SOME HISTOLOGICAL OBSERVATIONS ON THE CEREBELLAR CORTEX OF ADULT WISTAR RATS FOLLOWING ALUMINIUM CHLORIDE EXPOSURES

Ajibade A. J.*, Fakunle P. B. and Atanda O. M.

1Department of Anatomy, LAUTECH Ogbomoso, Nigeria.
2Department of Anatomy, LAUTECH Ogbomosu Nigeria.

*Corresponding Author: Ajibade A. J.
Department of Anatomy, LAUTECH Ogbomoso, Nigeria.

ABSTRACT
Aluminium, is regarded to be the most abundant element in the earth crust, has long been known to have no significant biofunction in the human body. However, research findings have shown that Aluminium chloride is implicated in several health complications within the body system. This study investigated the possible effects of aluminium chloride exposure on the cerebellar, cortex in adult rats. 32 adult Wistar rats were divided into four groups; group A was regarded as the control group, group B received 0.2g/kg, group C received 0.4g/kg, while group D received 0.6g/kg of Aluminium chloride for a duration of three weeks. At the end of three weeks of administration, the Wistar rats were sacrificed by cervical dislocation after which the brains were harvested, weighed and fixed immediately in formol calcium. The cerebellar tissues were processed for light microscopy. Results showed statistically significant decrease in the body weights but the brain weights of aluminium- treated -rats were not significantly different from the controls at P<0.05 . However, histological studies indicated that the cerebellar cortex of aluminum-treated showed neuronal degeneration and cellular loss which caused neuronal vacuolation and necrosis in treated rats compared with the cerebellar cortex of the controls that appeared normal. The study concluded that exposure to high levels of aluminium chloride in wistar rats resulted in neuronal degeneration which may ultimately result in compromise of cerebellar functions.

KEYWORDS: Aluminium, Cerebellar Cortex, Purkinje cells, Neuronal Degeneration.

INTRODUCTION
Concern has been raised from previous studies about the potentials danger associated with exposure to aluminium chloride as it has been clearly shown that aluminium accumulates in various mammalian tissues such as kidney, brain, liver and bone[1] and is similarly accompanied by renal failure[2] or associated with age factor.[3] Reports have indicated that aluminium, (AI), is ubiquitous in the environment and its extensive industrial utilization and applications have stimulated considerable interest in the possible environmental toxicity of this substance[4]. Aluminium chloride occurrence is not usually in its pure state but is always in association with other elements such as hydroxide, silicate, chloride, phosphate and sulphate. The wide distribution of this substance triggers the potential risk and hazard for human exposure and danger.[5,6] The distribution of Aluminium to the environment is by natural processes from various anthropogenic sources. Aluminium being a metallic substance with a melting point of 649.8°c, which is used for a different applications and uses[7] Report has indicated that human exposures to aluminium have been increasing over the last decade and consequently, patients on dialysis or on long-term treatment with total parenteral nutrition have been documented to be associated with accumulation of this metal in different organs Yokel and.[7,8] Furthermore, investigators have suggested that there is a relationship between high levels of aluminium and increased risk of a number of neurodegenerative disorders including dialysis, encephalopathy, Alzheimer’s disease (AD) and Parkinson’s disease (PD).[9,10,11] Similarly, Submissions from epidermological studies have indicated a link between aluminium in drinking water and Alzheimer’s disease and a variety of human and animal studies have indicated an association between learning and memory deficits after aluminium exposure.[4] Aluminium chloride has been reported to have adverse effects on anxiety-relative behavior of wistar rats as it could leads to increased rate of anxiety in aluminium treated rats.[4] Additionally, report has shown neurodegenerative effects of aluminium chloride on the microanatomy of cerebellar cortex of adult Wistar rats especially at higher close and also a reduced level of sperm count, but did not result into infertility.[12] Aluminium is known to a neurotoxic to the central nervous, skeletal and hematopoietic systems.[13] The primary source of oral aluminium exposure in the US for the typical human is foods.
representing approximately 95% of daily oral intake; drinking water contributes 1 to 2%. \(^{13}\) In particular, Aluminium both exerts direct neurotoxicity in primary human neural cells and induces neurodegeneration, through an increase in Fe accumulation and reactive oxygen species (ROS) production. Accumulation of aluminium in rabbits, rats, and rat pups, contributes to a variety of cognitive impairments.\(^ {14-15}\) (Muller et al, 1990; Mari, 2001). A recent study carried out in mice shows that there is an increase in inflammatory processes in the brain following chronic exposure to aluminium in drinking water. However, drinking water confuses the issue because it contributes only a minor portion of the total daily oral intake of aluminium.\(^ {16}\) Literature has shown different pathways in association with accumulation of aluminium in the brain of AD patients.\(^ {16}\) Furthermore, previous investigations have indicated that human exposure to Aluminium (Al) has been increasing over the decades, as it appears mostly in products of food and in drinking water derived from both natural sources and treatment methods.\(^ {17}\)

The cerebellum is known to be a region of the brain that performs an important role in the motor control integration of sensory neuronal perception. In order to coordinate motor control, there are many neural pathways connecting the cerebellum with the cerebral motor cortex (which sends impulses to the muscles causing them to move) and the spino-cerebellar tract (which provides proprioceptive feedback on the position of the body in space).\(^ {18}\)

The cerebellar cortex has three layers. At the innermost lies the thick granular layer, densely packed with granule cells, along with interneurons, mainly Golgi cells but also including Lugaro cells and unipolar brush cells. In the middle lies the Purkinje layer, a narrow zone that contains a row of cell bodies of Purkinje cells and Bergmann glial cells. At the outer part lies the molecular layer, which contains the flattened dendritic trees of Purkinje cells, along with the huge array of parallel fibers penetrating the Purkinje cell dendritic trees at right angles. This outermost layer of the cerebellar cortex also contains two types of inhibitory interneuron’s, stellate cells at the outer cycle and basket cells at the inner cycle.\(^ {19}\) In view of the neurotoxic effects of aluminium on the brain as previously reported, this study appraised the possible morphological effects of aluminium chloride on the cerebellar cortex in Wistar rats.

**MATERIALS AND METHODS**

**Location and duration of study:** This study was conducted at the Animal house of the Department of Human Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. The preliminary studies, animal acclimatization, actual animal administration of aluminium chloride to the test animals lasted for three weeks, while they were acclimatized for 2 weeks.

**Care of Wistar rat**

Wistar rats weighing from 110-200g at the first week of acclimatization were used for experimental design. A total number of 32 rats was used. The experimental rats were housed in standard plastic cages and maintained under standard laboratory conditions of temperature and under a controlled humidity condition, fed with chow and water daily. The experimental rats were separated into four groups of eight each. The wistar rats were also given adequate care in accordance with the principles of laboratory and animal care as indicated and published by Institute of Laboratory Animal Resources 1996.\(^ {20}\)

**The preparation of aluminium chloride solution**

Aluminium chloride preparation: 10g of Aluminium chloride was dissolved in 100mls of distilled water and administered to the rats daily for three weeks based on their body weights in relation to average human weight in mls.

**Experimental Design and Groupings**

Before acclimatization period, rat were weighed using a sensitive weighing balance and separated into groups based on their weights, comprising eight rats of four groups. The groups are as follows.

- **Group A**- control; this group has 8 male adult Wistar rats and was fed with rats feed and water was given to them also.

- **Group B**- low dosage; this group has 8 adult Wistar rats and 0.2mls of aluminium chloride solution was administered, for duration of 3 weeks.

- **Group C**- average dosage; this group has 8 adult Wistar rats and 0.4mls of aluminium chloride solution was administered, for duration of 3 weeks.

- **Group D**- high dosage; this group has 8 adult Wistar rats and was administered with 0.6mls of aluminium chloride solution for 3 weeks.

**METHOD OF ADMINISTRATION**

Aluminium chloride solution was administered orally, using an oral cannula every morning between the hours of 9am and 10am for 3 weeks. The weight of each rat was taken every week, throughout the period of administration, aluminium chloride solution was not administered to the control group, but just feed with rat feed and water. The aluminium chloride stock solution was always prepared daily so as not to lose it concentration.

**Weekly Body Weight Measurement**

The experimental rats were weighed and measured daily 0,7,14 and 21 respectively using a measuring weighing balance and the differences in weights in relation to the initial weight per group were calculated on each occasion.
Sample Collection
At the end of experimental period, the rats were sacrificed by cervical dislocation, the abdomen of each rat was carefully dissected and 2mls of blood was carefully collected into EDTA bottle which was immediately kept in the refrigerator. The brain from both control and test animals were removed and weighed to the nearest 0.01g and part of it were cut and pulverized using sterile laboratory mortar and pestle in which 5mls of acid phosphate was added to it and were collected in a plain bottle which was immediately place in the refrigerator for biochemical analysis and the remaining part of the brain was immediately fixed in 10% buffered formol calcium for histological analysis.

STATISTICAL ANALYSIS
Data were expressed as Mean ±SEM. The differences were compared for statistical significance by student’s t-test. Descriptive and inferential statistics was applied to the results. All statistical analysis were performed using SPSS version 6.0.

Table 1: Table Showing the Mean ± SEM of the Body Weight of Wistars Rats During Administration of Aluminum chloride
The table revealed the body weights of Wistar rats which started decreasing after exposure to aluminum chloride, except for control group (A) and the group that received a low dose of aluminum chloride 0.2g/kg(Group B). Wistar rats that received a medium dose 0.4g/kg of aluminum chloride (Group C), experienced a slight loss in weight. Wistar rats that received a high dose 0.6g/kg of aluminum chloride (Group D) also showed a significant weight loss (-15.43%).

Body weights of Wistar rats in Group A increased from mean value of 171 ± 9.70 at the beginning of the treatment to a mean value of 189.5 ± 1.09 at the end of the treatment denoting 26.43% weight gain.

Body Weights of Wistar rats in Group B increased from mean value of 148.5 ± 7.09 at the beginning of the treatment to a mean value of 159.5 ± 2.22 to the end of the treatment denoting 15.71% weight gain which shows a significance difference (P<0.05) when compared with the control (Group A).

Body weights of Wistar rats in Group C decreased significantly (P<0.05), from mean value of 162.3 ± 6.93 at the beginning of the treatment to a mean value of 159 ± 5.72 to the end of the treatment denoting -4.71% weight loss and it is statistically significant (P<0.05), compared to control group (group A).

Body weight of animals in Group D decreased significantly (P<0.05), from mean value of 163 ± 8.15 at the beginning of the treatment to a mean value of 152.5± 8.15 at the end of the treatment denoting -15.43 weight loss which is statistically significant (P <05) compared with the control (Group A).

Table 1: Initial And Final Body Weight Analysis Of Wistar Rats.
<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Weight (g)</td>
<td>171 ± 9.70</td>
<td>148.5 ± 7.09</td>
<td>162.3 ± 6.93</td>
<td>163.3 ± 8.15</td>
</tr>
<tr>
<td>Final Weight (g)</td>
<td>189.5 ± 1.09</td>
<td>159.5 ± 2.22</td>
<td>159 ± 5.72</td>
<td>152.5 ± 8.15</td>
</tr>
<tr>
<td>% Weight Gain or Loss</td>
<td>18.5%</td>
<td>6.5%</td>
<td>-3.3%</td>
<td>-10.8%</td>
</tr>
</tbody>
</table>

Values are represented as Mean ± SEM *P< 0.05 Significant when compared with Control group

Table 2: Showing The Mean ± SEM Of Weights Of Brain And Relative Brain Weights Of Rats After Sacrifice.
<table>
<thead>
<tr>
<th>Group</th>
<th>Brain Weights(g)</th>
<th>Relative Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.95 ± 0.04</td>
<td>1.03%</td>
</tr>
<tr>
<td>B</td>
<td>1.55 ± 0.07</td>
<td>0.97%</td>
</tr>
<tr>
<td>C</td>
<td>1.48 ± 0.09</td>
<td>0.93%</td>
</tr>
<tr>
<td>D</td>
<td>1.55 ± 0.10</td>
<td>1.02%</td>
</tr>
</tbody>
</table>

Values are represented as Mean ± SEM, P > 0.05, Not Significant when compared with Control group

From table 2., showing the brain weights in the various groups of animals. The brain weights in group A was 1.95± 0.04 which decreased insignificantly[ P > 0.05] to 1.55±0.07 in group B and 1.48± 0.09 in group C and decreased to 1.55± 0.10 in group D. Average brain weights of aluminum treated groups were reduced compared with the control.

**Fig 1. Brain Weight of Various Groups.**

Fig1: Graph showing the various brain weights in the various groups. Average brain weights of aluminum treated groups were reduced compared with the control group as shown above.
Histological Findings: The transitional region between the layers of the cerebellar cortex (molecular (M); purkinje (P) and granule (G) layers) was focused at higher-power magnification to study neuronal arrangements and morphology across study groups.

Normal morphological presentations of the cerebellar cortex are observable in group A with a mildly similar observation in group B. Cellular morphology in group A is characterised by purkinje cells (black arrows) with conspicuous cell bodies and dendrites that are projecting deep into the molecular layers (which has sparse nuclei), assuming the shape of a fan. Also, the granule layer in these groups consist of small granule neurons (black arrows), which are compactly disposed in contrast to the loosely arranged and cryptic (red arrows).

Cells in the granule layers of groups C and D. Degenerating purkinje cells (dotted red circles) with pyknotic cell bodies and short dendritic processes can be seen around the indistinctly demarcated cerebellar layers of groups C and D.

Furthermore, the neurophil in these two groups appear fragmented with irregular shaped and sized neuronal cells (red arrows). Groups B-D showed varying degrees of degenerative changes with severity progressing from groups B-D.

Plate 1: Photomicrograph of the cerebellar cortex of group A (control group) wistar rats with H&E staining, showing the different cell layers with proper identification by the arrows: Black arrow showing the medullary layer of white matter with normal cell orientation. The Green arrow shows the Purkinje cell layer with proper orientation. The white arrow indicates the granular cell layer. The Blue arrow shows the molecular cell layer.

Plate 2: Photomicrograph of the cerebellar cortex of group A (control group) wistar rats, with H&E staining showing a magnified view of the different cellular layers and orientations. The yellow arrow shows the purkinje cell layer which shows enlarged cells, while the white arrow shows the granular layer of cells which are somewhat scattered.

Plate 3: Photomicrograph of the cerebellar cortex of group B Wistar rats that received aluminium chloride with a low dose of 0.2g/kg for 21days showing the cell layers. On the molecular layer, are scattered molecular cells with diverse orientations. A reduction in cell number is noticed in the purkinje cell layer.

Plate 4: Photomicrograph of the cerebellar cortex of group B Wistar rats that received aluminium chloride with a low dose of 0.2g/kg for 21days Sparsely located clusters of granular cells on the granular cell layer. Here, the purkinje cell layer is not visible on this slide.
DISCUSSION

The brain weights in aluminium-treated group were insignificantly reduced compared with the control. The treatment appeared not to have significant effect on the brain weights of treated rats. The relative brain weights showed that group B rats gained less brain weights (0.97%) compared with the control group. Group C rats decreased in percentage brain weights (0.93%) when compared with the control groups and group D rats showed an increase in percentage brain weights (1.02%) compared to group C & D but decrease in the brain weights in aluminium-treated groups were insignificant compared with control group. The decreased in brain weight appeared to be dose-dependent in group B and C while in group D highest dose, there was insignificant increase in brain weight. It implies the effect did not follow a consistent dose-dependent pattern as reported in this study. The results of the histology findings in group B rats treated with aluminium chloride shows a neurodegenerative effect on the cerebellar cortex of an adult Wistar rats and this findings is consistent with the earlier findings as reported[4], whose work demonstrated that Wistar rats exposure to aluminium chloride for a duration of eight weeks resulted to neuronal vacuolation and necrosis of the cerebellar cortex in form of neurodegeneratio., He similarly observed extensive neuronal vacuolation and necrosis in the highest dose of aluminium treated groups, as the cerebellar cortex is known to play a key role in learning and memory, it then follows that neurodegeneration observed in the histology of cerebellar cortex of adult Wistar rats could go along way in affecting these specific brain functions such as Memory, learning new skills, consciousness.. The neurodegenerative changes observed in the cerebellar cortical layers of aluminium-treated rats in this study are in agreement with the neurodegenerative effect of aluminium on the histology of cerebral cortex of adult Wistar rats especially at higher dose as previously reported.[4,12]
Similarly, The histological changes in the cerebellar cortical layers of aluminium–treated rats in this study are in association with the observations that showed prominent Purkinje cell loss in the aluminium treated group when compared with the control. in the cerebellum of Wistar rats as reported[ Buraimoh, et al.,2014]. Aluminium- treated groups showed prominent loss and neurodegeneration of Purkinje cells in the treated wistar rats compared with the controls in this study. The result of the histology findings in group D showed a deviation from the normal histology of the cerebellar cortex as compared with the control group. Necrosis, vacuolation and degeneration of cells observed in this group of high dose of aluminium-treated rats and this agrees with the earlier research findings.[21] This study revealed neuronal loss and degenerative changes following aluminium exposure, consequently, his may support a hypothetical statement that aluminium exposure has neurodegenerating effect resulting in learning deficits in rats as previously observed[22] which indicated that in human aluminium inhibits learning. Cortical damage which resulted in gross neuronal loss particularly the Purkinje cells in the middle layer and cortical distortion in the treated rats might be due to the adverse effects of aluminium chloride. The weight loss or significant decrease in the body weights of aluminium –treated groups observed in this study is consistent with the previous report that aluminium chloride exposure resulted into weight loss in Wistar rats as indicated in their studies.[23]

This study concluded that aluminium chloride has adverse effect on the cerebellar cortex of adult Wistar rats which ultimately could impair some cerebellar functions.

CONSENT
It is not applicable.

ETHICAL APPROVAL
All authors hereby declare that the principles of laboratory animal care [NIH publication No. 85–23 revised 1985] were followed as well as specific national laws where applicable. All experiments have been examined and approved by the relevant ethics committee.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES