acid is absorbed in the body by both active transport and simple diffusion (Savini, et al., 2007). Sources of ascorbic acid are fruits, vegetables, liver, nutritional supplement, tablets, drinks (Wilson, 2005) and animal products (United Kingdom Food Standard Agency, 2007). **Aim:** The present work was aimed at evaluating the role of ascorbic acid on mercury induced hippocampal damage, learning and memory impairments in rats.

MATERIALS AND METHODS

Twenty five (25) Adult Male Wistar rats of average weight 185g 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 LD50 of 166 mg/kg body weight as adopted from ATSDR (2011). While; the LD50 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. The animals were dosed as follows: control group was administered with normal saline, group II with 30% mercuric chloride (HgCl₂, 49.8 mg/kg) only, group received HgCl₂ with distilled water only, group IV received HgCl₂ with 5% low dose ascorbic acid (595 mg/kg), while group V received HgCl₂ 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, number of rats, treatment and duration of administration of mercuric chloride and ascorbic acid

Groups	Dosage/kg body weight
(n=5)	
I	Distilled water (Control)
II	49.8mg/kg of mercuric chloride
III	49.8mg/kg of mercuric chloride

	and
	Distilled water
IV	49.8mg/kg of mercuric chloride
	and
	595mg/kg of ascorbic acid
V	49.8mg/kg of mercuric chloride
	and
	1,190mg/kg of ascorbic acid

Animal Sacrifice

After the administration, the animals were anaesthetized by inhalation of chloroform in the sacrificing chamber. The skull was opened with the aid of brain opener through a mid sagittal incision while brain tissues were removed and fixed in Bouin's fluid for fast fixation. The tissues were routinely processed for paraffin embedded histology and stained using Hematoxylin and Eosin (H&E) staining method.

Tissue processing technique

Brain tissues were allowed to stay in Bouin's fluid for 48 hours for proper fixation. The tissues were prepared using routine Hematoxylin and Eosine staining technique processing unit Histology laboratory of Human Anatomy Department, Ahmadu Bello University, Zaria. The brain tissues were processed routinely and stained using routine H and E technique.

Neurobehavioral test; spatial learning and memory test using Morris water maze

Morris water maze test was used to develop and test spatial learning and memory in the test animals according to the methods of Morris (1981), which was further modified by Mark et al. (2007) and Liu et al. (2011). According to this method, each animal was placed in a small pool of water which contained an escape platform, hidden a few millimeters


away and beneath the water surface. The animal task was to locate the hidden platform. The animal starting point was changed from time to time so as to build a cohesive spatial representative of the pool in order to find the platform during training trials and the latency to find the platform location was recorded during the training and weekly during the experimental periods. Animals were placed in circular pool of clear water which was partitioned into four quadrants. Each animal's starting point was in a random position and each animal swam from one quadrant to the other searching for an escape route. The time taken by each animal to locate the platform (escape route) was recorded as latency period in seconds.

Cell Count Analysis

Hippocampal CA1 and CA3 Pyramidal cells were counted using Digimizer image analysis software. Photomicrographs of hippocampal regions were uploaded into the image area of the software. A marker tool was used to mark and count cells in the aforementioned region. 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.

Statistical Analysis

Results were analyzed using the Statistical package for Social Scientist (SPSS version 20) and the results were expressed as Mean \pm 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 significant difference between control and experimental groups. A p-value < 0.05 was considered significant.

RESULTS:

Physical observation of the animals

On physical observation, the control group animals were physically, behaviorally and mentally stable while mercury treated animals were observed to be ataxic, agitated, distressed, apathetic with diarrhea during the first 3 weeks of administration. However, improved physical activity, agility, and behavioral stability were noted in animals treated with ascorbic acid in the last 3 weeks of administration.

Histological Observations

The brain sections stained with H&E stain obtained from

Group I rats reveal intact morphology and cytoarchitecture of hippocampal CA1 and CA3 region showing pyramidal neurons with normal profile, pale nucleus and prominent nissl granules in the cytoplasm without any shrinkage (Fig 1A, 2A). In the Group II and III rat brain sections the hippocampus demonstrated major alteration in the neuronal profile of CA1 and CA3 regions, increase in the number of dead, darkly stained shrunken pyramidal cells with pyknotic nucleus, the number of normal pyramidal cell population was drastically less. (Fig 1B, 1C, 2B, 2C). The brain sections of ascorbic acid treated rats (Group IV and V) demonstrate decreased number of shrunken cells, damaged neurons in the CA1 and CA3 regions of hippocampus and the proportion of normal cell in the pyramidal region was comparatively higher than the mercury only groups (Fig 1D, 1E, 2D, 2E).

Figure 1: Sections of cellular layers of hippocampal CA1 region (H&E × 250)

Fig.1A represents Group I (control), the arrow indicates the prominent pyramidal cells. Fig. 1B represents Group II (HgCl₂; 49.8mg/kg), arrow indicates the dead shrunken neurons with pyknotic nucleus. Fig. 1C represents Group III (HgCl₂;

49.8mg/kg and distilled water) showing degenerating pyramidal cell (white arrow) and some surviving pyramidal neurons (yellow arrow). Fig. 1D and 1E represents Group IV and V (HgCl₂; 49.8mg/kg and ascorbic acid; 595mg/kg and 1,190mg/kg treated), the arrow indicate pyramidal cells.

Figure 2: Sections of cellular layers of hippocampal CA3 region (H&E × 250)

Fig. 2A represents Group I (control), the arrow indicates the prominent pyramidal cells. Fig. 2B represents Group II (HgCl₂; 49.8mg/kg), showing dead shrunken neurons (DSN) with pyknotic nucleus (white arrow). Fig. 2C represents Group III (HgCl₂; 49.8mg/kg and distilled water) showing degenerating pyramidal cell (white arrow) and some surviving pyramidal neurons (yellow arrow). Fig. 2D and 2E represent Group IV and V (HgCl₂; 49.8mg/kg and ascorbic acid; 595mg/kg and 1,190mg/kg treated) showing few dead neurons (white arrow) and numerous surviving pyramidal cells (yellow arrow).

Table 2: Number of Hippocampal Pyramidal cells counted

Groups	Administration
GI	Control
GII	(HgCl ₂ alone)
GIII	(HgCl ₂ and Distilled H ₂ O)
GIV	(HgCl ₂ and Vit.C _{595mg/kg})
GV	(HgCl ₂ and Vit.C _{1,190mg/kg})

n= number of cells counted. SEM:Standard Error of Mean. HgCl₂: Mercuric Chloride. Vit. C: Vitamin C *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 *d indicates significant difference between Group IV and Group III.

Table 3: Mean latencies for spatial learning and memory using Morris water maze test

		End of Training
Groups	Administration	Mean ± SEM
		(s)

GI	Control	3.19 ± 0.32
GII	(HgCl ₂ alone)	3.59 ± 0.68
GIII	(HgCl ₂ and Distilled H ₂ O)	3.76 ± 0.63
GIV	(HgCl ₂ and Vit.C _{595mg/kg})	3.50 ± 0.47
GV	(HgCl ₂ and Vit.C _{1,190mg/kg})	3.85 ± 0.51

*p<0.05 indicates significant difference compared to Group I (Control). s = mean time in seconds.

SEM: Standard Error of Mean.

*a indicates significant difference between Group V and Group II.

*b indicates significant difference between Group V and Group III.

*d indicates significant difference between Group IV and Group III.

HgCl₂: Mercuric Chloride. Vit. C: Vitamin C

DISCUSSION

Many heavy metals such as mercury, lead, cadmium, manganese, solvents and other organic compounds have the capacity to damage the nervous tissues (Farina et al., 2011; Ibegbu et al., 2014). The administration of mercuric chloride in Wistar rats have induced a progressive damage to the CA1 and CA3 pyramidal neurons of the rat hippocampus and have significantly reduced the percentage of surviving pyramidal neurons. These neurodegenerative changes could invariably impair the activities of the hippocampus in learning, memory formation, storage and retrieval of information (Sadeeq et al., 2013). The present study showed a significant increase (p>0.05) in the mean time taken by rats to locate the hidden platform in Morris water maze cognitive test for spatial learning and memory during the weeks of mercuric chloride administration. The outcome of this present study correlates with the above data resulting in permanent selective damage to the CA1 and CA3 regions of the hippocampus with retarded performance in the Morris water maze cognitive task.

Ascorbic acid has shown improvement in the hippocampus histology and enhanced cognitive functions in rats induce with mercury neurotoxicity. Apart from cognitive enhancing property ascorbic acid has on various disease conditions, several neurological disorders were experimentally proved (Ibegbu et al., 2014; Animoku et al., 2018; Ekanem et al., 2020). This ameliorative role of ascorbic acid in mercury induced neurotoxicity in Wistar rats may be due to its rich antioxidant resource it posses (Ibegbu et al., 2014) which might have controlled the free radical generated during learning impairment and prevented neuronal death or its active compound might have evoked an effective resistance shield against various other mechanism which operates the neurotoxicity cascade.

CONCLUSION

In conclusion, this study has given a viable data that

ascorbic acid a well known antioxidant could also protect one of the most vulnerable population of brain regions, the hippocampus against the deleterious effect of mercury and retained the hippocampus mediated memory functions. The core mechanism of ascorbic acid which works against the neurotoxicity cascade operated by mercury was not completely understood through this study. Hence, a future detailed investigation could explore the unknown potentiality of ascorbic acid (Vitamin C).

Conflict of Interest: None declared.

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