Effect of Alpha-Lipoic Acid on Aluminium Induced Toxicity in the Prefrontal Cortex and Hippocampus of Wistar Rats

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Abstract
The aim of the present study was to determine the protective effect of Alpha lipoic acid (ALA) on Aluminium chloride (AlCl₃) induced cerebral and hippocampal toxicity in Wistar rat. The experimental design involved 5 groups of 5 rats that were treated with saline, Aluminium chloride and/or Alpha-lipoic acid; for 2 weeks. At the end of the treatment phase, Morris water maze test was conducted to estimate the spatial memory function before the animals were euthanized and the Prefrontal cortex (PFC) and Hippocampus tissues evaluated. The behavioral studies showed that AlCl₃ induces significant decline in spatial memory which was corroborated with histological features of loss of neural density and necrosis of pyramidal cells. Co-exposure of Aluminium with Alpha-lipoic acid was associated with sparing of spatial memory with maintenance of brain structure. The findings from the present study confirms that the structural and functional neurotoxicity of Aluminium can be ameliorated with the concomitant usage of Alpha-lipoic acid during the Aluminum exposure.

Keywords: Prefrontal Cortex, Hippocampus, Alpha-Lipoic Acid, Aluminium Chloride

INTRODUCTION
Exposure to Aluminum (Al) is frequently caused via food additives and water use (Sadeeq et al., 2013). Aluminum is known to be neurotoxic and triggers neurodegeneration by increasing the creation of reactive oxygen species and ions (Wu et al., 2012). The amount of Aluminum (Al) in the brain is significantly higher in Alzheimer's disease (AD). The Al was found to accelerate the accumulation of the hallmarks of AD disease called neurofibrillary tangle (Drago et al., 2007; Zaky et al., 2013). Chronic Al accumulation is also intimately linked to numerous other neurodegenerative conditions, including Parkinson's disease, dialyzed encephalopathy, and amyotrophic lateral sclerosis (Azeez et al., 2015; Mujittapha et al., 2022).

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Al is therefore frequently regarded as a key environmental pathogenic factor for neurological disease.

The toxic effect of Al on the brain is largely on the prefrontal cortex and Hippocampus. The regions are known to be responsible for processing information associated with emotional response, memory and learning (Walton, 2007; Wu et al., 2012; Ribe et al., 2008). Studies have shown that in Prefrontal cortex and Hippocampus, Al causes astrocytes to degenerate through apoptosis, which results in the death of neurons. Some studies have shown that Al makes neurons to atrophy and disintegrate. The toxic effect of Al in inducing neuronal degeneration and death leads to the manifestation of cognitive decline which can be measured in Morris water maze task (MWM) (Al-Amin et al., 2016). The test has been extensively employed in studying the relationship between hippocampal function of spatial learning and memory in rodents. Detailed analyses have shown that rats can solve the task using a minimal set of cues that involve angular separation and distance from the tank wall (Bannerman et al., 2002). The test is based on the rodents' inherent tendency to avert swimming in water, and it requires the animals to use visual cues to learn to identify the position of a platform submerged in water which provides a route to escape (Morris, 1981).

The inhibition of the toxic effect of Al is commonly achieved by antioxidants and has remained an important strategy against all forms of Al toxicity. Alpha-lipoic acid has been of great value in stroke and traumatic brain injury animal models and it restores the blood-brain barrier, and optimizes the glutathione levels (Schreibelt et al., 2006). ALA is a natural cofactor for mitochondrial enzymes and is critical in breaking down fatty acids and, increase the antioxidant capacity of the tissue. The aim of the current study was to assess the structural and functional impact of the neuroprotection of ALA on the development of an Al toxicity model.

MATERIAL AND METHODS

Drugs and Reagents
The Alpha-lipoic acid was purchased from Natrol LLC (Chatsworth, USA), normal saline and syringe were purchased from Haman Super Store and Pharmacy LTD, (CN 1628570 Kano, Nigeria). Aluminium chloride was collected from the Faculty of Medical science, Department of Biochemistry, Bayero University Kano.

Experimental Design
Twenty five 25 adult Wistar rats aged 8-10 weeks with average weight of 150g were procured from the Department of Anatomy, Faculty of Basic medical science, Bayero University Kano. They were acclimatized in animal treatment room for two weeks under standard conditions of animal housing (a 12-hour light/dark cycle, temperature 25° C, and humidity 55–60%). The animals had free access to feed and water ad libitum. All animal handling protocols were in accordance with the National Health Institute (NIH) and Institutional Animal Care and Use Committee (IACUC) guidelines and approved by Animal Care and Use Research Ethics Committee (ACUREC) of the Bayero University Kano, Nigeria.

Experimental Groups
After two (2) weeks of acclimatization, the Wistar rats were randomly divided into five (5) groups of five (5) rats per group. Different concentrations of Alpha-lipoic acid (ALA) and Aluminium chloride (AlCl3) were administered to different groups as stated below:
- Group I (control): 2mL/kg of saline
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- Group II: 500mg/kg of AlCl₃
- Group III: 200mg/kg of ALA
- Group IV: 100mg/kg of ALA followed immediately by 500mg/kg AlCl₃
- Group V: 200mg/kg of ALA followed immediately by 500mg/kg AlCl₃

All administrations were orally done on daily basis for two weeks.

Morris Water Maze Task

Learning and working memory
The rats were gently placed in the water, facing the Morris water maze tank wall, with their tail making contact with the water first, at one of the quadrants of the tank. The swimming activities of the rats in the MWM tank was synchronized with the filming software of the Any-maze downloaded on a personal computer linked with a recording camera. The recordings as well as timing for the duration of swimming were automatically captured by the software immediately the animal climb on the escape platform or when the total pre-programmed time setting was attained. The pre-programmed trial time was set at sixty (60) seconds per trial as a standard for acquisition. Any animals that failed to find the platform within this time limit was either placed on the platform or guided to it. All animals were left for 30s on the platform during the inter-trial interval and this was to ensure that the animals got properly oriented to its position in space in relation to the escape platform and the surrounding cues. A total of 4 trials per day for 4 days were carried out which usually last about 5 min per animal on the first day. It is expected that the time spent for the entire daily trial sessions must progressively decline for each animal with each day and this was taken as a pointer to both learning and good level of working memory.

Probe trial
Probe trial was conducted to examine the cognitive function of the rats by locating the platform. This was used to quantify the spatial memory. The platform was removed from the pool. The rats were placed in a novel start position in the maze, facing the tank wall. The number of platform-site crossover, time and distance spent in the target quadrant compared with the other quadrants were measured as evidence of memory.

Animal sacrifice
After assessment of spatial memory function of the animals, they were placed in a container (desiccators) with cotton wool soaked in chloroform. The inhalational container was tightly covered so as not to allow air in or out of the container. Shortly, the animals were anesthetized by the chloroform and killed by decapitation. The heads were carefully dissected and brains immediately removed and immerse into the 10% formalin for fixation.

Preparation of histological tissues slides
The fixed tissue was washed severally and undergo dehydration in ascending concentration alcohol; 30, 50, 70 and 90% over 3 hours. Pre-clearing was done using 50% of xylene and 50% of alcohol for 12 hours. Clearing was done using xylene I and II for 3 hours. Wax-infiltration was done using molten paraffin wax in a hot air woven for 6 hours. The tissue underwent pre-embedding in pure paraffin I for overnight. The tissues were embedded in tissue blocks using pure paraffin. The tissues were sectioned using rotatory microtome set at 5 microns and the sectioned tissues were transferred on to slides and xylene I and II for dewaxing and stained with Hematoxylin Eosin (H&E).
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Statistical Analysis
The statistical analysis was performed using “statistical package for social sciences (SPSS) version 25. The data from the study was expressed as mean standard deviation. Paired sample T test was presented for the comparison of means as appropriate. One-way ANOVA was performed to compare between the groups of Morris water maze task scores for the study animals. P-value less than or equal to 0.05 was considered as significant.

RESULTS

Morris Water Maze Task
Learning and working memory
The mean latency for group II (AlCl₃) groups on the first day was relatively higher when compared to the other four groups (52.35±3.06). The values observed for the rats in group V was the lowest (32.50±1.89). The mean values for the different groups of animals across each of the study days were similar to the values obtained on the first day. Specifically, there was a statistically significant increase in time (P < 0.01) needed to identify the hidden platform in rats exposed to Al only when assessment of the entire study groups was done using the three (3) days acquisition trial method of Morris water maze.

The difference in time spent in each quadrant during a 24hr-delayed probe trial as well as the number of platform site crossing of the animals from the different groups (seconds) were presented in Table 1.

Table 1: The difference in time spent in each quadrant during a 24-hour delayed Probe trial for reference memory evaluation and the number of platform site crossing (seconds).

<table>
<thead>
<tr>
<th>Quadrants</th>
<th>Group I (seconds)</th>
<th>Group II (seconds)</th>
<th>Group III (seconds)</th>
<th>Group IV (seconds)</th>
<th>Group V (seconds)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>13.4 ± 2.11</td>
<td>21.60± 2.09</td>
<td>7.20 ± 1.32</td>
<td>7.20 ± 1.43</td>
<td>3.00 ± 0.58</td>
<td>16.416</td>
<td>&lt;0.001&lt;sup&gt;b,f,g,h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Q2</td>
<td>5.40 ± 1.66</td>
<td>13.4± 2.73</td>
<td>14.80 ± 4.24</td>
<td>7.40 ± 1.53</td>
<td>10.33 ± 2.40</td>
<td>2.165</td>
<td>0.114</td>
</tr>
<tr>
<td>Q3</td>
<td>8.00 ± 1.87</td>
<td>13.00± 3.72</td>
<td>7.40 ± 1.56</td>
<td>20.40± 1.63</td>
<td>20.00 ± 2.08</td>
<td>6.626</td>
<td>0.00&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Q4</td>
<td>21.00±1.70</td>
<td>4.20 ± 1.35</td>
<td>23.00 ± 2.79</td>
<td>21.00± 1.37</td>
<td>25.00 ± 2.64</td>
<td>17.84</td>
<td>&lt;0.001&lt;sup&gt;b,f,g,h&lt;/sup&gt;</td>
</tr>
<tr>
<td>NPFSC</td>
<td>4.4 ± 1.14</td>
<td>0.6 ± 0.50</td>
<td>4.00 ± 1.58</td>
<td>3.6 ± 1.51</td>
<td>4.00 ± 1.00</td>
<td>7.63</td>
<td>0.00&lt;sup&gt;b,f,g,h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean±SEM, Q= Quadrant, NPFSC= Number of platform site crossing. The letters b, d, e, f, g, h, i and j depict post-hoc difference between group I & II, I & III, I & V, II & III, II & IV, II & V, III & IV and III & V respectively. The symbol asterisks (*) depicts the quadrant in which the escape platform was located.

Probe trial
There was a statistically significant (p< 0.01) to learn as well as a reduction in capacity for working in rats exposed to Al only when assessment of the entire study groups was done using a three (3) day acquisition trial method of Morris water maze. There was a statistically significant (p < 0.01) reduction in the time spent in the target quadrant and the number platform site crossing during the 24-hour delayed Probe trial for reference memory evaluation among animal exposed to Al only.

The mean time (seconds) spent in target quadrant as well as the number of platform site crossing for group II (AlCl₃) groups on the probe trial (Day 4) was relatively lower when compared to the other four groups (4.20±1.35) and (0.6±0.50) respectively. The mean values of time (seconds) spent in target quadrant for the animals in group V was observed to be relatively higher (25.00±2.64). However, the number of platform site crossing for group III was observed to be relatively higher when compared to the other treatment groups (4.00±1.58).
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Thus, there was a statistically significant (p < 0.01) reduction in the time spent in the target quadrant and the number platform site crossing during the 24-hour delayed Probe trial for reference memory evaluation among animal exposed to Al only.

**Histopathological Results**

The results of histopathological observation of the PFC and hippocampal neurons of animals in Group I (control), show a normal histo-architecture of the layers of the PFC and hippocampal neurons with Pyramidal cells appearing normal as shown in Figure 1a and 1b respectively. Animals in Group II showed neuronal necrosis in both PFC and hippocampus. Degenerated Pyramidal cells with dispersed layer of the hippocampus was also observed as shown in Figure 1c and 1d respectively. However, Animals in Group III showed a normal feature of the cerebral cortex and hippocampal neurons with Pyramidal cells appearing normal as shown in Figure 1e and 1f respectively. Animals in Group IV showed a slightly focal necrosis of neuronal cells in both cerebral cortex and hippocampus with slightly dispersed layer of the hippocampus as shown in Figure 1g and 1h respectively. Furthermore, Animals in Group V showed a normal feature of the cerebral cortex and hippocampal neurons with Pyramidal cells appearing normal as shown in Figure 1i and 1j respectively.

![Histopathological image of section of the cerebral cortex and hippocampus of Wistar rat.](image-url)

The image shows normal presentation of cerebral cortex with normal pyramidal cells containing centrally placed nuclei (PC) and Glial Cells (GC) in (a) & (b). Cerebral Cortex and hippocampus of rat treated with (500mg/kg AlCl₃) shows Neuronal Necrosis (NN) of pyramidal Cells in (c) & (d). Section of the hippocampus of rat treated with 200mg/kg Alpha-lipoic acid revealed normal feature of pyramidal cell layers in (e) & (f). Section of Hippocampus of AlCl₃ (500mg/kg) + Alpha-lipoic acid (100mg/kg) treated group showing slightly dispersed layer of neuronal cells of the hippocampus in (g) & (h). Section of hippocampus of AlCl₃ (500mg/Kg) plus Alpha-lipoic acid (200mg/Kg) treated demonstrate normal appearance of Neuronal Cells and normal pyramidal layer containing densely packed spherical pyramidal cell was also observed in (i) & (j).
DISCUSSION
The histopathological observation of the hippocampus from rats of the Al toxicity model was characterized with dispersion of the compact arrangement of pyramidal layer of cells (putatively due to edema) with their population appearing very sparse. The impaired learning, working and spatial memory functions observed from the Morris water maze task in rats of the Al toxicity model group were in tandem with the histopathological features observed.

The findings in this study were in tandem with the findings in an earlier study that also investigated the effect of astaxanthin on Al exposed male *Swiss albino* mice of 4 months old. The *Swiss albino* mice were assessed using radial arm maze and open field test following 42 days of treatment. The rats in the Al-only treatment group showed a significant reduction in spatial memory performance, an increase in anxiety-like behavior and corresponding histological features of neuro-degeneration (Al-Amin et al., 2016).

An earlier publication had revealed that deficits of memory and learning functions were common in direct or indirect (from toxic agents) lesions involving the hippocampus (Badamas and Esomonu, 2012; Sadeeq et al., 2013). Specifically, exposure to mercury chloride for 21 days as well as a long term exposure to cigarette smoke have been reported to have a degenerative effects on the hippocampus with associated deficits in memory and learning (Badamas and Esomonu, 2012; Sadeeq et al., 2013).

Although the toxicity of Al on neuronal tissue had already been established in the literature, the current study also provided additional evidence for the toxicity of Al based on duration of exposure - 2 weeks of exposure in this study unlike the 8 weeks exposure described in an earlier study (Buraimoh, et al., 2012).

The longer mean latency time for locating the hidden platform in the Morris water maze test observed during the 3 day acquisition trial among the rats from the models of the toxic Al groups points to poor learning and working memory capacity in the rats.

The results from the probe trial test on the 4th day was also similar to the test results from the acquisition trial especially when both assessments (probe or acquisition test) were compared with values from other rats from other study groups. These features of poor learning, impaired working memory as well as poor spatial memory could be as a result of the neurodegeneration and reduction in number of pyramidal cells of the hippocampus as observed earlier. There is clinical evidence regarding the effect of Aluminum on neuroinflammatory destruction of hippocampal (CA1 and DG region) neuronal spine with associated learning and memory deficits(Cao et al., 2016). The neuroinflammatory changes were characterized with variable molecular levels of pro-inflammatory as well as anti-inflammatory cytokines mRNA for -IL-1b, IL-6, TNF-a, MCH II, CX3CL1 and BDNF (Lee et al., 2010).

The role of oxidative stress in Al toxicity had already been highlighted in an earlier study in which an increase in the level of malondialdehyde level (oxidant) and a decrease in glutathione level (antioxidant) in both the hippocampus and cortex were reported after 45 days of Al exposure (Alghamdi, 2018). Animals that were treated with extract of virgin coconut oil (rich in anti-oxidents and anti-inflammatory) for 30 days did not have a significant reduction in the glutamate even after the 45 days exposure to Al (Alghamdi, 2018). In this study, assessment of memory function was performed using novel object recognition test which mainly assessed the time spent in exploring a new object in an environment that was
previously familiar (Alghamdi, 2018). A negative discrimination index was reported for the Al only exposed groups, thus suggesting a deficit in short term memory. It is pertinent to note that contributions of spatial location, sequences, and temporal durations mainly coordinated by different areas of the brain, like the temporal, parietal, prefrontal cortices and the hippocampus are vital in the development of short term memory (Ma et al., 2014; Eriksson et al., 2015; Manohar et al., 2017). Another literature report suggested that exposure to Aluminium for 21 days followed by oral administration of virgin coconut oil for 21 days restored the structural and functional integrity of the PFC (Olanrewaju et al., 2018). Thus, the current finding as well as the literature reports suggest that regardless of the exposure factor, features of cerebral and hippocampal damages were associated with alteration in learning and memory function.

In the present study, treatment using ALA appeared to protect neurons of the hippocampus and cerebral cortex from the toxic effect of Aland this is similar to an earlier report regarding neuroprotection effect of a combination of quercetin and Alpha-lipoic acid on Aluminum toxicity (AL-Otaibi, et al., 2018). In the study, it was reported that the combination of the two agents gave a better antioxidative effects compared to two weeks of intraperitoneal ALA alone. Thus, ALA plays an antioxidant effect when administered via the oral route as well as parenteral route.

CONCLUSION
The findings from the present study suggests that Alpha-lipoic acid has a neuroprotective effect against Aluminium chloride induced neuro-toxicity.

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Conflict of interest
The authors do not have any conflict of interest to declare.

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