EFFECT OF ASCORBIC ACID ON MERCURY EXPOSURE ON HIPPOCAMPUS OF ADULT WISTAR RATS

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ABSTRACT

Aim: The effect of ascorbic acid on mercury exposure on hippocampus of Wistar rats was investigated.

Methods: Twenty five adult Wistar rats of average weight of 185 grams were randomly grouped into five with five animals per group. The animals in Group I (Control) were administered with distilled water, Group II were administered with 49.8mg/kg body weight of mercuric chloride. Groups III and IV were administered with 49.8mg/kg body weight of mercuric chloride and distilled water, and 49.8mg/kg body weight of mercuric chloride and 595mg/kg body weight of ascorbic acid respectively while Group V was administered 49.8mg/kg body weight of mercuric chloride and 1,190mg/kg body weight of ascorbic acid. Mercuric chloride was given for 3 weeks, while ascorbic acid was also given for 3 weeks. Morris water maze navigation test was done to test for spatial learning and memory.

Results: Histological result showed normal architecture in the Control Group while distortion of CA3 region cells of the hippocampus, reduction in number of cells and vacuolation of cells were observed in experimental Groups. The mean latency time taken by the animals to locate the hidden platform in Morris water maze test increased significantly (p<0.05) in mercury treated Groups when compared with the animals in the Control and ascorbic acid treated Groups.

Conclusion: Administration of ascorbic acid has been shown to ameliorate induced degenerative changes in the hippocampus caused by mercury exposure in Wistar rats.

Key words: Mercuric chloride, Hippocampus, Ascorbic acid

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. These interactions with the environment occur through food, air and water (Wade et al., 2002; 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. Mercury can also be present as a result of mining of mercury, gold and other metals such as Copper, Zinc, Lead and Silver (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 (Valko et al., 2005; Farina et al., 2011). 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 (Brian and Fred, 1995; Wright and David, 2001). 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). Tilapia fishes from Lagos Lagoon were characterized with relatively high level of mercury concentration in the Lagoon (Fodeke, 1979), while Sates 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, 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, (Grant and Lipman, 2009; WHO, 2005) while a person suffering from mercury poisoning may also experience profuse sweating, tachycardia, frequent urination, increased salivation, and hypertension, muscle weakness, kidney dysfunction, memory impairment and insomnia (Horowitz et al., 2002; Liuji et al., 2002; WHO, 2005; ATDRS, 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 (Sies et al., 1992; Padayatty et al., 2003; WHO, 2003; Ibegbu et al., 2014). 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 dehydroascorbic acid (Valko et al., 2005; UKFSA, 2007; Gemma et al., 2010). The present work aimed at evaluating the effect of ascorbic acid on mercury exposure on Hippocampus of Adult Wistar rats.

**Experimental Chemicals**

Mercuric chloride manufactured by May and Bakers limited, Degenham England with batch number X-N202 was used for this study. The LD50 of Mercuric chloride was adopted from ATDRS, (2011) as 166mg/kg body weight. 30% (49.8mg/kg) of the LD50 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 LD50 of Ascorbic acid was adopted from MSDS, (2008) as 11,900mg/kg body weight. 5% (595mg/kg) and 10% (1,190mg/kg) of the LD50 per kg body weight of ascorbic acid was used in this study.

**Experimental Procedure**

Twenty five rats were grouped into five (5) Groups of five (5) animals per Group as GI, GII, GIII, GIV and GV respectively. In addition to normal diet, the animals in GI received distilled water from 1st to 42nd day, GII animals were administered with 49.8mg/kg body weight of mercuric chloride from 1st to 21st day, GIII animals were administered with 49.8mg/kg body weight of mercuric chloride from 1st to 21st day and distilled water from 22nd to 42nd day, GIV animals were administered with 49.8mg/kg body weight of mercuric chloride from 1st to 21st day and 595mg/kg body weight of ascorbic acid from 22nd to 42nd day, while GV animals were administered with 49.8mg/kg body weight of mercuric chloride from 1st to 21st day and 1,190mg/kg body weight of ascorbic acid from 22nd to 42nd day (Table1). The administration was by oral route daily and lasted for 3-6 weeks while the animals were feed and drinking water was allowed ad libitum.

**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.

**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 with five animals per group.
Table 1: The administration of mercuric chloride and ascorbic acid

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (Dosage/kg body weight)</th>
<th>Duration (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled water (control)</td>
<td>1st – 42nd</td>
</tr>
<tr>
<td>1</td>
<td>49.8mg/kg of mercuric chloride</td>
<td>1st – 21st</td>
</tr>
<tr>
<td>11</td>
<td>49.8mg/kg of mercuric chloride</td>
<td>1st – 21st</td>
</tr>
<tr>
<td>+</td>
<td>Distilled water</td>
<td>22nd – 42nd</td>
</tr>
<tr>
<td>1V</td>
<td>49.8mg/kg of mercuric chloride</td>
<td>1st – 21st</td>
</tr>
<tr>
<td>+</td>
<td>595mg/kg of ascorbic acid</td>
<td>22nd – 42nd</td>
</tr>
<tr>
<td>V</td>
<td>49.8mg/kg of mercuric chloride</td>
<td>1st – 21st</td>
</tr>
<tr>
<td>+</td>
<td>1,190mg/kg of ascorbic acid</td>
<td>22nd – 42nd</td>
</tr>
</tbody>
</table>

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 Liu et al, (2011). According to this method, the animal was placed in a small pool of water which contained an escape platform, hidden a few millimeters away and below the water surface. The animal task is 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 was recorded as latency period in seconds.

Cell count analysis

Pyramidal cells of the Hippocampus involving the CA3 regions were counted with the aid of Digimizer image analysis software. Photomicrographs were uploaded into the image area of the software and 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; Turkey’s 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

The result of physical observation of the animals showed that animals in Group I were very active while animals in Groups II, III, IV and V were observed to be using their forelimbs to scratch their mouth, gnawing, restlessness and watery feces on mercury exposure. The animals got weakened which was observed due to the reduction of their physical activities. However, improvements in physical activity of the animals were observed during the period of ascorbic acid treatment.

The effect of Ascorbic acid on Mercuric chloride exposure on Morris Water Maze test

The result on spatial learning and memory using Morris water maze navigation test showed neither significant increase nor decrease in the mean time taken by the animals in Groups I to V to complete Morris water maze task at the end of the training period and week 1. However, significant increase (p<0.05) in the mean time was observed in Group III at week 6 when compared to Groups IV and V and animals in the Control Group as shown in Table 2.

Cell count analysis

The result of the analysis of the Pyramidal cells of the hippocampus counted showed significant decrease (p<0.05) in the number of Pyramidal cells of the hippocampus among animals in Groups II and III when compared to those in Groups I, IV and V as shown in Table 3.
Histological Observations
The results of histological observation of the hippocampus of animals in Group I, showed normal cytoarchitecture of CA3 region of the hippocampus with Pyramidal cells appearing normal as shown in Plate I, while animals in Group II showed disorientation and degenerated Pyramidal cells of the CA3 region of the hippocampus as shown in Plate II. The hippocampus of animals in Group III showed disorientation of CA3 region, degenerated Pyramidal cells, clumping of cells and some normal Pyramidal cells as shown in Plate III while the animals in Groups IV and V showed some evidence of degenerated cells with some Pyramidal cells appearing normal as shown in Plates IV and V respectively.

Table 2: The effects of ascorbic acid administration on mercuric chloride induced changes on spatial learning and memory using Morris water maze test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Administration</th>
<th>At end of Training</th>
<th>Week 1</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM (s)</td>
<td>Mean ± SEM (s)</td>
<td>Mean ± SEM (s)</td>
</tr>
<tr>
<td>I</td>
<td>(Control)</td>
<td>3.19 ± 0.32</td>
<td>3.83 ± 0.39</td>
<td>2.55 ± 0.53</td>
</tr>
<tr>
<td>II</td>
<td>(HgCl₂ 1st-3rd Wk)</td>
<td>3.59 ± 0.68</td>
<td>7.26 ± 1.18</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>(HgCl₂ + Distilled H₂O)</td>
<td>3.76 ± 0.63</td>
<td>6.46 ± 1.01</td>
<td>17.66 ± 2.44*</td>
</tr>
<tr>
<td>IV</td>
<td>(HgCl₂ + Vit.C595mg/kg)</td>
<td>3.50 ± 0.47</td>
<td>7.77 ± 1.40</td>
<td>7.69 ± 1.71*a,b</td>
</tr>
<tr>
<td>V</td>
<td>(HgCl₂ + Vit.C1905mg/kg)</td>
<td>3.85 ± 0.51</td>
<td>8.06 ± 2.22</td>
<td>3.35 ± 0.40*a</td>
</tr>
</tbody>
</table>

*p<0.05 indicates significant difference compared to Group I (Control). *a indicates significant difference between Group V and Group III. *b indicates significant difference between Group IV and Group III. s = mean time in seconds, SEM: Standard Error of Mean. HgCl₂: Mercuric chloride

Table 3: Number of Pyramidal cells counted from Hippocampus sections of adult Wistar rats following administration of mercury and ascorbic acid

<table>
<thead>
<tr>
<th>Group</th>
<th>Administration</th>
<th>Hippocampus (Pyramidal cell) Mean ± SEM (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>30.33 ± 0.88</td>
</tr>
<tr>
<td>II</td>
<td>(HgCl₂ 1st-3rd Wks)</td>
<td>7.33 ± 0.33*</td>
</tr>
<tr>
<td>III</td>
<td>(HgCl₂ + Distilled H₂O)</td>
<td>10.33 ± 1.45*</td>
</tr>
<tr>
<td>IV</td>
<td>(HgCl₂ + Vit.C595mg/kg)</td>
<td>20.67 ± 1.20*</td>
</tr>
<tr>
<td>V</td>
<td>(HgCl₂ + Vit.C1905mg/kg)</td>
<td>26.00 ± 2.08*</td>
</tr>
</tbody>
</table>

n= number of Pyramidal 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. *ab 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.

Plate I: Hippocampus of Group I (Control). Normal CA3 region with Pyramidal cells (PC) (H&E X250).
Plate II: Hippocampus of Group II. Disorientation of CA3 region, area of degenerating neurons (ADN) and some pyramidal cells (PC) (H&E X250)
Plate III: Hippocampus of Group III. Disorientation of CA3 region, area of degenerating neurons (ADN) and some pyramidal cells (PC) (H&E X250).
Plate IV: Hippocampus of Group IV. Normal CA3 region with Pyramidal cells (PC) H&E X250
Plate V: Hippocampus of Group V. Normal CA3 region with Pyramidal cells (PC) H&E X250
DISCUSSION
The findings from the present study revealed that the CA3 region cells of the hippocampus showed neuronal changes ranging from disorientation, degeneration and reduction in number of cells when compared to animals in the Control Group suggesting the toxic effect of mercury exposure. These neurodegenerative changes could invariably impair the activities of the hippocampus in memory formation, learning, storage and retrieval of information. The findings in this study agree with that of Wolf et al., (2009) and Sadeeq et al., (2013) who observed that rats exposed to mercury vapor, showed neurodegenerative changes in the hippocampus which was related to impaired memory deficit in the animals. 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 (Farina et al., 2011; Ibegbu et al., 2014). The present study showed a 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 (Wolf et al., 2009; Quirino, 2012; Sadeeq et al., 2013). 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 damages caused by mercury to the brain (Ibegbu et al., 2013; 2014). 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; Ibegbu et al., 2014).

CONCLUSION
The findings from the present study justify the ameliorative effect of ascorbic acid against mercury induced toxicity and hence should be encouraged for use by consumers of mercury containing cosmetics, lipsticks, ointments and other mercury containing products. 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 mercury exposure.

REFERENCES


