PREVENTIVE ACTIVITY OF ASCORBIC ACID ON LEAD ACETATE INDUCED CEREBELLAR DAMAGED IN ADULT WISTAR RATS

Among the environmental contaminants, lead is one of the most hazardous to living matter. In mammals, the main target is the central nervous system, particularly in the young. Ascorbic acid is an antioxidant which is a substance that may protect your cells against the effects of free radicals.

This study investigated the protective effect of ascorbic acid on the cerebellum of adult Wistar rats following oral administration of different doses of Lead acetate. Thirty adult Wistar rats of average weight of 215g were used in this study. The animals were divided into five (5) groups of six animals per group and were administered different doses of lead acetate (60mg/kg bwt of 1/10th LD₅₀ and 30mg/kg bwt of 1/20th of LD₅₀) and ascorbic acid (4.3mg/kg bwt) orally over a period of three (3) weeks.

Group 1 (control) was administered distilled water and Group 2 and 3 were administered 30mg/kg and 60mg/kg of Lead acetate respectively while Group 4 and 5 were given co-administration of 30mg/kg of Lead acetate+4.3mg/kg of ascorbic acid and 60mg/kg of Lead acetate+4.3mg/kg of ascorbic acid respectively. Histopathologically, Lead acetate induced cellular damage in the cerebellum of adult Wistar rats and it was also observed that ascorbic acid prevents or minimize lead-induced cellular damage in the cerebellum of adult Wistar rats.

**Keywords:** Lead acetate, ascorbic acid, cellular, cerebellar damage

**UDC:** 574:543.9+613.632:612.015

Introduction

Human and animal populations throughout the world are exposed on daily basis to low levels of environmental contaminants (Bolognesi and Morasso, 2000). The human race is burdened by an increasing load of environmental contaminants, affecting not just health and behavior, but finally survival of the species itself. Among these chemicals, lead (Pb) is one of the most hazardous to living matter. The metal is primarily found in leaded gasoline, (Srianujata, 1997; Tong, Von Schirnding, and Prapamontol, 2000). Automobile emissions have been an important source of lead exposure for urban residents, particularly in areas with congested traffic. The main source of adult human exposure is food, which is believed to account for over 60% of blood levels; air inhalation accounts for approximately 30% and water of 10% (John, Cheryl, Richard, and Christine, 1991).

Lead is highly toxic and can interrupt the body’s neurological, biological and cognitive function. Children are particularly susceptible, and according to the World Health Organization, high levels of lead exposure can cause brain, liver, nerve, and stomach damage, as well as permanent intellectual and developmental disabilities (Elombah and HRW, 2012).

Lead poisoning is rarely fatal, but medical workers in Nigeria reported that the lead concentration in Zamfara State ore is so toxic that in 2010 in seven villages the mortality rate was estimated as high as 40 percent among children who showed symptoms of lead poisoning (Elombah and HRW, 2012). In a study conducted in Jos, Nigeria, risk factors...
such as the use of lead-containing eye cosmetics, chipping paint in the home and proximity to battery smelters and gasoline sellers have been identified as the major sources of high blood lead levels amongst the inhabitants (Wright, Thacher, Pfitzner, Fischer, and Pettifor, 2005).

The involvement of the nervous system in lead toxicity is well known. Encephalopathy with Seizures and coma is one of the most striking and serious complication of lead poisoning (Martin, Paul, and Thomas, 1970). Cerebellar dysfunction may occur in association with exposure to a wide variety of toxins including heavy metals (such as mercury, lead, thallium, and manganese), drugs and solvents. These toxins may adversely affect the cerebellum directly or as part of a more generalized encephalopathy. Practically, all toxins in high dose can cause neurologic sign and symptoms (Fredericks, 2011). Permanent abnormalities are induced only by sustained use of exposure of these chemicals in a greatly excessive dosage and the most sensitive elements of the cerebellar cortex to these chemicals are the Purkinje cells (Maurice and James, 1972).

The toxic effects of lead are treated with by chelation therapy which also depletes the body store of essential cations (Ruff, Markowitz, and Bifur, 1996). Therefore there is need to look for an alternative solution to lead poisoning.

Ascorbic acid is an antioxidant. Antioxidants are substances that may protect our cells against the effects of free radicals. It is a water-soluble vitamin that is found intra- and extracellularly as ascorbate (Chihuailaf, Contreras, and Wittwer, 2002). It is a natural antioxidant that prevents the increased production of Free Radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues (Sies, Stahi, and Sundqvist, 1992). It has been shown to react directly with superoxide (Nishikimi, 1975; Hemila, Roberts, and Wikstrom, 1985), hydroxyl radicals (Bielski, 1982) and singlet oxygen (Bodannes and Chan, 1999). It is generally regarded as a primary first-line protective agent that repairs or nullifies Free Radicals by donating a single electron, followed by a proton to yield a chemically dehydroascorbic acid (Carr, Zhu, and Frei, 2000; Halliwell, 2001). Ascorbic acid plays a significant role in the toxicity inversion of lead by forming inert complexes and inhibiting its toxicity on the dopaminergique neurons (Satija and Vij, 1995).

In this present study we aimed to evaluate the protective effect of ascorbic acid on the cerebellum of adult Wistar rats following oral administration of Lead acetate.

**Materials and methods**

*Purchase of Chemicals:* 500g of Lead acetate of 99% to 103% purity, manufactured by BDH Chemicals Ltd England and with the product No 29021 was purchased from a reputable Chemical supplier, Cardinal Scientific, No.11/12 Samaru/Sokoto Road, Opposite Longman Nig. Plc. The chemical was taken to the Department of Chemistry Ahmadu Bello University Zaria for identification and it was confirmed to be Lead acetate. Ascorbic acid (Vitamin C) tablets manufactured by a reputable Pharmaceutical Company Cinnamon Drugs Ltd. Plot C9 Emene Industrial layout, Emene Enugu, Enugu State, were purchased from a licensed Pharmaceutical Store, Samaru, Zaria, Nigeria.

*Experimental Animals:* Thirty (30) healthy adult Wistar rats (14-16 weeks old) of average weight of 215g reared in the Animal House of the Department of Human Anatomy, Ahmadu Bello University, Zaria, Nigeria, were used for this study. The rats were acclimatized to experimental condition for a period of two weeks in the same Department and fed with rat chow and clean tap water.
Experimental design: Based on the reported oral LD$_{50}$ of Lead acetate for Wistar rats by Sujatha et al., 2011, we administered 1/10$^{th}$ of LD$_{50}$ (60mg of lead acetate/kg body weight) and 1/20$^{th}$ of LD$_{50}$ (30mg of lead acetate/kg body weight) in this study.

Route and duration of administration: Lead acetate and ascorbic acid were administered orally by gastric gavage once per day for three weeks.

### Table 1. Showing the Grouping and the Treatment of the Animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>No of animals</th>
<th>Treatment (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Distilled water (control)</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>30mg/kg Lead acetate</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>60mg/kg Lead acetate</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>30mg/kg Lead acetate + 4.3mg/kg Ascorbic acid</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>60mg/kg Lead acetate + 4.3mg/kg Ascorbic acid</td>
</tr>
</tbody>
</table>

Animals Sacrifice: the weights of the animal were taken with a laboratory weighing balance. The animals were then anaesthetized through chloroform inhalation. Incision was made through the skin and muscle of the skull. The skull was opened through the mid sagittal suture and the cerebellum was removed and fixed in Bouin’s fluid and the tissues were process for histological study using bench model Automatic Tissue Processor, available in the histology laboratory of Human Anatomy Department, Ahmadu Bello University, Zaria. The processed tissues were embedded in paraffin and stained in Haematoxylin and Eosin and viewed under a light power microscope and photomicrographs were taken using digital optical eyepiece.

Results

Photomicrographs

**Figure 1. Photomicrograph of Group 1 (Control), Transverse section of cerebellum. Showing the Molecular layer (ML), Purkinje layer (PL) and Granular layer (GL). H & E, Mg X 250**

**Figure 2. Photomicrograph of Group 2 (30mg/kg BW of Lead acetate), Transverse section of cerebellum. Showing the Molecular layer (ML), Purkinje layer (PL) and Granular layer (GL). H & E Stain X 250**
Histopathological studies

No histopathological changes were observed in control group 1, and all the layers of the cerebellar cortex were intact as seen in Figures 1 above. In experimental group 2 which was administered 30mg/kg BW of Lead acetate; there was cellular degeneration of cells in the Purkinje layers. In experimental group 3 administered 60mg/kg BW of Lead acetate, there was more pronounced cellular damaged of the Purkinje and granular layers than in group two. While in the co-administration groups 4 and 5 there were lesser cellular damage of Purkinje and granular layers in these groups when compare with group two and three. In group 5 there was more protection than group 4. The molecular layer seems not to be affected at all in group 5; this is due the protective of ascorbic acid.
Discussion

In this study, cellular damage of the cerebellar layers of adult Wistar rats was induced by different dosages of lead acetate as showed above in Photomicrographs. This observation agrees with the reports of Maurice and James (1972), that stated that many heavy metals such as lead, mercury, etc and other organic compounds have the capacity to damage nervous system and the most sensitive elements of the cerebellar cortex to these chemicals are the purkinje cells in the middle purkinje layer; The report was also supported by the work of Fredericks, 2011. Our study further confirmed these two findings. Cerebellar dysfunctions may occur in association with exposure to a wide variety of toxins including heavy metals and Martin et al., 1970: the involvement of the nervous system is well known. Encephalopathy with Seizures and coma is one of the most striking and serious complication of lead poisoning as earlier documented. Even at low BPb levels, lead exposure has been associated with deficits in the early developmental years. Canfield et al.’s study (2003) on the effects of low level lead poisoning (<10 µg/dL) on cognitive functioning in children and infants, found that IQ declined 7.4 points as average blood lead concentrations increased from 1 µg/dL to 10 µg/dL, and then declined 4.6 points for every 10 µg/dL increase after that. Lead exposure has also been linked to behavioral problems. In his landmark study, Needleman et al., (1979) measured dentine Pb levels in 312 first-and second-grade students (mean age 7.3 years), and administered neurobehavioral tests. The results showed that behavioral and performance problems in primary school children were more prevalent with increasing dentine (teeth) lead levels. These behavioral problems included hyperactivity, distractibility, impulsivity, disorganization, non-persistence, inability to follow simple instructions and overall poor functioning. Lead elicits it’s activity through the generation of free radicals which are very harmful to the cells in the living system. Ascorbic acid is generally regarded as a primary line protective agent that repairs or nullifies Free Radicals by donating a single electron, followed by a proton to yield a chemically dehydroascorbic acid (Carr et al., 2000; Halliwell, 2001). Ascorbic acid plays a significant role in the toxicity inversion of lead by forming inert complexes and inhibiting its toxicity on the dopaminergique neurons (Satija and Vij, 1995). The scavenging activities of ascorbic acid was demonstrated in these present study. The groups co-administered with ascorbic acid show some degree of protection to the cells of the cerebellum.

In conclusion, it is also established in this study that administration of ascorbic acid has a clear improvement in preventing the degeneration of brain cells when compare with rats exposed to lead acetate. Lead exposure induced a significant degeneration in the cerebellum of adult Wistar rats and the need to prevent the neurotoxicity induced by lead acetate using ascorbic acids has been established. And the preventive effect of ascorbic acid was dose dependent. The damage that lead poisoning can have on the neuropsychological functioning of the central nervous system can be devastating. It can affect anybody, at any age, although children are most often the worst affected, as their cognitive development can be disrupted. Now we are beginning to realize that there are consequences beyond what was once thought the neurological endpoint for this harmful substance, and hopefully we will one day fully understand the mechanisms by which lead destroys the mind. It is also established in this study that administration of ascorbic acid in groups four and five have a clear improvement in preventing cerebellar degeneration when compare with rats exposed to lead acetate only (group two and three). Our study further validate the antioxidant activity of ascorbic acid as earlier reported by other studies.

Recommendations

From our findings in the study we are recommending that the preventive activity of ascorbic and other antioxidants on Lead acetate induced cerebellar degeneration should further be investigated on a large sample size of experimental animals. The use of Lead should be minimize or avoided, especially in materials such as petroleum fuel. Also analysis of the components of natural ores should be carried out before extraction; consumers of lead containing products such as paints, house dusts, pesticides, and some industrial products such as glazes, colorings for ceramics, hose and pipes should always be conscious of the percentage of lead in them and people in urban residents particularly in
area with congested traffic and those using products containing Lead should eat food rich in ascorbic acid and other antioxidants.

**References**


Elombah.com and HRW. (2012). Nigeria’s Child Lead Poisoning Crisis is ‘worst in modern history’ HRW.


