CEREBRAL CORTICAL DAMAGE IN ADULT WISTAR RATS FOLLOWING ALUMINIUM CHLORIDE ADMNISTRATION

ABSTRACT

This study investigated the histomorphological effect of aluminium chloride on the cerebral cortex. Aluminium chloride as one of the toxic metal have been known to be the major environmental pollutant across the world which has led to the discovery of diverse Neurodegeneration diseases (ND) associated with metallic intoxication. It is present in many pharmaceutical drugs, food products and also used in treatment of drinking water being involved in skeletal, hematological and neurological diseases.

Thirty two adult wistar of both sexes weighing between 143g-189g were randomly grouped into grouped into four groups, group A,B,C and D each group containing 8 rats. Group A rats which was the control and was maintained on standard feed (grower mesh) and water for 21 days, group B rats were treated with 0.2g of aluminium chloride for 21days, group C rats were treated with 0.4g of aluminum chloride for 21days, group D rats were treated with 0.6g of aluminium chloride for 21days. The aluminium chloride solution was administered orally on daily basis.

The weight of the wistar rats was recorded on weekly basis (before and at the end of each week of administration). On the 22nd day the wistar rats in group A, B, C and D were sacrificed by cervical dislocation, blood was collected through cardiac puncture, the brain was removed and weighed immediately using sensitive balance, part of the brain of all wistar rats in each group was collected and homogenized for biochemical analysis, it was then fixed in 10% formol saline, the tissue was processed and sectioned at 5µm and stained with hematoxylin and eosin for histological study.

Results showed that the mean body weights of the wistar rats significantly increased in the treated groups when compared with the control group. The mean brain weights of the aluminium- treated groups showed a significant decreased when compared to the control group. In the biochemical analysis there was statistically significant increase in the level of MDA in the aluminium-treated group, and a significant decrease in the level of SDH and SOD in the aluminium treated group. Histological study of brain (cerebral cortex) revealed that the cerebral cortical layers of the aluminium treated groups appeared distorted and degenerated, in a dose dependent manner. The study concluded that aluminium chloride has a

neurotoxic effect on the cerebral cortex of adult wistar rats which invariably may alter some cerebral functions.

Key word: Aluminium chloride, cerebral cortex, histomorphogy neurodenegeration, SOD, MDA.

INTRODUCTION

Increasing concern has been raised to the effect that the human organism is constantly and inevitably exposed to aluminium, a ubiquitous metal which is known to be the third most abundant element in the Earth's crust, representing 8% of total components[1]. Report has shown that aluminium is a toxicant substance that is implicated in dialysis encephalopathy [2] osteomalacia [3], non-iron responsible anemia [4], and also associated with many other diseases including Alzheimer's disease[5] (Gupta *et al.* 2005), Parkinson's disease[6] and amyotrophic lateral sclerosis[7].

Previous investigation has indicated that aluminium entry into the brain primarily occurs through the blood–brain barrier (BBB). Additionally, the mechanism(s) responsible for aluminium transport across the BBB is not fully understood, it has been reported from other studies that aluminium can penetrate into the brain as a complex with transferrin by a receptor-mediated endocytosis[8] and bound to citrate via a specific transporter, the system Xc^- (l-glutamate/l-cysteine exchanger) being the most recently accepted principles[9]. The apparently long half-life of aluminium in brain tissue has been advanced to explain its possible accumulation in the brain [10-11], which coupled with the long life of neurons may be responsible for the elevated levels of aluminium found in the brain of some patients suffering PD [6] and Alzheimer's disease [12].

Report from previous studies has shown Increasing evidence which demonstrated that oxidative stress is the primary and leading cause of pathogenesis in metabolic, inflammatory,

partial ischemia and denatured cranial nerve disease [13]. It has been documented that the brain tissues are highly vulnerable and susceptible to oxidative damage, probably due to high oxygen consumption rate (20%), the availability of abundant polyunsaturated fatty acids in cell membranes, high iron (Fe) content coupled with low anti-oxidative enzyme activities[14]. Additionally, reactive oxygen species may also cause cellular damage, by oxidizing amino acid residues on proteins, resulting ultimately in protein carbonyls [15]

Findings from Several studies have demonstrated that oxidative stress induced by aluminium leads to modification of the peroxidation of lipids and the activities of anti-oxidative enzymes. Julka and Gill [16]. However, reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens [17]. Short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis[18]. Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses, such as glutathione[19].

Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include free radicals and peroxides. Most long-term effects of oxidative stress are caused by damage to DNA [20] (Evans, Cooke,2004). Oxidative stress is suspected to be important in neurodegenerative diseases including Lou Gehrig's disease (aka MND or ALS), Parkinson's disease, Alzheimer's disease, Huntington's disease, Depression, Autism and Multiple sclerosis[21]. Aluminium chloride as one of the toxic metal have been known to be the major environmental pollutant across the world which has lead to the discovery of diverse Neurodegeneration diseases (ND) associated with metallic intoxication[22]. The causes of neurodegeneration seem to involve susceptibility genes and environmental pollutants. Toxic metal exposure on human can cause damages to number of organ systems. The nervous

system is vulnerable target for toxicant due to specific voltage which must be maintained in the cells and all the responses when voltages reach threshold level[23]. Aluminium is a trivalent cation found in its ionic form in most kinds of animals and plant tissues and in natural waters everywhere[24]. Aluminium (AL), is ubiquitous in the environment and its extensive industrial utilization has stimulated considerable interest in the possible environmental toxicity of this metal. However, little is known about possible effects of Aluminium as a trace element in animals and human in normal conditions. It has recently become clear that when Aluminium (Al) is mobilized from soil by acid rain, it poses a hazard to all exposed organism[25]. Aluminium has the capacity to be neurotoxic both in human and animals. It is present in many pharmaceutical drugs, food products and also used in treatment of drinking water being involved in skeletal, hematological and neurological diseases. Aluminium is widely used in antacid drugs as well as in food additives and tooth paste [26]. Aluminium compound have been used for 30 years to control phosphate level in patients undergoing haemolysis, the toxic effect arising from absorption and accumulation of Aluminium have well been documented and includes a progressive cerebral cortex which eventually leads to dementia. Environmental pollution with different aluminium containing compounds, especially those in industrial waste exposes human and animals to higher than normal levels of Aluminkium[22].

Particulate matters distributed by cement – producing factories contain, high amount of Aluminium, and animals and populations residing in the vicinity are exposed to the pollution[27]. In the past, toxic levels of Aluminium have been associated with neurodegenerative diseases including Alzheimer's disease, Parkinsonism, Dementia complex and causes extensive damage to the nervous system. Aluminium is a risk factor in Alzheimer disease [28]. However ,epidemiological investigation revealed a link between Aluminium in drinking water and AD and a variety of human and animal studies have implicated learning

and memory deficits after Aluminium exposure.[10,29]. Furthermore, other researches have revealed that there are possible adverse effects of aluminium on human health with no known physiological role for aluminium within the body. Aluminium neurotoxicity has been a matter of serious concern to scientists in view of several investigations conducted in relation to that. Chronic exposure of animals to aluminium is associated with behavioral, neuropathological and neurochemical changes including alter behaviors, anxiety, depression, weakness etc. Aluminium toxicity has been implicated in many neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AL), Parkinsonism-Dementia, amyotrophic lateral sclerosis, dialysis encephalopathy etc [28,30]. A possible link between Aluminium and Alzheimer's disease has been highlighted, and a variety of animals and human studies have implicated learning and memory deficit after aluminium exposure [33].

MATERIALS AND METHODS

This study was conducted at the animal house of Department of Human Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. The premilinary studies animal acclimatization, actual animal experiment and evaluation of results, lased for a period of three months. However, the actual administration of Aluminum chloride lasted for three weeks. The animals were housed in serene and conducive cross…ventilated room in the Animal Holding Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria and treated in accordance with 'Guide for the care and use of Laboratory Animal' prepared and compiled by the National Academy Of Science and published by the National Institute of Health[31].

Wistar rats weighing 110-240g were used for the experiment design, a total number of 32 rats (males and females) were involved. The experimental animals were housed in

standard plastic cage, fed with rat chow, and water daily. The experimental animals were divided into four groups. After acclimatization period, rats were weighed and randomly divided into four groups comprising eight animals in each group. Animal were administered with aluminum chloride 0.2mls/kg, 0.4mls/kg and 0.6mls/kg for low dose, medium dose and high dose respectively.

GROUP A: rats were given stock diet and water, they served as control.

GROUP B: Experimental animals were given stock diet and 0.2mls of aluminum chloride (low dose) orally for 3weeks

GROUP C: Experimental animals were given stock diet and 0.4mls of aluminum chloride (medium dose) orally for 3weeks

GROUP D: Experimental animal were given stock diet and 0.6mls of aluminum chloride (high dose) orally for 3weeks. The animals were sacrificed by cervical dislocation on the 22^{nd} day, Blood were collected from the heart for biochemical analysis of enzymes and the tissue (brain) for histological analysis after the $Alcl_3$had been administered for 21 days. The brain from each groups were fixed separately in 10% formo-saline.

The Statistical analysis of the results in this study was carried out and tested for significance using student T-test. Data were expressed as means ± SEMs of the three independent experiment and also by using 2-ways ANOVA of the graph prism 5 for window version 5.02 trial (1992-2009). If the p value is greater than 0.05 ($P>0.05$) this means that the effect is not significant, if the P value less than 0.05 ($P<0.05$) this means the effect was significant.

RESULTS

Table 1: Showing The Mean± Sem Of Brain Weight Of Adult Wistar Rats After Administration Of Aluminium Chloride

GROUPS	MEAN ± SEM OF BRAIN WEIGHT	RELATIVE BRAIN WEIGHT %
GROUP A (CONTROL)	1.95 ±0.035	1.03%
GROUP B LOW DOSE (0.2g/kg)	1.55 ±0.068	0.97%
GROUP C MEDIUM DOSE (0.4g/kg)	1.48 ±0.091	0.93%
GROUP D HIGH DOSE (0.6g/kg)	1.55 ±0.096	1.02%

From table 1, the weight analysis for brain shows an insignificant difference ($P>0.05$) in weight, comparing control group to group B, also there was an insignificant difference ($P>0.05$) in weight of brain when group C and D were compared with the control Group A. Group D which received the highest dose has the highest brain weight compared with other aluminium-treated groups, after the Group A which is the control, followed by group B which received the low dose and group C which received the medium dose.

Which shows that the effects of aluminum is not dose dependent on the brain weight.

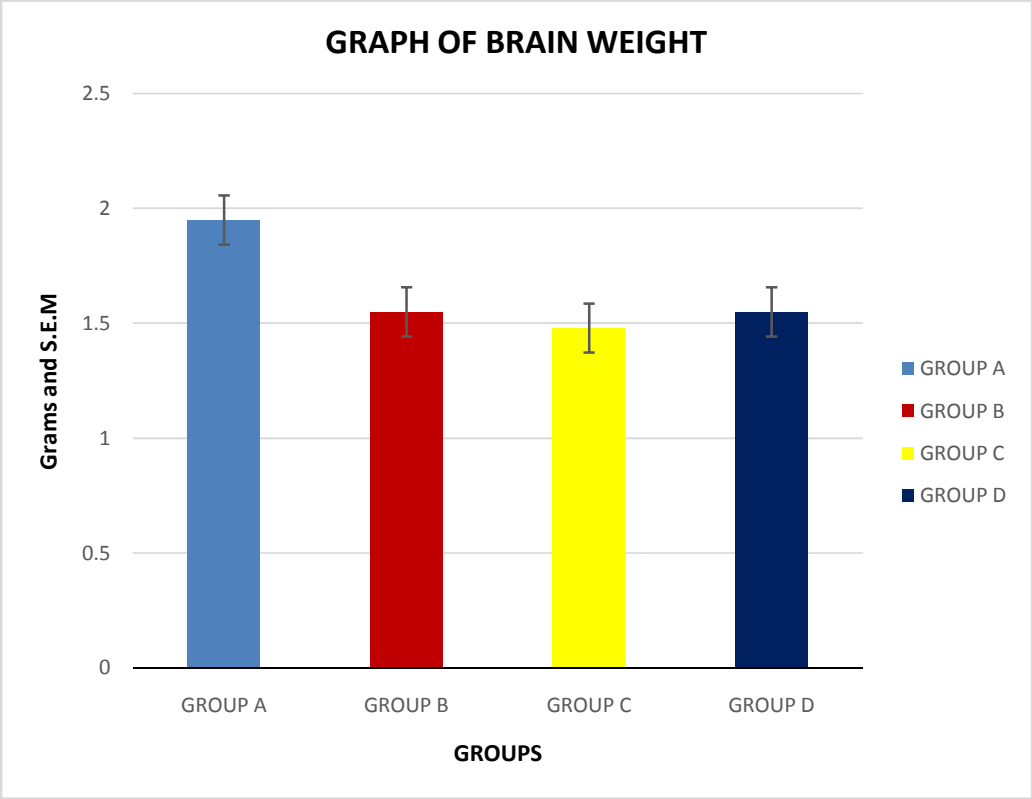


Fig.1:Bar Chart Showing Brain Weights of Wistar Rats

The graph showing the effect of aluminum chloride on the brain weight, general decrease in brain weight occur in all the group when compared with the control.

The graph also shows that Rats in Group B shows a decrease in brain weight compared to brain weight of group A (control), brain weight of rats in group C show a decrease in weight compared to group A also group D shows a decrease in brain weight compared to group A. However the graph also shows that there was a decrease in group C compared to group B and D and decrease in group D compared to group B

BOCHEMICAL ANALYSIS

Table 2: Effect Of Alumnium Chloride On The Activities Of Sod, Mda And Alp In The Brain

	GROUP A ± S.E.M (n=5)	GROUP B ± S.E.M (n=5)	GROUP C ± S.E.M (n=5)	GROUP D ± S.E.M (n=5)
SOD (ηmol/gtIssue)	78.34 ± 7.81	33.07 ± 1.37*	60.42 ±4.48*	45.63 ± 9.96*
MDA (ηmol/gtIssue)	33.06 ± 1.37	39.74 ± 2.06*	60.42 ± 4.48 *	51.42 ± 9.65*
SDH (µmol/gtissue)	2.91 ± 0.24	2.23 ± 0.44	1.37 ± 0.15*	1.17 ± 0.11*

Data were represented as Mean ± SEM * P<0.5 statistically different from the control;

SOD: Superoxide dismutase

MDA: Malondialdehyde

SDH: Succinate Dehydrogenase

Table 2 reveal rapid decrease in the activity of SOD in the aluminium-treated rats when compared with the control, it decreased significant (P<0.05) from 78.34 ± 33.0 to 33.07 ± 1.37 in group B, 60.42 ± 4.48 in Group C and 45.63 ± 9.96 in group D.

The level of MDA (malondialdehyde) increased significantly (P<0.05) in the treated groups compared with the control. It increased from 33.06 ± 1.37 in group A to 39.74 ± 2.06 in group B, 60.42 ± 4.48 in group C and 51.42 ± 9.65 in group D.

There was a decrease in the level of SDH among the aluminium-treated group compared with the control, it decreased from 2.91 ± 0.24 in group A to 2.23 ± 0.44 in group B, 1.37 ± 0.15 in group C and 1.17 ± 0.11 in group D.

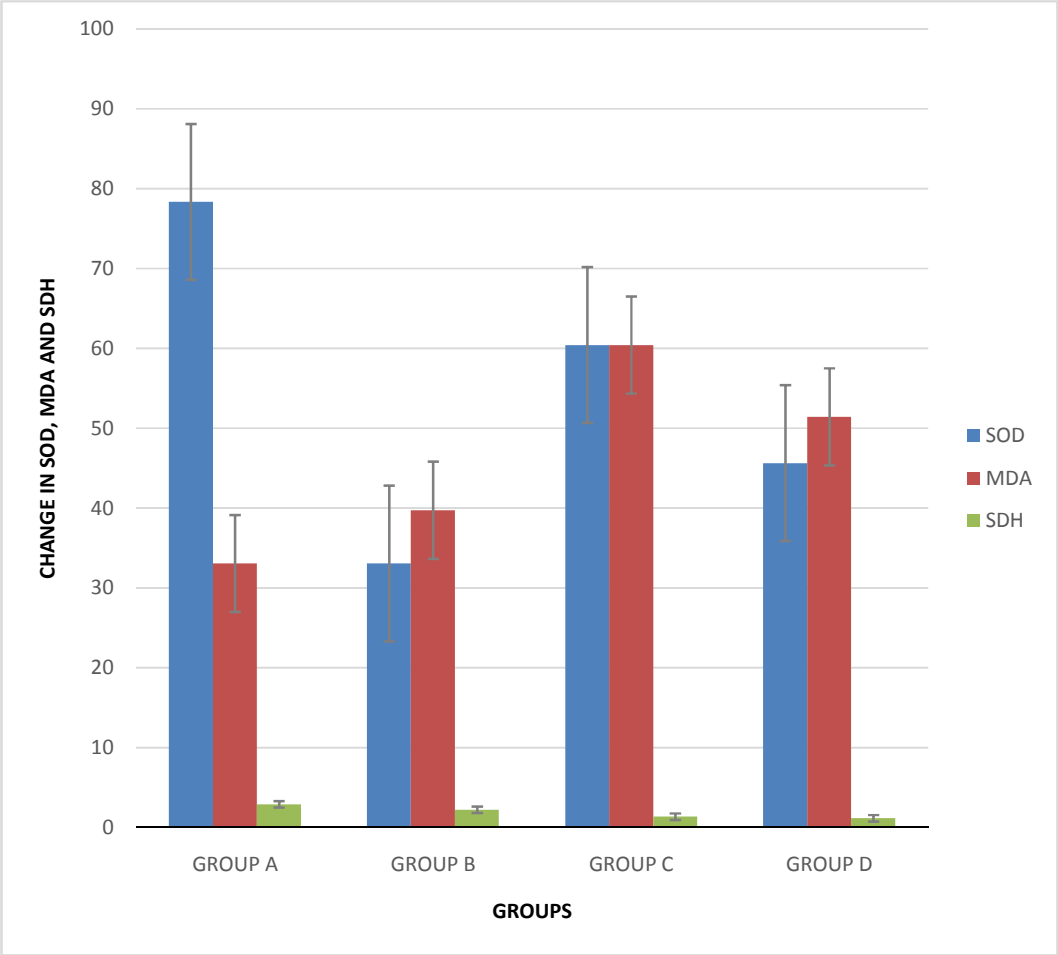


Fig 2: Histogram of Changes in SOD, MDA and SDH Per Group

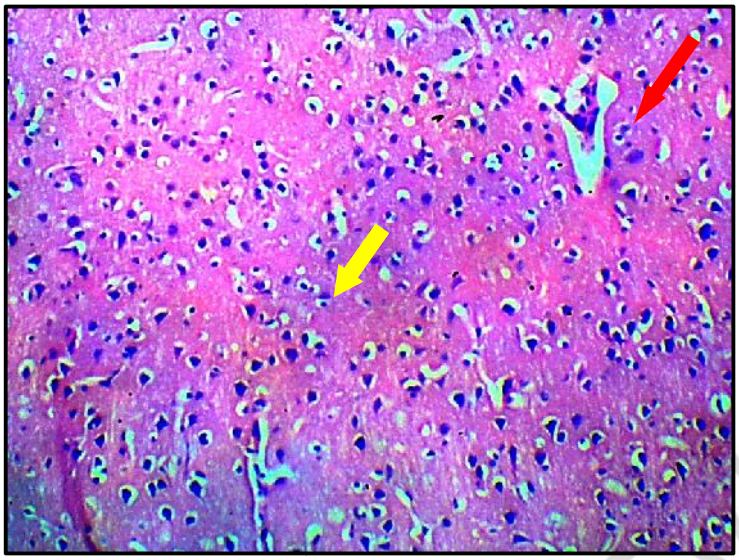
Graph of effect of aluminum chloride on the brain

Decrease in the level of**SOD**

Increase in the level of **MDA**

Decrease in the level of **SDH**

HISTOLOGICAL OBSERVATION (Photomicrograph of the Histology)



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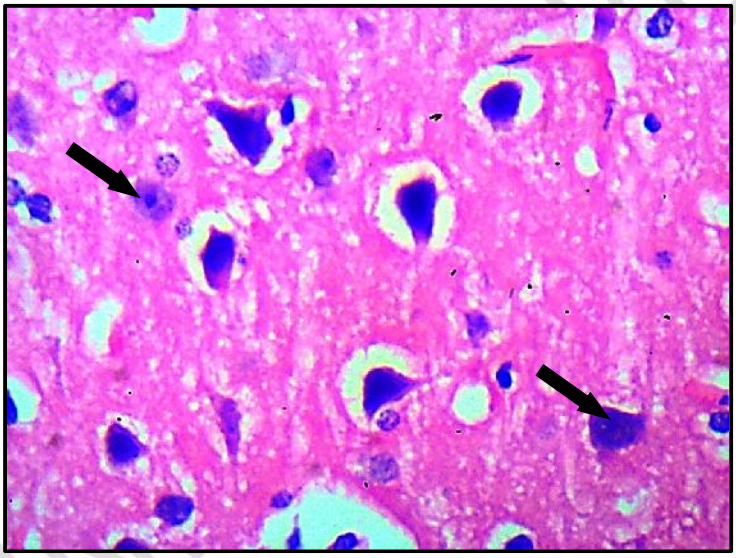

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GROUP A: CONTROL GROUP

Plate A: Photomicrograph control group showing a normal histological feature of the cerebral cortex, characterized by large pyramidal cell (black arrow), with long axons (white lines) that extends well from the delineated soma of the pyramidal neurons, normal molecular layers (yellow arrow) and external granular layer (red arrow) also appear normal. (H & E X100 X400)

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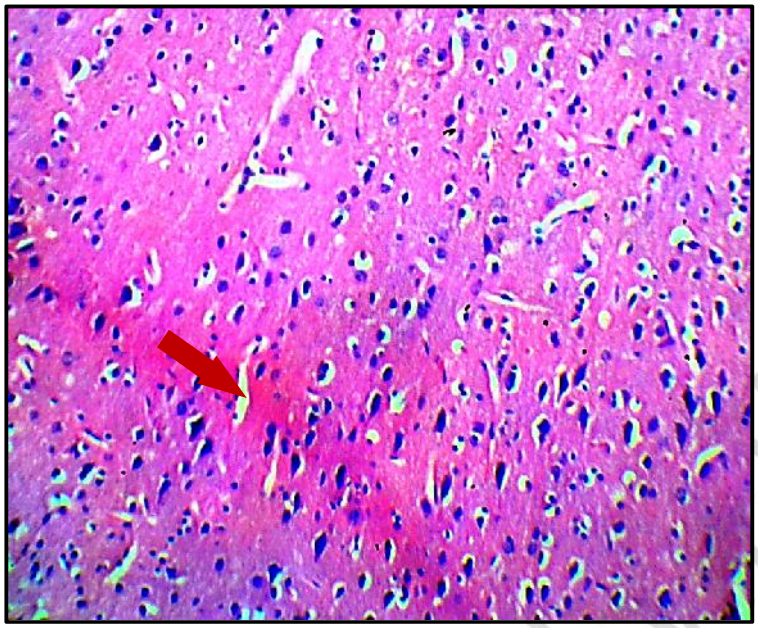

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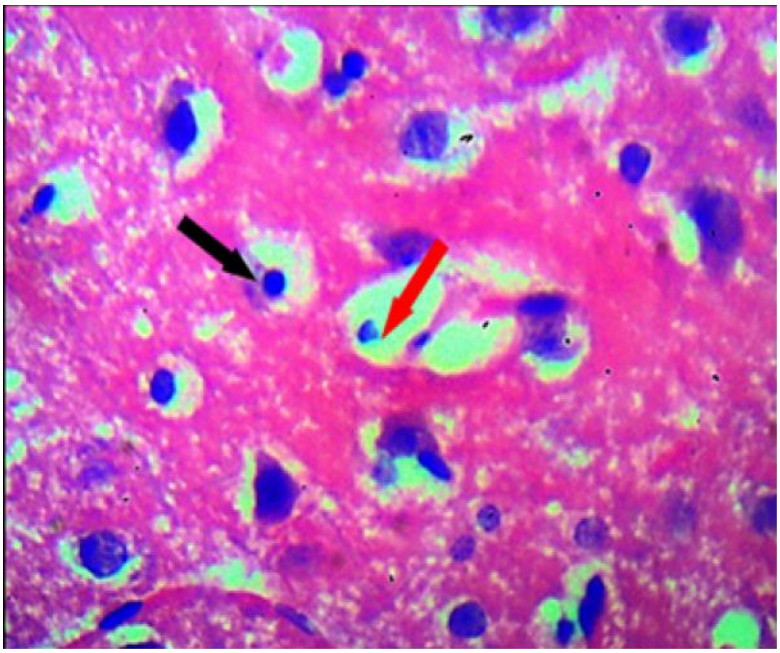

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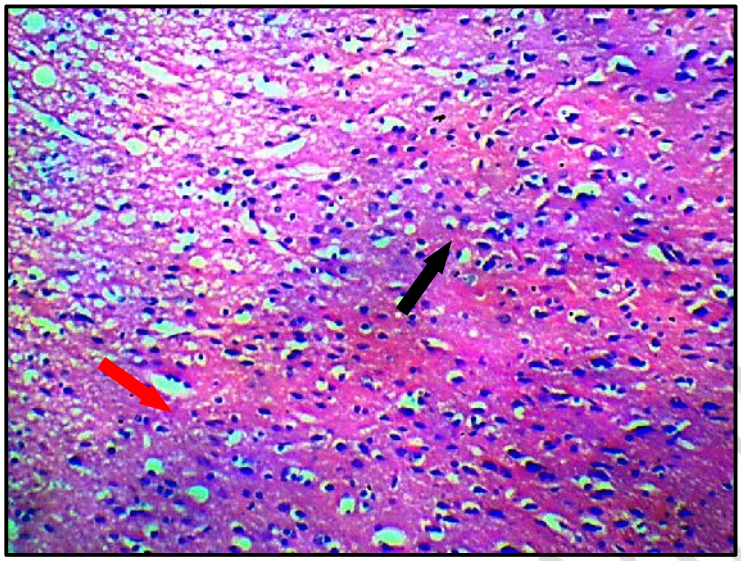
X 400GROUP B: EXPOSED TO 0.2 MLS OF ALUMINUM CHLORIDE

Plate B: Photomicrography of group B administered 0.2mls/kg of Aluminium chloride, showing slightly mild generative changes in the pyramidal cell, which appear slightly distorted with loss of their process (black arrow), mild generative changes occur in the cytoplasm and condensed nuclei is seen (red arrow). Morphology (molecular layer) is similar to that of group A. (H & E X100 X400)



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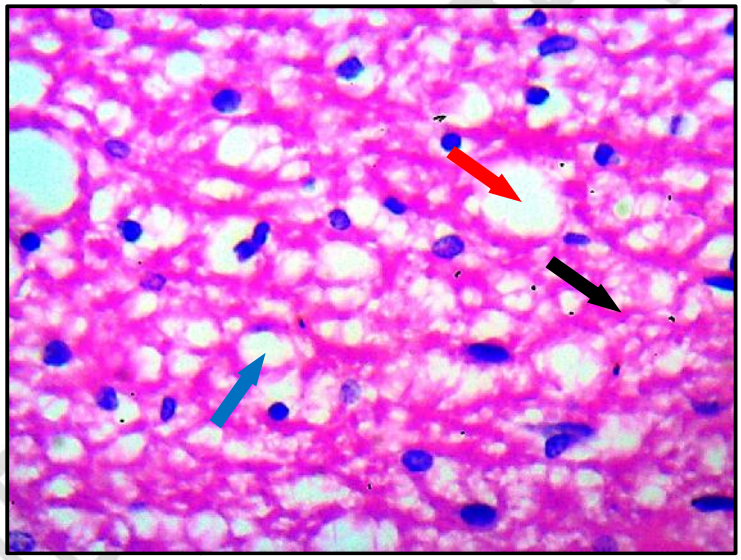

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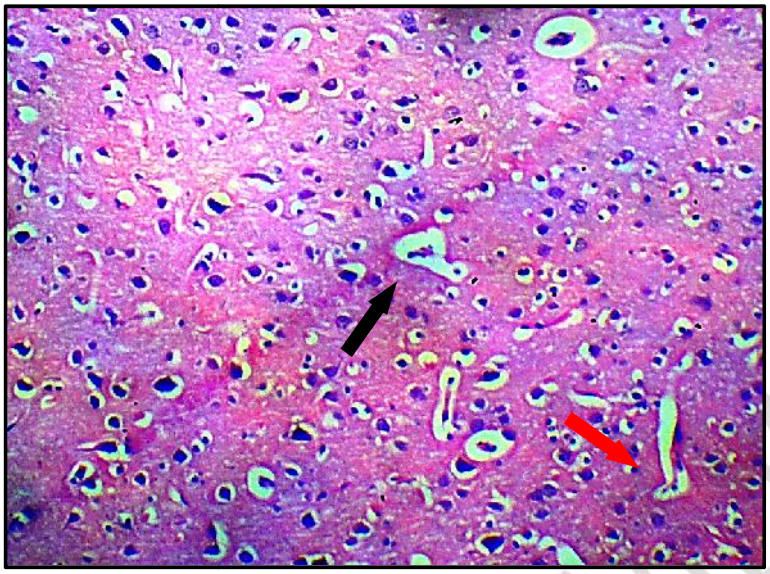
GROUP C: ADMINISTERED 0.4MLS OF LEAD CHLORIDE

Plate C: Photomicrograph of group B, administered 0.4mls/kg of Aluminium chloride showing loss of pyramidJal cells due to degeneration, leading to plenty of perineural spaces (red arrow), molecular layers appear unorganized with lots of spaces (black arrow) , cell distortion was very obvious (blue arrow). (H & E X100 X400)



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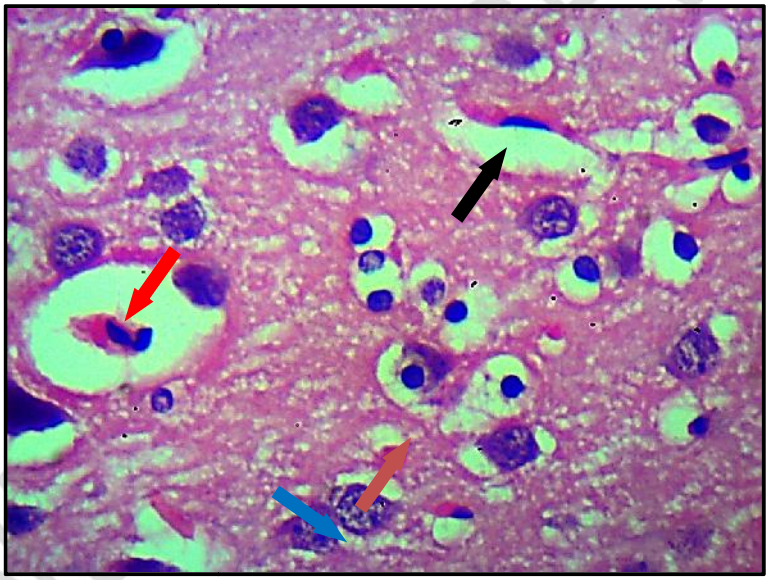

X 400

GROUP D: EXPOSED TO 0.6MLS OF ALUMINUM CHLORIDE

Plate D: photomicrograph of group D administered 0.6 mls/kg of aluminium chloride showing severe degeneration in fragmented cytoplasm, condensed nuclei within soma (black arrow), very large and numerous Perineural space can surrounding degenerating neurons (red arrow), neurofibril tangle (brown arrow), spaces within the pyramidal cells and granular layer (blue arrow). (H & E X100 X400).

HISTOLOGICAL FINDINGS

GROUP A (CONTROL): the histological examinations show a normal cerebral cortex histological morphology, the cells are normal as it could be seen in the photomicrograph (plate A). The cells are well arranged with normal nucleus, pyramidal cells and organized molecular layers there is no sign of degenerations or distorted cells.

GROUP B (LOW DOSE): this group received 0.2mls of aluminium chloride.The histological examination shows slightly distorted cell with loss of their process, mild generative changes occur in the cytoplasm and condensed nuclei is seen. Morphology (molecular layer) is similar to that of group A.

GROUP C (MEDIUM DOSE): this group received 0.4mls of aluminium chloride. The histological examination as it could be seen in the photomicrography shows loss of pyramidal cells due to degeneration, leading to plenty of perineural spaces, molecular layers appear unorganized with lots of spaces, cell distortion was very obvious, compare with plate A and plate B.

GROUP D (HIGH DOSE): this group received 0.6mls of aluminium chloride. The histological examinations shows a severe degeneration in fragmented cytoplasm, condensed nuclei within soma, very large and numerous Perineural space can surrounding degenerating neurons, neurofibril tangle, spaces within the pyramidal cells and granular layer, axon and dendrite are scarcely appreciable around neurons.

DISCUSSION

Findings from previous studies have indicated that the cortex is region known to be particularly susceptible in Alzheimer's disease and performs an important role in learning and memory functions[33] Reports from Several studies have suggested a general decline in learning abilities which are mediated by aluminium toxicity[34]. Results from the present e study, demonstrated a significant increase ($P<0.05$) in lipid peroxidation following

aluminium exposure in treated adult wistar rats, measured in terms of TBARS levels in the rat brain. Similarly, other investigators have also reported a significant increase ($P<0.05$) in whole brain thiobarbituric acid reactive substances after treatment with aluminium salts. Additionally, it has been reported from previous investigations, that aluminium is a non-redox metal whose accumulation in the brain has been implicated in various neurodegenerative diseases [35,3]. Several hypotheses from various investigators have been written to explain the potentials ability of aluminium to promote biological oxidations [37]. Thus, it has been shown to facilitate iron-induced lipid peroxidation [38], non-iron-induced lipid peroxidation[39]. non-iron-mediated oxidation of NADH [40] and non-iron-mediated formation of the hydroxyl radical[41]. Additionally, aluminium also appears to inhibit several antioxidant enzymes in different parts of the brain[42]. The significant variations and reductions in antioxidant enzyme activities in this study is adequately supported by findings from previous reports. Furthermore, The result of variations in antioxidant status is consistent with similar behavioural patterns of a significant decrease in the enzyme activity of most antioxidant systems, and agree with previous studies[43,44,42,45] (Dua and Gill 2001; Abubakar *et al.* 2004a; Nehru and Anand 2005; Jyoti *et al.* 2007). Findings from previous studies on adult animals have shown that Aluminium induced the production of ROS and caused oxidative damage in the brain[46]. Additionally, reactive oxygen species can also cause cellular damage, by oxidizing amino acid residues on proteins, forming protein carbonyls, Similarly, it has been reported that aluminum (Al) is a relatively low redox mineral, which has the potential to indu ce oxidative damage through multiple mechanisms. It has the tendency to bind negatively charged brain phospholipids, which possess polyunsaturated fatty acids and are readily attacked by reactive oxygen species (ROS) such as $O_2^{\cdot -}$, H_2O_2, $OH^{\cdot}$, and OH^-[46]. Furthermore, it has been suggested that oxidative stress caused by high Al content is greater than the protection provided by the anti-oxidizing

system; subsequently leading to high possibility of oxidative damage to brain tissue[47]. It has been reported that Al concentration in the brain tissue increased with increasing Al intake, but not in a dose-dependent manner and consequently oxidative damage occurred in specific brain areas of adult rats [48]. Aluminium has been shown by studies to be bound by the Fe^{3+} carrying protein transferrin thus reducing invariably the binding of Fe^{2+}. Moreover, it has been observed that the increase in free intracellular Fe^{2+} causes the peroxidation of membrane lipids and thus causes membrane damage [49]. Similarly, Aluminium (Al) is a widely known to be a neurotoxin that inhibits more than 200 biologically important functions in organisms[49]

This project studied the histomorphological effects of aluminium chloride on the cerebral cortex, result of weight analysis showed a significant increase in the final total body weight of animal in group B there treated with 0.2gof aluminium chloride (15.71%) initially but reduced in the bod weight when compared with the control group (26.43%).

Rats induced with Aluminium chloride in various groups has been reported to have their brain weight decrease compared to the control group although not dose dependent, in some research it was report that Aluminium chloride increase the brain weight of a wistar rats after the animals were induced for 30 days[49]. Microscopic examination of the cerebral cortex In the group B (induced 0.2 mls $Alcl_3$) only slight and mild distortions were observed in the architecture of the brain, the architecture of the brain in group C and D animals that were treated on higher dose (0.4 mls and 0.6mls respectively), a more prominent and significant damage was observed in the brain, it was also observed that animals in various groups demonstrated a dose dependent damage in the cerebral cortex of aluminium-treated rats as observed in this study. The histological alterations and distortions in the histo-architecture of the cortical layers in the treated rats and these findings are consistent with and corroborate the

359 reports of previous studies [50, 51,52] The alteration in the histological layers may have been
360 the reason behind the reduction in weight of the brain across the aluminium treated group,
361 which correlate with[53],

362 The finding from this study supports the hypothesis that Aluminium has potential
363 role in neurodegenerations[54] [Gupta *et al…,)*. The results of biochemical parameters
364 investigated showed elevated level of MDA activity in the aluminium treated groups (group
365 B,C and D) when compared with the control group(group A), in group C treated with 0.4g of
366 aluminium chloride has the highest elevated level of MDA (60.42 ± 4.40), followed by rat in
367 group D treated with 0.6g of aluminium chloride (51.4±9.05) and group B treated with 0.2g
368 of aluminium chloride when compared with the control group (group A) which has the MDA
369 level of (33.06), lipid peroxidation generates MDA which is major indicator for oxidative
370 damage initiated by reactive oxygen species (ROS) and causes impairment in cell membrane
371 function[55]. The increase in lipid peroxidation observed in this study may be attributed to
372 the direct effect of increase in generation of reactive oxygen species (ROS) resulting from
373 aluminum chloride administration similar observation here earlier been reported in studies
374 involving the brain[56]. Several studies have implicated oxidative stress in the pathogenesis
375 of a number of disorders and the severity of damage is generally associated with an increase
376 or decrease of one or more free radical scavenging enzymes [57].

377 The increased lipid peroxidation in aluminum- treated rats in this study, may be due
378 to an inhibition of SOD activity in the brain. The result is a substantial increase in the rate of
379 phospholipid peroxidation in brain cells, leading to membrane damage and neuron death. The
380 activity of SOD decreased significantly in the treated group when compound with the control.
381 Group B treated with 0.2g of aluminium chloride has the most decreased level of SOD (33.06
382 ± 1.37) and group D (45.63+ 9.96) followed by Group C (60.42+ 4.48), the decreased in the
383 activity of these enzyme could be a result from their inactivation by reactive oxygen species

resulting in a significantly decreased activity, similarly, a decreased activity of this enzyme is also an indication of the increased level of lipid peroxidation caused by the effect of aluminum chloride. The activities as SDH also decreased across the aluminium-treated Group when compared with the control group (group A) which may have been due to the toxicity of aluminium chloride leading to changes in metabolism of non-essential animal acid, which could lead to decrease function of mitochondrial cell respiration and energy generation which correlate with the findings of research carried out by previous investigator [53]. This project has presented consisted information from all result including the histological and biochemical analysis confirming the damaging effect of Aluminium on the cerebral cortex.

The study concluded that exposure to aluminium chloride could lead to neurodegenerative induced oxidative cerebral cortical damage in wistat rats as observed in this study which invariably may result in compromise of cerebral functions.

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