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Ameliorative Effects of Ascorbic Acid on Mercury-Induced Learning and Memory Impairment in Rats

Abdulrazaq Animoku[1], **Adebayo Buraimoh**[1], **Wilson Hamman**[1], **Augustine Ibegbu**[1], **Yunusa Y. Pala**[2] **and Iliyasu Musa**[1]

[1]Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria
[2]Avian Influenza Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

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ABSTRACT

Mercury is a hazardous heavy metal and the nervous system has been shown to be the main target. The present work was aimed at evaluating the effects of ascorbic acid on mercury exposure on spatial learning and memory of adult Wistar rats. Twenty five adult Wistar rats (average weight 185 g) were randomly divided into five groups of five rats per group; control group administered normal saline, mercuric chloride ($HgCl_2$; 49.8 mg/kg), $HgCl_2$ with distilled water, $HgCl_2$ with low dose vitamin C (595 mg/kg) and $HgCl_2$ with high dose vitamin C (1,190 mg/kg). The animals were each orally administered daily for three weeks. Morris water maze test was carried out to test for spatial learning and memory. The results from Morris water maze test showed significant increase ($p<0.05$) in mean time taken by the animals to locate the hidden platform in mercury treated groups compared to animals in the control and ascorbic acid treated groups, suggestive of neurological toxicity of mercury to learning and memory loss. Histological result showed distortion of CA3 region cells of the hippocampus and vacuolation of cells were observed in all other groups compared to the control. Therefore, ascorbic acid seems to ameliorate memory deficit in the hippocampus caused by mercury exposure in adult Wistar rats.

Keywords: *Mercuric Chloride, Hippocampus, Memory, Ascorbic acid, Adult Wistar rats*

INTRODUCTION

Human and animal populations interact with their environment on daily basis and as such are exposed to a range of chemicals and heavy metals such as mercury, lead, thalium, aluminum and cadmium (WHO 2005). These interactions with the environment occur through food, air and water (Burger et al. 2011). Mercury occur in the environment owing to natural processes like degassing from earth crust, emissions from volcanoes and evaporation from water bodies and anthropogenic processes, particularly from coal-fires, power stations, residential heating systems and waste incinerators (WHO 2005). There is a growing appreciation of the effects that exposure to heavy metals such as lead, cadmium, aluminum and mercury may have on the nervous system. The toxic effects of these compounds are variable and diffuse, involving different parts of nervous system (Volko et al. 2005).

Mercury 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; Ibegbu et al. 2014), mental impairment, behavioral anomalies

Correspondence: Abdulrazaq Animoku, M.Sc., Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, P.M.B. 81006, Zaria, Nigeria. Email: animokuaa@gmail.com; +2348135492316

(Sadeeq et al. 2013), neuromuscular weaknesses, hearing problems, impaired cognitive functions and coma (Liuji et al. 2002; Flora et al. 2007; Verina et al. 2007). This is because some of these heavy metals can cross the blood brain barrier and accumulate in brain tissues thus causing damage to these tissues (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 (European Commission 2005).

Tilapia fishes from Lagos Lagoon were characterized with relatively high level of mercury concentration (Fodeke 1979), while states like Katsina, Sokoto, Gombe and other Northern States are associated with the use of Kohl; a traditional cosmetic which had been reported to predispose people to mercury toxicity (Onyeike et al. 2002). Some of the symptoms of mercury poisoning include irritability, excitability, restlessness and irrational outburst of temper, depression, headache and dizziness, itching, burning, pain, fingertips and toes swelling, and shedding of the skin, profuse sweating, tachycardia, frequent urination, increased salivation, and hypertension, muscle weakness, kidney dysfunction, memory impairment and insomnia (ATSDR 2011).

Ascorbic acid is an antioxidant that prevents the production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues (Ibegbu et al. 2013). Antioxidants have been shown to react with superoxide (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 (Gemma et al. 2010). The study seeks to evaluate the ameliorative effects of ascorbic acid on mercury-induce learning and memory impairment in rats.

MATERIALS AND METHODS

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 with five animals per group.

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

Table 1: Animal Groupings, Treatment and Duration of Administration of Mercuric Chloride and Ascorbic Acid

Groups (n=5)	Dosage/kg body weight	Treatment Duration (day)
1	Distilled water (control)	1-42
2	49.8 mg/kg of mercuric chloride	1-21
3	49.8 mg/kg of mercuric chloride and distilled water	1-21 22-42
4	49.8 mg/kg of mercuric chloride and 595 mg/kg of ascorbic acid	1-21 22-42
5	49.8 mg/kg of mercuric chloride and 1,190 mg/kg of ascorbic acid	1- 21 22- 42

Twenty five rats were grouped into five (5) groups of five (5) animals per group; control group administered with normal saline, a mercuric chloride ($HgCl_2$; 49.8 mg/kg), $HgCl_2$ with distilled water group, $HgCl_2$ with low dose vitamin C group (595 mg/kg) and $HgCl_2$ with high dose vitamin C (1,190 mg/kg). However, administrations of distilled water and vitamin C from 22nd day were done so as to observe for possible natural recovery and ameliorative potential of vitamin C 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*.

Spatial Learning and Memory Test using Morris Water Maze Test

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 millimetres away and below 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.

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. Tissue sections were viewed under light microscope at ×250 magnification while photomicrographs were taken with the aid of MD900 Amscope microscope digital camera.

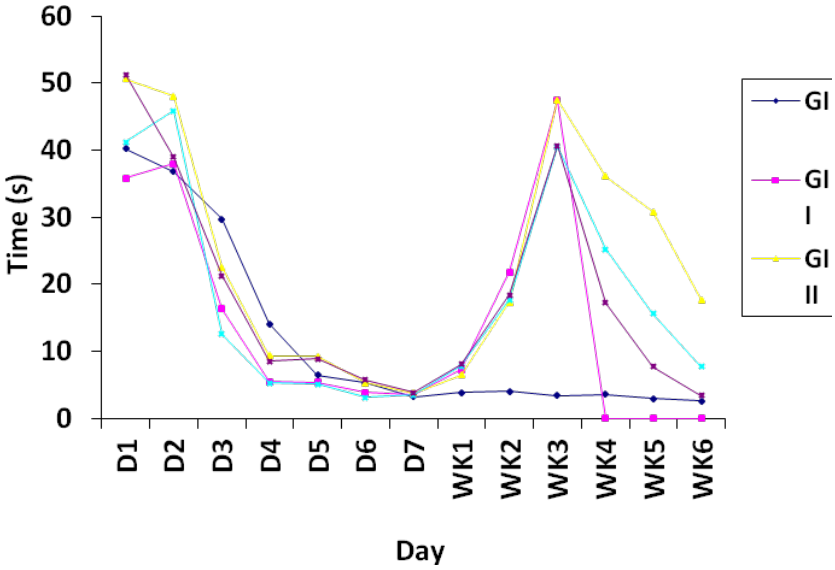

Fig. 1: Spatial learning and memory assessment measured in time (s) taken to locate the platform in Morris water maze navigation test.

Statistical Analysis

All the results were analyzed using the Statistical Package for Social Sciences (SPSS version 20) and the results were expressed as Mean ± SEM. The Statistical significance between means was analyzed using one-way analysis of variance (ANOVA) followed by post HOC test; Tukey multiple comparison test to test for statistically 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 while animals that received mercuric chloride only ($HgCl_2$; 49.8 mg/kg), $HgCl_2$ with distilled water, $HgCl_2$ with low dose Vitamin C (595 mg/kg) and $HgCl_2$ with high dose Vitamin C (1,190 mg/kg) were observed to be using their forelimbs to scratch their mouth, gnawing, restlessness and watery faeces on mercury exposure. The animals got weakened progressively and this observation could be related to reduction in their physical activities. However, there were improvements in physical activity of the animals during the period of ascorbic acid treatment.

Table 2: Mean Latencies for Spatial Learning and Memory using Morris Water Maze Test

Groups	At end of Training	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
	Mean ± SEM (s)	Mean ± SEM (s)	Mean ± SEM (s)	Mean ± SEM (s)	Mean ± SEM (s)	Mean ± SEM (s)	Mean ± SEM (s)
Control	3.19 ± 0.32	3.83 ± 0.39	3.96 ± 0.54	3.38 ± 0.51	3.55 ± 0.41	2.94 ± 0.37	2.55 ± 0.53
$HgCl_2$ 1st-3rd Weeks	3.59 ± 0.68	7.26 ± 1.18	21.82 ± 3.74*	47.45 ± 8.72*			
$HgCl_2$ & Distilled H_2O	3.76 ± 0.63	6.46 ± 1.01	17.34 ± 3.78	47.53 ± 7.67*	36.06 ± 6.13*	30.76 ± 6.55*	17.66 ± 2.44*
$HgCl_2$ & $Vit.C_{595mg/kg}$	3.50 ± 0.47	7.77 ± 1.40	17.69 ± 2.74	40.51 ± 8.78*	25.19 ± 6.71*	15.56 ± 5.01	7.69 ± 1.71*d
$HgCl_2$ & $Vit.C_{1,190mg/kg}$	3.85 ± 0.51	8.06 ± 2.22	18.42 ± 5.61	40.67 ± 9.95*	17.26 ± 3.25	7.73 ± 1.71*b	3.35 ± 0.40*a

*p<00.05 indicates significant difference compared to control group (normal saline). s = mean time in seconds. SEM: Standard Error of Mean. *a indicates significant difference between $HgCl_2$ with high dose Vitamin C (1,190mg/kg) group and mercuric chloride only ($HgCl_2$; 49.8mg/kg) group. *b indicates significant difference between $HgCl_2$ with high dose Vitamin C (1,190mg/kg) group and $HgCl_2$ with distilled water group. *d indicates significant difference between $HgCl_2$ with low dose Vitamin C (595mg/kg) group and $HgCl_2$ with distilled water group. $HgCl_2$: Mercuric chloride (49.8mg/kg). Vit. C: Vitamin C

Spatial Learning and Memory of Rats

The results of the spatial learning and memory test showed no difference in the mean time taken by the animals that received mercuric chloride only ($HgCl_2$; 49.8 mg/kg), $HgCl_2$ with distilled water, $HgCl_2$ with low dose Vitamin C (595 mg/kg) and, $HgCl_2$ with high dose Vitamin C (1,190 mg/kg) to complete Morris water maze task at the end of the training period and week 1 after. However, significant increase ($p < 0.05$) in the mean time were observed in mercuric chloride only group ($HgCl_2$; 49.8 mg/kg) at the end of week 2, but in groups $HgCl_2$ with distilled water, $HgCl_2$ with low dose vitamin C (595 mg/kg) and $HgCl_2$ with high dose vitamin C (1,190 mg/kg) at the end of week 3. Significant increase ($p < 0.05$) in $HgCl_2$ with distilled water and $HgCl_2$ with low dose vitamin C (595 mg/kg) groups was observed at week 4 as well as in $HgCl_2$ with distilled water group at the end of week 5 when all compared to the control group. This significant increase

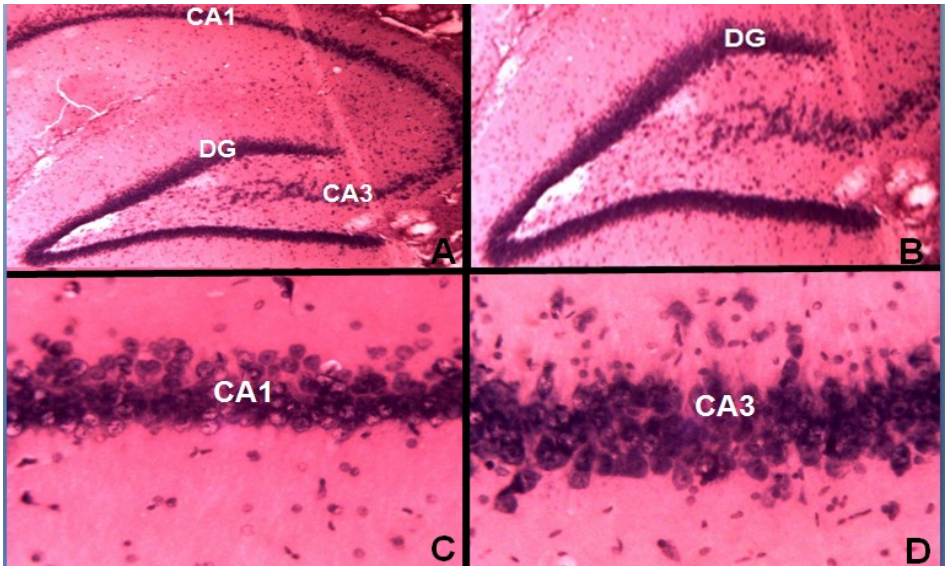

Fig. 2: Hippocampus of control group (normal saline) showing; A (architecture of hippocampus), B (Dentate gyrus; DG), C (CA1 region) and D (CA3 region), (H&E, Magnifications X 40, 100 & 250).

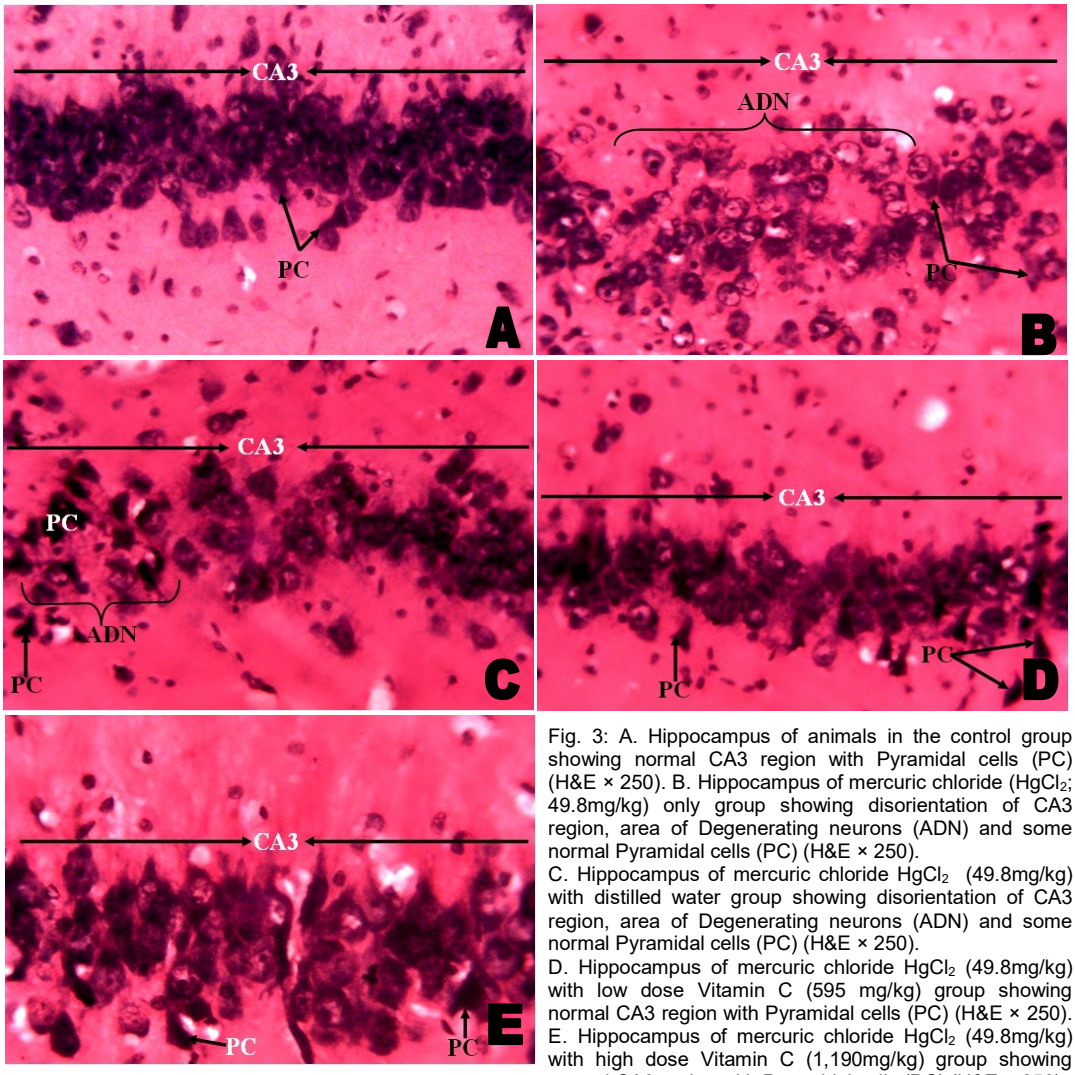

Fig. 3: A. Hippocampus of animals in the control group showing normal CA3 region with Pyramidal cells (PC) (H&E × 250). B. Hippocampus of mercuric chloride ($HgCl_2$; 49.8mg/kg) only group showing disorientation of CA3 region, area of Degenerating neurons (ADN) and some normal Pyramidal cells (PC) (H&E × 250).
C. Hippocampus of mercuric chloride $HgCl_2$ (49.8mg/kg) with distilled water group showing disorientation of CA3 region, area of Degenerating neurons (ADN) and some normal Pyramidal cells (PC) (H&E × 250).
D. Hippocampus of mercuric chloride $HgCl_2$ (49.8mg/kg) with low dose Vitamin C (595 mg/kg) group showing normal CA3 region with Pyramidal cells (PC) (H&E × 250).
E. Hippocampus of mercuric chloride $HgCl_2$ (49.8mg/kg) with high dose Vitamin C (1,190mg/kg) group showing normal CA3 region with Pyramidal cells (PC) (H&E × 250).

($p<0.05$) in the mean time was only observed in $HgCl_2$ with distilled water group at week 6 when compared to control group, $HgCl_2$ with low dose vitamin C (595mg/kg) and $HgCl_2$ with high dose vitamin C (1,190mg/kg) groups (Table 2 and Figure 1).

Histological Observations

The results of histological observation of the hippocampus of animals in control (normal saline) group, showed normal cytoarchitecture of CA3 region of the hippocampus with Pyramidal cells appearing normal as shown in Figure B, while animals in mercuric chloride only ($HgCl_2$; 49.8 mg/kg) group showed disorientation and degenerated Pyramidal cells of the CA3 region of the hippocampus as shown in Figure C. The hippocampus of animals in $HgCl_2$ with distilled water group showed disorientation of CA3 region, degenerated Pyramidal cells, clumping of cells and some normal Pyramidal cells as shown in Figure D while the animals in $HgCl_2$ with low dose Vitamin C (595 mg/kg) group and $HgCl_2$ with high dose Vitamin C (1,190 mg/kg) group showed some evidence of degenerated cells with some Pyramidal cells appearing normal as shown in Figure E and F respectively.

DISCUSSION

The present study showed significant increase ($p < 0.05$) in the mean time taken by the experimental animals to locate the hidden platform in Morris water maze test for spatial learning and memory during the weeks of mercuric chloride administration. This could be associated to memory loss which could be as a result of neuronal degeneration, distortion in the general morphology of the pyramidal cells and CA3 region of the hippocampus as observed from the present study. These alterations imply that, activity such as memory and learning abilities from the brain region that projects into the pyramidal layer and CA3 region of the hippocampus will be lost (Sadeeq et al. 2013) and these could invariably impair the activities of the hippocampus in memory formation, learning, storage and retrieval of information (Wolf et al. 2009). However, Mutter et al. (2010), had reported that short term occupational exposure to high levels of mercury induced slight cognitive deficits and that, mercury has no effect on memory as observed from his Y-maze cognitive test for memory, which conversely disagrees with the present study. The findings from the present study also agreed with the work of other researchers who reported that many heavy metals such as mercury, lead, cadmium, thallium, manganese, drugs, solvents (Jomova and Valko 2011) and other organic compounds have the capacity to damage the nervous tissues (Ibegbu et al. 2014) 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). Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system responsible for development, learning, memory and response to injury (Feather-Stone 2010). However, glutamate at high concentrations at the synaptic cleft acts as a toxin, inducing neuronal injury and death (Meldrum 2000). Glutamate-mediated neurotoxicity has been dubbed as "excitotoxicity", referring to the consequence of the over activation of the N-methyl D-aspartate (NMDA)–type glutamate receptors, leading to increased Na+ and Ca^{2+} influx into neurons (Pivovarova and Andrews 2010). Hence, increased intracellular Ca^{2+} levels are associated with the generation of oxidative stress and neurotoxicity (Farina et al. 2011).

The present study showed significant decrease ($p<0.05$) in the mean time taken by the animals to locate the hidden platform in Morris water maze test for spatial learning and memory during the weeks of ascorbic acid administration. Thus, the study has revealed the ameliorative effects of ascorbic acid on spatial learning and memory in the experimental animals induced with mercuric chloride toxicity. Administration of ascorbic acid has shown some improvement in the hippocampus of animals when compared with animals exposed to mercuric chloride only 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). 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, Ascorbic acid serves as antioxidant which plays significant role in the reversion of the toxicity of mercury by forming inert complexes and inhibiting their toxicity (Burger et al. 2011).

CONCLUSION

The findings from the present study justify significantly the ameliorative effect of ascorbic acid against mercury induced neurotoxicity on spatial learning and memory of adult Wistar rats. As such, people exposed to mercury poison should consume food rich in ascorbic acid along with other antioxidants.

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