Histomorphological Studies of the Cerebellum in Mercury Exposed Rats and the Role of Ascorbic Acid (Vitamin C)

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ABSTRACT

Mercury is a heavy metal contaminant of known toxicity with potential for global mobilization following its give off through air, soil, water and food. Aim: The present study was aimed at investigating the role of ascorbic acid on mercury induced alterations in cerebellum and body weights of Adult Wistar rats.

Methods: Twenty five (25) Adult Wistar rats with average weight 185 g were randomly divided into five groups of five rats per group (n=5); The animals in Group I (Control) were administered with normal saline, Group II received 49.8mg/kg body weight of mercuric chloride. Animals in Groups III and IV were treated 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 animals were administered with 49.8mg/kg body weight of mercuric chloride and 1,190mg/kg body weight of ascorbic acid. All administration was carried out orally for a period of three to six weeks and the animals were humanely sacrificed. Results: Results revealed alterations in cerebellum histoarchitecture involving necrosis, neuronal vacuolation, neuronal degeneration, cytoplasmic shrinkage and reduction in the number of Purkinje cells (p<0.05) in HgCl₂ intoxicated groups. However, ascorbic acid administration significantly ameliorated the induced degenerative changes in the cerebellum caused by mercury exposure in Wistar rats suggestive of its neuroprotective potentials against HgCl₂. The results also revealed significant decrease (p<0.05) in weight gain by mercury intoxicated rats when compared to weights gain by rats treated with ascorbic acid and rats in the control groups. Conclusion: The administration of ascorbic acid remarkably ameliorated HgCl₂ induced changes in the cerebellum histoarchitecture and body weights alterations in Wistar rats.

Key words: Cerebellum, Mercuric Chloride, Ascorbic acid, Body Weight, Wistar rats.
INTRODUCTION

Mercury intoxication has been a public health problem for many decades (Berlin, 2007; Burger et al., 2011). Consideration of the role of environmental factors in determining the susceptibility to mercury has recently been renewed by evidence from epidemiological studies (Wang, et al., 2007). Many populations Worldwide have been exposed to mercury through the consumption of fishes and sea foods (European Commision, 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). Mercury readily crosses the blood-brain barrier due to limited lipid solubility and can result in neurological symptoms; mental retardation, seizures, vision and hearing loss, delayed development, language disorders and memory loss (WHO, 2003, 2007; Animoku et al., 2018). It is a potential factor in brain damage (Ibegbu et al., 2014), mental impairment, behavioral anomalies (Farina et al., 2011), impaired cognitive functions and coma (Flora et al., 2007) while, mild subclinical signs of central nervous system toxicity can be seen in workers exposed to an elemental mercury level in the air (WHO, 2007). Mercuric salts can be absorbed through the skin of animals (Altmann et al., 2008) and distribute to all tissues reaching peak levels within hours or days (WHO, 2003). The urine and feces are the main excretory pathways of mercury compounds in humans (WHO, 2005; 2007). 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). Presenting symptoms include depression, headache, dizziness, itching, burning, irritability, excitability, restlessness, irrational outburst of temper, profuse sweating, tachycardia, frequent urination, increased salivation, and hypertension (Grant and Lipman, 2009; ATDRS, 2011). Ascorbic acid is an essential nutrient for humans and some other animal species. Vitamin C functions as an antioxidant that scavenges free radicals (Padayatty et al., 2003), prevents scurvy (WHO, 2001), pneumonia (Hemila and Louhiala, 2007) and may be useful in lowering the incidence of gout (Choi et al., 2009). Antioxidants are substances that nullify the effect of free radical by either inhibiting the initial production or inhibiting the preparative phase of free radicals (Sujatha et al., 2011). Vitamin C is found in high concentration in immune cells and is consumed quickly during infections (Preedy et al., 2010). Examples of antioxidants are Ascorbic acid (Vitamin C), Vitamin E and Vitamin A (Vasudevan and Sreekumari, 2007). 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 (UKFSA, 2007; Gemma et al., 2010). The aim of the study was to determine the role of ascorbic acid on mercury induced cerebellum and body weight impairments in Wistar 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 LD\textsubscript{50} of 166 mg/kg body weight as adopted from ATSDR (2011). While, the LD\textsubscript{50} 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 with reference Number ABU/FVM/EAUC/2015/12. The animals were dosed as follows: control group was administered with normal saline, group II with 30% mercuric chloride (HgCl\textsubscript{2}, 49.8 mg/kg) only, group III received HgCl\textsubscript{2} with distilled water only, group IV received HgCl\textsubscript{2} with 5% low dose ascorbic acid (595 mg/kg), while group V received HgCl\textsubscript{2} 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

Animal Sacrifice

After the administration, the animals were weighed and 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. The tissues were routinely processed for paraffin embedded histology and stained using H&E and Cresyl violet staining methods.

Tissue Processing Procedure

The fixed tissues were removed from the Bouin’s fluid and dehydrated using ascending grades of alcohol. This method involved dehydration of tissues in two (2) changes of 70% alcohol and two (2) changes of 90% alcohol, three (3) changes of 95% alcohol and three (3) changes of absolute alcohol, each of which lasted for 30 minutes. The dehydrated tissues were further cleared in two (2) changes of chloroform for two (2) hours each. The cleared tissues were infiltrated by
immersion into molten paraffin wax. The embedded tissues were blocked in rectangular blocks, while tissues were sectioned coronally using the rotary microtome at 5 µm per section. The tissue sections were allowed to float in water bath at 30°C to help the spreading of the paraffin ribbons. The clean slides were used to pick the tissues from the warm water bath. The slides were left to dry and later stained using H&E and Cresyl violet solutions.

**Cresyl violet staining method**

The tissue sections were deparaffinized and hydrated to distilled water and then stained for 5 minutes in Cresyl violet solution. The stained sections were rinsed in two changes of distilled water and placed in 95% alcohol for 30 seconds. Sections were transferred to absolute alcohol for 30 seconds and then placed in xylene for 1 minute and 2 minutes sequentially. Differentiations were made in absolute alcohol, two changes for 10 and 30 seconds each. The sections were then taken through several changes of xylene and mounted with synthetic resin. Digital photomicrographs were made from all the experimental groups with the aid of MD900 Amscope microscope digital camera.

**Cell Count Analysis**

Purkinje cells involving the cerebellum was counted using Digimizer image analysis software.

Photomicrographs of cerebellar cortical regions were uploaded into the image area of the software. This was followed by the utilization of marker tools to mark and count cells in the aforementioned regions. The numbers of counted cells were automatically indicated on the statistics area of the software, while results obtained were further subjected to statistical analysis.

**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 significant difference between control and experimental groups. A p-value < 0.05 was considered significant.

RESULTS:

**Physical observation of the animals**

On physical observation of the animals, the control group animals were very active and behaviorally stable while mercury treated animals were observed to be ataxic, apathetic, agitated, distressed, with diarrhea for the first 3 weeks of administration. However, there were improvements in activity, agility, and behavioral stability as observed in animals treated with ascorbic acid in the last 3 weeks of administration.

**Histological observation of the cerebellum**

The results revealed normal histoarchitecture and cellular layers of the cerebellum in the control group (Fig.1A and 2A), while animals in the HgCl₂ (49.8 mg/kg) only and HgCl₂ with distilled water groups revealed necrosis of cells, disorientation of Purkinje cells, vacuolation and congestion of cells (Fig.1B, 1C, 2B and 2C). However, HgCl₂ with low dose vitamin C (595 mg/kg) and HgCl₂ with high dose vitamin C (1,190 mg/kg) groups showed minimal
1. Control Group showing normal histoarchitecture of the Molecular layer (ML), Purkinje cell layer (PCL), Purkinje cell (PC) and Granular layer (GL).

2. Group II (48.9mg/kg mercuric chloride) showing the Molecular layer (ML), Separated Purkinje cells (SPC) and Granular layer (GL).

3. Group III (48.9mg/kg mercuric chloride and Distilled Water) showing the Molecular layer (ML), Vacuolated Purkinje cell (VPC) and Granular layer (GL).

4. Group IV (48.9mg/kg mercuric chloride and 595mg/kg ascorbic acid) showing the Molecular layer (ML), Separated Purkinje Cell (SPC), some Normal Purkinje cells (NPC) and Granular layer (GL).

5. Group V (48.9mg/kg mercuric chloride and 1,190mg/kg ascorbic acid) showing the Molecular layer (ML), Degenerating Purkinje Cells (DPC), some normal Purkinje cells (PC) and Granular layer (GL).

Cellular degeneration with some normal cerebellar cortical cells (Fig. 1D, 1E, 2D and 2E).
Figure 2: Photomicrographs of the Cerebellar cortices (Cresyl violet × 250)

1. Control Group showing normal histoarchitechture of the Molecular layer (ML), Purkinje cell layer (PCL), Purkinje cell (PC) and Granular layer (GL).
2. Group II (48.9mg/kg mercuric chloride) showing the Molecular layer (ML), Separated Purkinje cells (SPC), Degenerating Purkinje cells (DPC) and Granular layer (GL).
3. Group III (48.9mg/kg mercuric chloride and Distilled Water) showing the Molecular layer (ML), Degenerating Purkinje cell (DPC) and Granular layer (GL).
4. Group IV (48.9mg/kg mercuric chloride and 595mg/kg ascorbic acid) showing the Molecular layer (ML), Granular layer (GL), Normal Purkinje Cells (NPC) with some evidence of Degenerating Cells (DC).
5. Group V (48.9mg/kg mercuric chloride and 1,190mg/kg ascorbic acid) showing the Molecular layer (ML), Granular layer (GL), Numerous Purkinje Cells (NPC) with some evidence of Degenerating Cells (DC).

Cell Count Analysis

Number of Purkinje cells in the Cerebellar cortex There was significant decrease \((p<0.05)\) in the number of cerebellar cortical Purkinje cells in HgCl\(_2\) (49.8mg/kg) and HgCl\(_2\) (49.8mg/kg) with distilled water groups compared to the control, HgCl\(_2\) (49.8 mg/kg) with low dose vitamin C (595 mg/kg) and HgCl\(_2\) (49.8 mg/kg) with high dose vitamin C (1,190 mg/kg) groups. This decrease \((p<0.05)\) in the number of
cerebellar Purkinje cells was also observed in HgCl₂ (49.8 mg/kg) with low dose vitamin C (595 mg/kg) compared to the control group (Table 2).

### Table 2: Number of Cerebellar Purkinje cells counted

<table>
<thead>
<tr>
<th>Groups</th>
<th>Administration</th>
<th>Cerebellum (Purkinje cells) Mean ± SEM (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>Control</td>
<td>19.00 ± 1.18</td>
</tr>
<tr>
<td>GII</td>
<td>(HgCl₂ alone)</td>
<td>6.20 ± 0.97*</td>
</tr>
<tr>
<td>GIII</td>
<td>(HgCl₂ and Distilled H₂O)</td>
<td>7.20 ± 0.37*</td>
</tr>
<tr>
<td>GIV</td>
<td>(HgCl₂ and Vit.C595mg/kg)</td>
<td>14.80 ± 1.01*cd</td>
</tr>
<tr>
<td>GV</td>
<td>(HgCl₂ and Vit.C1,190mg/kg)</td>
<td>16.40 ± 0.75*ab</td>
</tr>
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</table>

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.

### Body weight assessment of Animals

The results of body weight assessment showed that there was progressive increase in the mean body weights of the animals in Groups I-V throughout the duration of the experiment. However, the degree of weight gain by the animals in Groups II and III decreased significantly (p<0.05) when compared to the weights gain by animals in Groups I (Control), IV and V as shown in Table 3 and Figure 3.

### Table 3: Body weight assessment following administration of mercury and ascorbic acid.

<table>
<thead>
<tr>
<th>Initial Week</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Final Week</th>
<th>% Weight Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
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<td>(g)</td>
<td></td>
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<td>SEM</td>
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</tr>
<tr>
<td></td>
<td>I (Normal saline)</td>
<td>II (HgCl₂ alone)</td>
<td>III (HgCl₂ and Distilled H₂O)</td>
<td>IV (HgCl₂ and Vit.C 595mg/kg)</td>
<td>V (HgCl₂ and Vit.C 1190mg/kg)</td>
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<tr>
<td></td>
<td>150 ± 10.99</td>
<td>147 ± 6.06</td>
<td>163 ± 11.27</td>
<td>142 ± 4.73</td>
<td>161 ± 3.41</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>11.34</td>
<td>12.56</td>
<td>12.38</td>
<td>5.90</td>
<td>5.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.54</td>
<td>3.74</td>
<td>3.78</td>
<td>2.74</td>
<td>5.61</td>
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<tr>
<td></td>
<td>9.31</td>
<td>14.32</td>
<td>16.69</td>
<td>7.26</td>
<td>8.58</td>
<td></td>
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<tr>
<td></td>
<td>12.03</td>
<td>.</td>
<td>17.97</td>
<td>9.55</td>
<td>2.85</td>
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<tr>
<td></td>
<td>10.33</td>
<td>.</td>
<td>19.52</td>
<td>9.64</td>
<td>3.67</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>7.76</td>
<td>.</td>
<td>20.24</td>
<td>10.89</td>
<td>3.14</td>
<td></td>
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<tr>
<td></td>
<td>6.09</td>
<td>.</td>
<td>7.13*</td>
<td>4.88*cd</td>
<td>1.75*ab</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 indicates significant difference compared to Group I (Normal saline). g = mean weight in grams. 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.

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

GI= Control, GII=HgCl₂ (alone), GIII =HgCl₂ and Distilled H₂O, GIV =HgCl₂ and Vit.C 595mg/kg, GV=HgCl₂ and Vit.C 1190mg/kg.

**Figure 3:** The % body weight change of animals throughout the experiment.
DISCUSSION

Mercury is a heavy metal contaminant with potential for global mobilization following its give off through air, soil, water and food from anthropogenic activities or natural processes (Gochfeld, 2003). The present study revealed histological changes ranging from degeneration of Purkinje cells, necrotic features, clumping and disorientation of cerebellar cortical cells in HgCl₂ exposed rats with or without ascorbic acid treatment while, the control group showed normal histology. These neurodegenerative alterations could actively affect the cerebellum resulting in cerebellar syndromes which include: muscular hypotonia, tremor, nystagmus, scanning speech and ataxic gait (Farina et al., 2011). In addition, degeneration and decrease in the number of Purkinje cells which result from exposure to HgCl₂ implies that activities associated with the cerebellum in fine movement, coordination posture, equilibrium will be impaired while motor learning function could also be lost (Fine et al., 2002; Ibegbu et al., 2014).

This study agree with the findings of several authors who reported that many heavy metals such as mercury, lead, cadmium and other organic compounds have the capacity to damage nervous system (Mahmoud, 2007; Ibegbu et al., 2014) because this system is very sensitive to mercury and permanent damage to the brain can occur from exposure to sufficiently high levels of mercury (ATSDR, 1999). After crossing into the brain, mercury may affect many different areas of the brain and their associated function, resulting in a variety of symptoms. These include personality changes, irritability, shyness, nervousness, tremors, changes in vision, deafness, muscle incoordination, loss of sensation and difficulties with memory (ATSDR, 1999; WHO, 2003) while the most sensitive elements of the cerebellar cortex to HgCl₂ are the Purkinje cells which react to this heavy metal by undergoing degeneration and as such disappear from their relative positions in the Purkinje cell layer (Farina et al., 2011) resulting in cerebellar dysfunction (Wolf et al., 2009). The present study also revealed progressive increase in the mean body weights of the animals throughout the period of administration of mercuric chloride and ascorbic acid. However, the degree of
weight gain by the animals treated with mercuric chloride alone throughout the experiment was less than that of the weight gain by animals treated with ascorbic acids and animals in the control groups. This difference in decreased body weight gain was significant (p<0.05). This agrees with the findings of Mohammad, (2009) who reported that the body weight of mercuric chloride treated rats was less than their controls significantly during the 8 weeks of exposure while Thomas et al., (2001) observed that exposure of rats, rabbits and dogs to metallic mercury vapour for 7h/day, 5 days/week for 72-83 weeks, resulted significantly in weight decrease. However, the present study showed that animals administered with ascorbic acid both at low and high doses showed significant improvements (p<0.05) when compared with animals intoxicated with mercury alone and this agrees to the fact that ascorbic acid can improve the oxidative stress effect of mercury substances (Bernhoft, 2012) which can result in reduced superoxide dismutase, catalase, glutathione and increased Lipid peroxidase levels (Farina et al., 2013). Hence, ascorbic acid as an antioxidant plays significant ameliorative role (p<0.05) in the reversion (to certain level) of mercury induced cerebellum impairment possibly 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 temporal lobe neurotoxicity and hence populations exposed to mercury poison should consume foods rich in ascorbic acid (vitamin C) along with other antioxidants.

**Conflict of Interest** None declared.

**Reference:**


