Review on Histological and Functional Effect of Aluminium Chloride on Cerebral Cortex of the Brain

Birhane Alem Berihu[1], Mekbeb Afwerk[2] , Yared Godefa Debeb[3], Adhanom Gebreslassie[4]

[1,4]Department of Anatomy and Histology, Institute of Bio-Medical Sciences, College of Health Sciences, Mekelle University, P.O. Box: 1168, Mekelle, Ethiopia.
E-mail: alem.birhane12@yahoo.com
[2]Addis Ababa University College of Health Sciences,
School of Medicine; Department of Anatomy
[3]Department of physiology, Institute of Bio-Medical Sciences, College of Health Sciences, Mekelle University, Mekelle, Ethiopia.

Abstract.

Various findings are give emphasis to Aluminium has more and more obvious disturbance of the brain other body organs. The purpose of this review is to give a comprehensive report of the existing data on Aluminium induced brain toxicity in different animal models. Along with, we also have made an attempt to present the possible mechanism related to aluminium induced brain toxicity suggested by various researchers. We used 62 different published materials for the compilation of this review article. Google search engine was used for accessing published materials from databases like google scholar, pubmed and hinari. The focus is on Al levels in brain, region-specific and subcellular distribution, mechanism of aluminium on neurotoxicity, histological change and neurobehavioral alternations. The present analysis indicated that $AlCl_3$ showed to be neurotoxin chemical by affecting the biochemical content of brain, histological alternation of cerebral cortex of the brain, disrupting behavioral activities. However, whether aluminium is a sole factor in neurodegeneration, histological alternation of cerebral cortex of the brain still needs to be understood.

Key words: Aluminium; oxidative stress; neurodegeneration; lipid per oxidation; glutathione peroxides; superoxide dismutase.

Introduction

Aluminum (Al) is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere [1]. Al occurs naturally in the environment and is also released due to anthropogenic activities such as mining and industrial uses, in the production of Al metal and other forms of Al compounds. A variety of Al compounds are produced and used for different purposes, such as in water treatment, papermaking, fire retardant, fillers, food additives, colors and pharmaceuticals. Al metal mainly in the form of alloys with other metals has many uses including in consumer appliances, food packaging and cookware [16, 46].

The major route of exposure to Al for the general population is through food (breads, cakes and pastries, glace fruits, dairy products, sausages, shellfish, sugar-rich foods baking mixes, and Also, foods with very high mean concentrations included tea leaves, herbs, cocoa and cocoa products, and spices [16]. Under normal and typical conditions migration of Al from food contact materials contributes to increase the total dietary intake. In the presence of acids and salts, the use of Al-based pans, bowls, and foils for foods such as apple puree, tomato puree or salted herring could result in increased Al concentrations in such foods [19]. Al containing over the counter medications such as antacids and buffered aspirin are assumed to be safe in healthy people at recommended doses based on historical use.

The ground-breaking studies on Al neurotoxicity in experimental animals were initially described in 1886 by Siem and Dollken [58]. Most of the understanding of Al toxicity in humans was established as a result of studies of disorders experienced by dialysis patients when the dialysis fluid contained Al at or above 0.5*m*mol/l In such patients, Al accumulated in various tissues, including kidney, liver, bone and brain; giving rise to pathological conditions such as brain disease that can lead to dementia and death and dialysis osteomalacic osteodystrophy. The main carrier of the Al ion in plasma is the iron binding protein, transferrin and it can enter the brain and reach the placenta and fetus [16].

The Al hypothesis in neurotoxicity came to light following the extraordinary discoveries that showed administration of Al salts into to adult or weanling animals brain led to the impaired performance on neurobehavioral tests of motor and cognitive function [33, 39and 55]. Since numerous reports have prompted the suggestion that Al is a possible cause of formation of first chronic neurotoxicity [9]. In these annotations, we

have tried to argue that the Al hypothesis continues to survive for the following reasons: there is a definite toxic action of Al in brain; Al levels are elevated in the brain of patients with brain disorders; and the incidence of brain disorders is increased in regions where people are more exposed to Al.

Various studies of Al on neurotoxicity showed that there is no single unifying mechanism has but it is likely that more than one mechanism is involved. The main sites of action of Al are difficult to discern because the studies have been performed using a variety of exposure methods (including a number of different *in vivo* injections and *in vitro* systems) and animal species, and a number of typical effects are not common to all species and exposure circumstances (i.e., are only expressed using certain models of neurotoxicity)[1].

After decades of research towards resolving Al toxicity, the exact mechanism of Al neurotoxicity and its complex biology still remain unanswered. A superfluity of studies on Al toxicity and neurodegeneration are still being undertaken with a promise to generate more scientific controversies in the future. Al hypothesis in various brain disorders (oxidative stress, histological disturbance, behavioral disorders): putting together pieces of puzzles;

The relevance of Al in neurotoxiccity is highlighted by discussing the Al load in the brain and Al in relation to other metals. Here, we review different scientific literatures to evaluate histological and functional effect of aluminium chloride on cerebral cortex of the brain.

Load of Al on brain

Nervous system is a vulnerable target for toxicants due to critical voltages which must be maintained in the cells and all responses when voltages reach threshold levels[33]. Many studies have reported that Al has the potential to be neurotoxin metal in human and animals. Although Al is present in trace amounts in the biological material, it does not appear to be essential element and usually considered to have harmful effects on general health[1]. Environmental pollution with different Al containing compounds, especially those in industrial waste exposed people to higher than normal levels of Al [16].

Different studies have been reported that Al causes extensive damage to the nervous system by accelerate oxidative damage to biomolecules like lipid, protein and nucleic acids [33, 55]. Al accumulates mainly in the bone, liver, testes, kidneys and brain [1]. Exposure to Al could occur through different principal routs which include inhalation of air contaminated with Al compounds and oral ingestion of Al dusts or with food and drinking water [48]. The ingestion pathway is the most significant route of transfer of Al from the environment to animals and humans [16,48]. Despite its ubiquitous presence, very small amount of ingested Al is absorbed across the gastrointestinal tract. Al uptake is limited by the presence of certain other dietary components such as citrate, which forms a complex with it, and its competition with other elements such as Ca, Mg, and Si [16]. In industrial settings, inhalation is the most important rout of Al entry into the body. This leads to absorption of Al into the blood with possible systemic intoxication [48].

Studies of pathogenesis of brain victims inconsistently showed elevated brain Al, contributing to the controversy concerning a possible role of Al in the causing brain disease. The possible role of Al in pathogenesis of brain gives rise to the important question whether Al can enter the brain, and if it does what the mechanisms of entry are. Blood-Brain barrier (BBB) has been proposed to be rout by which Al could enter the brain from systemic circulation [51]. This appears to occur by two processes. Yokel *et al*, provided evidence that transferrin can mediate Al transport across the blood-brain barrier by transferrin-receptor mediated endocytosis (TfR-ME) of Al transferrin, the predominant Al species in plasma (Table-1) [52].

The brain has lower Al concentrations than many other tissues Considering the Al species in plasma (Table -1); it is likely that Al transferrin and Al citrate account for the majority of the Al that distributes to tissues from the vascular compartment [51]. Yokel *et al* gave to rats Al intravenously either as an Al-transferrin complex, to model the predominant chemical species of Al in the plasma where greater than 90% of Al is bound to transferrin, or as Al citrate, the predominant small molecular weight Al species in plasma [52]. The appearance of Al in brain extracellular fluid was too rapid to be mediated by TfR-ME. This suggests involvement of a genetic variant (Tf) and citrate has been found to be responsible for the excess transport of Al in to the brain [52]. Sanchez-Iglesias *et al* have also identified Al accumulation in the cortex, hippocampus, striatum, cerebellum, and ventral midbrain of rats following treatment with intraperitoneal injected or orally drinking with aluminum chloride [56].

Table 1: The predominant binding ligands for Al *in vivo*, their effective equilibrium constants with Al, their concentrations in plasma and brain extracellular fluid (based on values in cerebrospinal fluid), and the percentage of Al predicted to be associated with that ligand [52].

Ligand	Effective equilibrium constant with Al	Plasma		Brain extracellular fluid	
		Concentration (mmol/l)	% of A1 species	Concentration (mmol/l)	% of A1 species
Transferrin	13.7, 12.6	30	91	≤0.25	4
Citrate	11.6	99	7–8	180	90

The above finding is evidenced by Buraimoh *et al* who found that a significant relationship existed between concentration of Al and brain uptake showed that dose dependant graded increase in brain Al uptake across the groups[9]. This finding is in line with that of Yuan *et al* who demonstrate that excess Al accumulates in specific areas of the brain, (the hippocampus, diencephalon, cerebellum, and brain stem) in neonatal rats following intraperitoneal injection of high levels of Al [55].

Al and oxidative stress

The first recognition of Al neurotoxicity in humans was recorded as encephalopathy in haemodialysis patients [57]. Related changes are found in the same regions of brains exposed to Al [50]. Al has also been recognized as a neurotoxin and is implicated in a number of neurodegenerative diseases, such as Alzheimer's disease and encephalopathy [3]. Alzheimer's disease (AD) is a neuropsychiatric disorder affecting elderly people, as described by Alois Alzheimer in 1906 [3]. Individuals with impaired renal function are particularly vulnerable due to their poor ability to excrete Al, as the kidneys are a major route of Al elimination. AD is a progressive mental deterioration manifested by memory loss, inability to calculate, visual-spatial disturbances, confusion and disorientation [22]. amyloid deposition and neurofibrillary tangles, the main neuropathological features of AD, occur in selectively vulnerable brain regions such as the hippocampus and the cerebral cortex [58]. Although the causative agents of Alzheimer's disease have yet to be fully delineated, metals, such as copper, zinc, and Al have been proposed to participate in the pathogenesis of this disease [22, 32, 42]. The disruption of the intracellular redox environment appears to be an important contributing factor [5].

The role of oxidative stress in neuronal degeneration is a widely discussed concept, and understanding on the role of Al in mediating neuronal oxidative stress may help to clarify the role of Al in brain damage. Although Al has no redox capacity in biological systems it can induce oxidative damage through multiple mechanisms [48]. It can bind to negatively charged brain phospholipids, which contain polyunsaturated fatty acids and are easily attacked by reactive oxygen species (ROS) such as O^{-2}, H_2O_2, and OH^- [60, 61]. Further, this element stimulates iron-initiated lipid peroxidation in the redox reaction, which causes ROS production and Fe^{+3} formations [60]. Reactive oxygen species may also cause cellular damage, by oxidizing amino acid residues on proteins, forming protein carbonyls suggesting for Al to have catalytic activity to produce free radicals [48]. Furthermore, the main mechanism of Al toxicity involves the disruption of the homeostasis of metals, such as magnesium (Mg), calcium (Ca), and iron (Fe) [28]. The physical and chemical properties of Al allow it to effectively mimic these metals in their respective biological functions and trigger biochemical abnormalities. Al has been shown to replace Mg and bind to phosphate groups on the cell membrane, DNA and ATP [45]. In particular, there is evidence to suggest that nanomolar concentrations of Al can induce genotoxicity in primary human neural cells, promoting the up-regulation of proinflammatory and pro-apoptotic genes [31]. However, the effect of Al on Fe homeostasis is the pivotal factor that renders this metal toxic [41, 49]. This interaction generates labile Fe from Fe-containing enzymes and proteins. The intracellular pool of free Fe increases, a situation conducive to the formation of reactive oxygen species (ROS). Indeed, elevated levels of ROS have been shown in various systems exposed to Al [35,55].

An Al-induced oxidative environment is characterized by increased oxidized lipids, oxidized proteins, and a sharp decrease in mitochondrial activity. Oxidative damage in the brains of animals exposed to Al has been observed [21, 30, 37]. These findings correlated well with *in vitro* studies, implicating Fe-mediated ROS production under Al stress in nerve tissue [24, 25, 26, 38]. The intracellular genesis of ROS in response to an Al challenge in various cellular models has also been reported [7, 54]. Increased lipid peroxidation, decreased membrane fluidity, oxidized high-density lipoprotein, and altered redox status are hallmarks of an oxidative environment, and these dysfunctions are all linked to the Al toxicity [15]. Therefore, Al inflicts its toxic influence by creating an intracellular oxidative environment, a situation conducive to major biological complications and diseases.

Various studies reported that high Al concentrations cause oxidative stress. This condition was defined by Sies and Jones ‘‘an imbalance between oxidants and antioxidants, in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage’’ [44]. According to Sies and Jones the nervous system is particularly sensitive to oxidant-mediated damage because of: (i) Its high oxygen consumption rate (approximately 20% of total oxygen consumed), (ii) Brain membranes are enriched in highly oxidizable polyunsaturated fatty acids, (iii) Brain antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) activities are comparatively lower than those found in other tissues. (iv) The brain content of iron is high. The latter is particularly important because iron is a redox-active metal, that can interact with molecular oxygen to generate superoxide anion (O^{-2}), which in turn, generates hydroxyl radical (OH^{-}), a highly reactive oxygen species (ROS). As a consequence of Fe^{+2}reaction with O_2, $Fe^{+3}$is generated, that can trigger lipidoxidation through its reaction with lipid hydroperoxides normally present in biological systems [44]. Evidence of an oxidative stress status has been found in association with most neurodegenerative disorders in which Al is present in relative high amounts. These findings led to an extensive investigation on the possible link between Al and the promotion of oxidative stress.

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues and lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds [40]. Al^{+3} has been reported to induce lipid peroxidation, and to alter physiological and biochemical characteristics of biological systems [44]. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) upon decomposition and measurement of the concentration of malondialdehyde used as an indicator of lipid peroxidation [40]. Research, conducted on oral administration of Al (0.1 mmol/kg/d) also suggested that high accumulation of Al in the hippocampus increased the lipid peroxidative products [24]. Similarly, Yuan *et al* reported that administration of $AlCl_3$ at dose levels of (0, 7, and 35 mg/kg/day; control, low Al, high Al respectively) through intraperitoneal injection for 14 days revealed positive correlation between Al content and levels of lipid peroxidative products which was measured by lipid peroxidation Colorimetric Assay kit and spectrophotometer [55]. Several other studies have shown that accumulation of Al in the cerebellum increased the lipid peroxidative products.

In one study, oral administration of Al (100 mg/kg/d) for 2 mo increased lipid peroxidation in the cerebellum of adult rats [6]. The cerebellar TBARS levels also increased in rats intraperitoneally injected with aluminum lactate (7 mg Al/kg/d) for 11 wk [45, 37]. In addition, identified significant increases in lipid peroxidation in the cerebrum and cerebellum of pup brains following exposure of developed and developing rat brains to oral aluminum chloride (100 mg/kg/ d) for 6 wk or 8 wk . Aluminum can also stimulate iron-initiated lipid peroxidation in the Fenton reaction, which causes ROS production and Fe3+ formation. Superoxide (O2˙-) is neutralized by Al3+ to form an Al-O2˙- complex, which increases the oxidative capacity of O2˙- [60, 61].

Investigations on the brain stem have also demonstrated increased lipid peroxidation in the medulla oblongata [38] ventral midbrain [43] of adult rats. Results, therefore, suggest that increased lipid peroxidation in the brain stem was associated with high Al. The possible mechanism for elevation of lipid per oxidation suggests to be binding of Al to negatively charged brain phospholipids, which contain polyunsaturated fatty acids and are easily attacked by reactive oxygen species (ROS) such as O^{-2}, H_2O_2, and OH^{-} which leads to increase in lipid peroxidation. In this complex, the negative charge of phospholipids is neutralized by the positive charge of Al, increasing the oxidant capacity of O^{-2} and H_2O_2 [48]. Similar result were mentioned by Nedvetsky *et al* who stated that Al induced significant increase in MDA concentration in hippocampus and frontal cortex of rats administered daily $AlCl_3$ via drinking water for six weeks [36]. Nearly similar findings were obtained by manal *et al* who demonstrated that administration of $AlCl_3$ at dose level of (1600 mg/L/day) in drinking water for a month induced elevation of LPO in brain of Al- treated rats [33]. This finding is evidenced by the increased production of MDA which may be attributed to the direct neurotoxic effect of this metal or perhaps a disarrangement of the cell membrane caused by increased lipid per oxidation. Yang *et al* reported that intraperitoneal injection of $AlCl_3$ solution for 60 days at different dose can accelerate lipid peroxidation in rat's brain which may be one of the most important intoxication mechanisms [50]. This elevation of MDA could be attributed to the ability of Al itself and its different salts to accelerate oxidative damage to biomolecules like lipids, proteins and this element can be able to cross the blood brain barrier and is deposited in to brain and increases LPO [48]. Therefore, the estimation of free radical generation and antioxidant defense has become an important aspect of investigation in mammals. Ahkam and El-Gendy showed that one month administration of $AlCl_3$ at dose level of 53.5mg through drinking water induced increase lipid peroxidation in liver, kidney and brain of rat which is evidenced by increase in production of MDA^2. The Al induced group had an increase in malondialdhyde (MDA) associated with a significant elevation in brain glutathione (GPX and SOD) activities [2, 33 and 55]. Furthermore, neurons appear to be particularly vulnerable to free radicals as the important natural antioxidant glutathione content is low, they have higher membrane content of polyunsaturated fatty acids and brain requires substantial quantities of oxygen for metabolism [44].

Glutathione peroxidase (GPX) is an enzyme family with peroxides activity whose main biological role is to protect the organism from oxidative damage and helps to prevent lipid peroxidation of cellular membrane by removing the free peroxides in the cell [27]. Oxidative stress has been implicated in the pathogenesis of a number of disorders and its extent of injury is generally related to an increase or decrease of one or more free radical scavenging enzymes of which Gpx is one [65]. This finding is evidenced by Yuan *et al* who demonstrate that the positive correlation between Al content and GPX activity in neonatal rats administered daily $AlCl_3$ at dose level of (0, 7, and 35 mg/kg body wt; control, low Al (LA), and high Al (HA), respectively) via traperitoneal injection for duration of 14days via drinking water for six weeks. High Al significantly increases activity of GPX in specific brain regions such as, hippocampus, diencephalon, cerebellum, brain stem as compared with LA and control groups, which was measured by GPX Colorimetric Assay kit and spectrophotometer. Gpx activities confirmed Al induced production of the ROS free radical H_2 O_2 and OH which participated in oxidative stress in the brain [55]. However, this finding is inconsistent with Manal *et al* who demonstrated that negative correlation between Al exposure and GPX activities, which was measured by GPX Colorimetric Assay kit and spectrophotometer. The levels GPX activities in adult rats' brain were significantly low in the high Al animals compared to the control [33]. This finding is consistent with Ahkam and El-Gendy who demonstrated that one month administration of $AlCl_3$ at dose level of 53.5mg through drinking water to adult rats induced significantly decreased in brain GPX activities. Although they used different dose of $AlCl_3$; the duration of exposure and experimental animals used was the same and obtained similar result [2]. This suggest that the difference obtained in GPX activities by Yuan *et al* could be due to maturity (age) of rats which leads to abnormal increasing of this antioxidative enzyme [55].

Superoxide dismutase (SOD) belongs to the members of enzymatic antioxidative defense mechanisms against reactive oxygen species (ROS), and protects macromolecules, cells and cell membranes from peroxidative damage [27]. SOD catalyses the dismutation of superoxide anion radical into oxygen and hydrogen peroxide, which in turn, can be removed by GPX. The imbalance between production and neutralization of reactive oxygen species, which may occur when anti oxidative system is not efficient enough, leads to peroxidative damage of macromolecules and in consequence the disturbances of metabolic pathways, injury of tissues and clinical symptoms of illnesses [27]. Ivana *et al*, showed that injection of $AlCl_3$ (3.7×10^{-4} g/kg) into rat hippocampus and forebrain cortex for duration of 30 days resulted increasesO^{-2} production, MDA concentrations and decreased in SOD activities [23]. This finding is in line with that of Yuan *et al* who demonstrate that presence negative correlation between Al content and SOD activities in rats administered daily $AlCl_3$ at dose level of (0, 7, and 35 mg/kg body wt; control, low Al (LA), and high Al (HA), respectively) via traperitoneal injection for duration of 14days via drinking water for six weeks [55]. High Al significantly decreased the activities of SOD, leads to peroxidative damage of brain tissues that may result to oxidative stress. Comparable finding were also obtained by Ahkam and El-Gendy who demonstrated that one month administration of $AlCl_3$ at dose level of 53.5mg through drinking water significantly decreased the activities of SOD [2]. Decreased in SOD activities suggests that Al to have catalytic activity for production of ROS which leads to peroxidative damage of brain tissues. Furthermore, Manal *et al* reported that the exposure to $AlCl_3$ resulted in significant increase in neuronal lipoperoxidation damage with concomitant alteration in the antioxidant defense status thus having serious bearing of the functional and structural status of CNS [33]. The reduction in activities of SOD and GPX may be attributed to the elevated level of protein and lipidoxidative products due to oxidative damage effect of Al on biomolecules. This inhibition of these enzymes activities also may be referred to the effect of Al in neuronal death due to high production of reactive oxygen species such as O^{-2}, H_2O_2 which leads to oxidative damage on brain tissues.

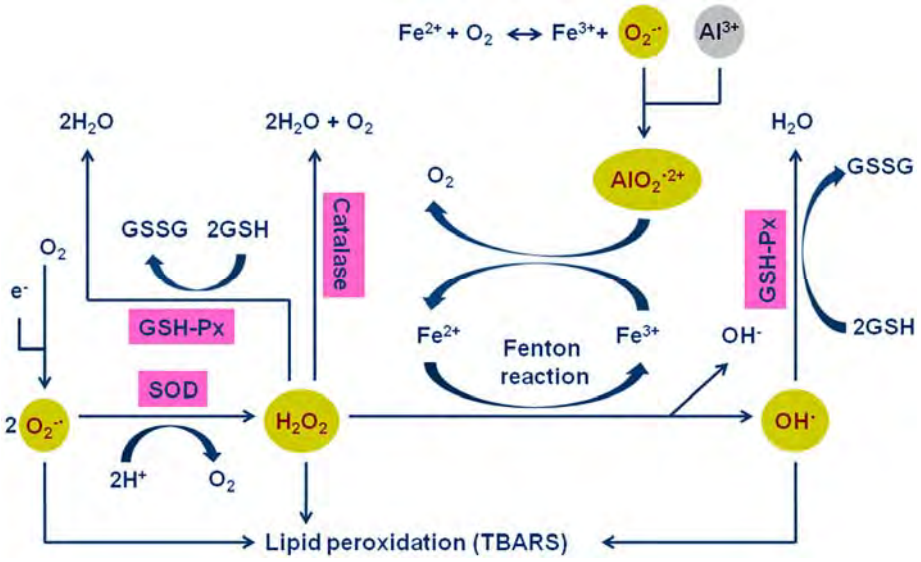

Figure 1: Diagrammatic representation of the relation among the aluminum (gray), reactive oxygen species (yellow), and anti-oxidative enzymes (pink) and lipid peroxidation. TBARS = thiobarbituric acid reactive substances; SOD = superoxidase dismutase; GPx. (It is adapted from the research findings of Exley, 2004; Halliwell, 2007).

Histological effect of aluminium on cerebral cortex

The cerebral cortex plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness. It also integrates higher mental functions, general movement, visceral functions, and behavioral reactions [9]. Al exposure also has neurodegenerative effects on the histology of cerebral cortex which is evidenced by learning and memory deficits and impairment of motor activities as well as in behavioral alternation [9, 13, 33 and 39]. Buraimoh *et al* demonstrated that exposure of rats' to $AlCl_3$ for duration of eight weeks induced neuronal vacuolation and necrosis of the cerebral cortex as a sign of neurodegeneration [13]. Since the cerebral cortex is said to play a key role in memory, attention, perceptual awareness, thought, language, and consciousness, then the neurodegenerations observed in the histology of the cerebral cortex of rats could go a long way in affecting these functions (memory, attention, perceptual awareness, thought, language, consciousness, etc).

Different studies have also revealed that at higher dose of exposure, extensive neuronal vacuolation and necrosis of cerebral cortex was evident. Based on these observations, they reported that $AlCl_3$ exposure has neurodegenerative effects on the histology of cerebral cortex of rats, especially at higher doses. Similar result were reported by Ouafa *et al* who demonstrated that three months administration of different concentration of $AlCl_3$ at a dose of 50 mg/kg/day through drinking water has induced lesions in the temporal and parietal cortex of poisoned mice as evidenced by cellular hyperplasia and congestion of blood vessels [39]. Comparable findings were also obtained by Manal *et al*. who demonstrated that one month administration of $AlCl_3$ at a dose of 1600 mg/l through drinking water cause marked histophathological alterations in the brain tissue which were represented by focal as well as diffuse gliosion in cerebral cortex, odema and inflammatory cell infiltration and pericellular odema in the cerebral cortex, encephalomylacia with neuronal degeneration [33]. Finally they reported that $AlCl_3$ has neurodegenerative effects on histology of cerebral cortex.

Buraimoh *et al*. also conducted a study in order to evaluate the possible effects that $AlCl_3$ exposure could have on the histology of cerebral cortex of rats' offspring showed normal histological appearances of the cerebral cortex [14]. Based on this observation, they reported that the neurodegeneretative effects of $AlCl_3$ exposure on the cerebral cortex of adult wistar rats are not transferable to the offsprings.

Additionally, Buraimoh *et al*. have done expermental study to assess the possible effects of oral administration of $AlCl_3$ on the histology of the hippocampus of rats showed that 12 week administration of different dose of $AlCl_3$ induce clumpy of cell neurons, or reduced pyramidal cells and scanty neurofibrillary tangle as indication of neurodegeneration in the treated groups when compared to the control group [10]. These lead to the conclusion that oral administration of $AlCl_3$ could induce brain damage which may impair memory and learning as seen in Alzheimer disease. This supports a hypothetical statement by Yokel *et al* that Aluminium exposure has neuro-degenerating effect resulting in learning deficits [53] and also the documentation

compiled by Frank *et al* who stated that in human aluminium inhibits learning [17]. This finding is evidnced by Ouafa *et al* who demonstrated that administration of $AlCl_3$ at a dose of 50 mg/kg/day through drinking water for duration of three months cause a massive cellular depletion in the hippocampal formation with neurofibrillary degeneration and showed numerous ghosts like neurons with cytoplasmic and nuclear vaculations, which were thought to be due to the accumulation of Al in these regions which resulting in behavioral modification leading to cognitive impairment and enhanced anxiety of mice to unfamiliar environment as discuss in detail below [39].

Effect of Al in behavioral alternation and brian modification

In an attempt to model human neurobehavioral changes in rodents, a wide range of behavioral testing paradigms have been developed. Many of these tests induce a fearful response like maze test for learning and memory, rota rod, anxity effect and general motor activity etc. Ouafa *et al*. done experimental study to assess the effect of $AlCl_3$ associated with behavioral and brain modifications. Additionally, histological study of the brain of mice was designed to assess the effects of $AlCl_3$ exposure on the viability of cells in the hippocampus and cerebral cortex structures known to contribute significantly to spatial learning and memory function. The experimental study was carried out on 40 albino mice. The results of this study showed that administration of $AlCl_3$ at a dose of 50 mg/kg/day through drinking water for a duration of three months resulted in behavioral and morphological alternation of the brain [39].

Behavioral measurement was performed using batteries of tests such as locomotor activity, hole-board test, forced swimming, black and white Test box and Morris water maze [39]. Locomotor activities were tested in acrylic cages (45 x 25 cm) divided into 16 equal squares. The number of crossed squares was recorded for each mouse per time of 5 min for 20 min. Results showed that Al increased the activity scores of locomotor and head-dipping, this was tested by Holeboard an apparatus which is consist of a grey wooden box (50×50×50 cm) with four equidistant holes of 3 cm in diameter in the floor. Head-dipping behaviors' were checked for 20 min with sample intervals of 5 min. The hyperactivity observed in these tests were considered to be the result of stress conditions, which has suggested that there may be an important link between Al and oxidative stress [33, 55] indicating that Al facilitates oxidative stress and may be the cause for Al-induced learning and memory deficits observed before severe neurodegeneration can be identified. Based on these results, it seems likely that enhancement in exploratory behavior in Al mice during the holeboard test and locomotor activity may reflect the anxiety response of an animal to an unfamiliar environment. This change may be dueto drugs with diverse pharmacological properties alter head dipping suggesting that many neurotransmitter systems are involved in the expression of exploratory behavior [29].

Additionally, Ouafa *et al*. also used Black and White Test Box to permit simple and quick evaluation of the anxious behavior. The test was based on the adversive properties of the open field in which anxiolytic drug-induced ease of exploratory activity is compared between an illuminated and dark compartment. Both compartments are separated by a wall with a 70x70 mm opening in its base. Total time spent in each compartment was recorded for 20 min with sample intervals of 5 min. Results showed that Al exposed mice spent less time in dark than light, which has suggested for the anxiety of intoxicated animals [39].

In addition to these batteries of behavioral tests these researchers also used forced swimming test to assess the activity and behavior of the mice. Mice were placed in a Plexiglas cylinder with 10 cm internal diameter and 50 cm high filled with 25-26°C water (10 cm height). This experiment was carried out with in 6 min and the behavior of the mice was evaluated between the first and the sixth minute for 5 min. The immobility time was measured, and a mouse was judged to be immobile when it remained floating in the water, making only those movements necessary to keep their head above the water. The result of forced swimming test showed a significantly increased immobility time of Al-treated mice, this suggested that Al induced the deterioration of hippocampal and cerebral cortex function which is also evidenced by extensive neuronal vaculation and necrosis of cerebral cortex and hippocampus which leads to impaired physiological (learning and memory) functions and changes in motor performance [12, 14, 33, 39]. This test is a common behavioural test for assessing depression in which animals have given up the hope of escape and depression remains controversial [18], drugs with antidepressant activity reduce the time during which the animals remain immobile.

In addition to these batteries of behavioral tests, Ouafa *et al*. used an apparatus Morris Water Maze (MWM) to assess learning and memory of Al treated mice over course of 4 days of behavior. They used a circular pool (1.2 m in diameter) made of white plastic and filled to a depth of 20 cm with water (24°C-25°C) that was made opaque by addition of non toxic white paint. A clear platform (10 cm in diameter), was submerged 0.5 cm under the water level so that the animal has to swim until it finds the hidden platform. A video camera recorder was anchored to the ceiling directly above the circular pool to record the swimming pattern of each mouse. Two experimenters standing around the edge of pool and objects on the walls of the room served as visual cues. The mice were to use the visual cues (remind) to learn the position of the submerged plate form. Four trials were run for four days. The parameters measured in Morris water maze were the distance (in cm) and time to reach the plate form. Significant difference between Al treated mice and control mice was observed. The control mice covered significantly more distance than the Al treated mice. This could indicate

that the control knew a source of refuge existed and when they could not find it, they kept swimming to look for it [39].

The treated mice covered less distance either because they did not sufficiently learn where the platform was and so swam at a normal pace or knew there was a platform but could not find it and gave up their search for it, knowing they would be removed from the pool after a certain amount of time. The time took to reach the submerged platform on all days (4 days) to reach the platform and they stated that there were significant difference between control and treated mice; treated mice took longer to reach the platform. It was assumed that over the course of training, mice would learn the position of platform using the visual cues [39]. Comparable finding were obtained by Buraimoh *et al*. who demonstrated that eight week administration of $AlCl_3$ at dose level of (475 mg/kg, 950 mg/kg, 1,425 mg/kg and 1,900 mg/kg respectively) through oral intubation and tested in MWM induced negative effects on behavioral endpoints of wistar rats (i.e., alters behavior) and impair learning and memory functions [11]. These findings are consistent with previous reports that have demonstrated a spatial memory deficit in mice and rat after Aluminum exposure [62]. The memory impairment in AD patients is often difficult to specify precisely because of the heterogeneity of psychopathology, confounding impairments in other faculties, or difficulties in determining the duration of illness, but the general consensus is that the working memory system is compromised first at early stages of the disease development [4]. In this respect, the impairment in working memory observed in the Al exposed mice may likely correspond to early clinical stages of the disease.

These findings are also supported by Buraimoh *et al* who used an apparatus the elevated plus maze (EPM) to measure anxiety levels in laboratory rats under Al toxicity. The EPM apparatus consists of a plus shaped ("+") maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms and a center area. The maze was kept in a room and elevated 50 cm above the floor. Rats were placed individually in the centre of the maze, facing an enclosed arm, allowed to freely explore the maze. Their behavioral profiles were then recorded simultaneously for 5 min by means of a video camera mounted above the maze and the records analyzed. The preference for being in open arms over closed arms was calculated to measure anxiety-like behavior and an increase in open arm activity (duration and/or entries) reflected anti-anxiety behavior. Results of this experimental study showed that the rats treated with $AlCl_3$ had, increased faecal boli, increased number of time crossing close arm entries and increased average time spent in close arms. However they show decreased time (lesser time) spent in the open arm of the maze when compared with the control group. This implied that the Al treated groups were more anxious than the control groups in exploration of their activities on the elevated plus maze[11]. Finally they concluded that $AlCl_3$ exposure has negative effects on anxiety related behavior of rats as indicated by increased rate of anxiety in Al treated rats.

Additionally the above findings were also supported by Tripathi *et al.* who used another apparatus Y maze (techno co. 40 cm long x 13 cm height x 10 cm width) to assess the short term memory in vivo [47]. This test was used to see if the mouse remembers the arm it had just explored and therefore enters in one of the other arms of the maze. Rats were placed at the bottom (middle arm) in the Y maze and are allowed to explore freely all three arms for an eight minute session. The first two minutes are for habituation and the last six minutes the alteration between arms was recorded via photo beam breaks. The acquisition time was noted to determine the short-term memory. Learning and memory test showed that there was a gradual decreased in acquisition time in Al treated rats in comparison with the control rats.

Tripathi *et al*. also used rota rod to detect the motor coordination of the $AlCl_3$ treated rats in comparison to the control rats. Rats were placed on the rota rod perpendicular to the long axis of the rod. The rota rod rotated at speed of 10 rpm for at least 2 seconds for two consecutive days. On day 1 and 2, treated rats displayed no differences in time spent on rota rod. On day 3, treated rats were significantly different from controls in time took to fall off. As a result, the researchers that the time the controls stayed on was much longer. The control rats learned to stay on the rota rod and had better coordination as the testing days progressed. However the $AlCl_3$ treated rats also learned to keep place; however, they did not learn as well and found to be increased Muscle in-coordination [47].

The hippocampus and the cerebral cortex are the key structures of memory formation. Because the hippocampus is especially indispensable in the integration of spatial information, a decline in learning ability may be induced by the deterioration of hippocampal function [34]. The brain regions particularly affected by Al neurotoxicity include those involved in memory and learning. This may be due to the specific distribution of transferrin receptors (tfrs) and neuroanatomical connections between brain regions important for cognitive processes [59]. Anatomical and neurochemical connections between brains structures involved in memory processes are of great importance to understand the involvement of cholinergic system in AD and Al-induced neuropathology.

Table: 2 aluminium induced brain toxicity in various laboratory animals

No.	Animal model	Route of administration	Dose	Duration	Observations	Reference
1.	Male wistar rats	Oral intubation	Aluminium chloride (475mg/kg; 950mg/kg; 1,425mg/kg 1,900mg/kg).	Eight weeks	There was a dose dependant graded increase in brain al uptake across the groups.	[9]
2.	Male wistar rats	Oral intubation	Aluminium chloride (475mg/kg; 950mg/kg; 1,425mg/kg 1,900mg/kg).	Eight weeks	Aluminium chloride exposure has negative effects on behavioural endpoints and can impair learning and memory.	[11]
3.	Postnatal rats	Intraperitoneal injection	0, 7, and 35 mg/kg	14days	Excess al accumulates in specific areas of the brain, including the hippocampus, diencephalon, cerebellum, and brain stem in neonatal rats following intraperitoneal injection of high levels of al which induce oxidative damage of brain	[55]
4.	Male albino rats	Gavage	1600 mg/l/day	One month	revealed a significant increase in acetylecholinesterase (ache) activity and malondialdhyde content (mda) while the enzymatic antioxidant activities as glutathione-s-transferase (gst), glutathione peroxides (gpx) and glutathione reductase (gr) were significantly decreased in aluminum treated group.	[33]
5.	Wistar rats	Oral intubation	Alcl 3 100 mg. / kg. B.wt	90 days	Serum concentration of total t3 and t4 was decreased and ft4 was insignificant changed while Vitamins c and e were markedly changed on 60 and 90 days of treatment with the behavioral change	[47]
6.	Albino mice	Oraly	Alcl 3 (50 mg/kg/day)	3 months	Al-treated mice had impaired spatial working memory, with lower performance at morris water maze. The brains of experimental animals, studied by optical microscopy, have revealed damage In the hippocampus and cortex, including neurofibrillary degeneration, which can be due to the accumulation of	[39]

					aluminium in these regions.	
7.	Male wistar rats	Oral intubation	Aluminium chloride (475mg/kg; 950mg/kg; 1,425mg/kg 1,900mg/kg).	Eight weeks	Wistar rats treated with aluminium chloride had, increased faecal boli, increased number of time crossing close arm entries and increased average time spent in close arms; but decreased time (lesser time) spent in the open arm of the maze when compared with the control group. This in turn implies that the aluminium treated groups were more anxious than the control groups in exploration of their activities on the elevated plus maze.	[12]
8.	Male wistar rats	Oral intubation	Aluminium chloride (475mg/kg; 950mg/kg; 1,425mg/kg 1,900mg/kg).	Eight weeks	Histological examinations hippocampus showed clumpy of cell neurons, or reduced pyramidal cells and scant,y neurofibrillary tangle which was an indication of Neurodegeneration in the treated groups when compared to the control.	[10]
9.	Male wistar rats	Oral intubation	Aluminium chloride (475mg/kg; 950mg/kg; 1,425mg/kg 1,900mg/kg).	Eight weeks	The histological observations of the aluminium treated Groups revealed extensive neuronal vacuolation and necrosis (neuro-degeneration) of the Cerebral cortex of wistar rats.	[13]
10	Male wistar rats	Oral intubation	Aluminium chloride (475mg/kg; 950mg/kg; 1,425mg/kg 1,900mg/kg).	Eight weeks	Effects of aluminium chloride exposure on the cerebral cortex of adult wistar rats were not transferable to the offspring.	14
11	male albino rats	Intra Peritoneally	(53.5 mg AlCl3/litre drinking water , 5 mg	30 days	in al- toxicated group ,serum glucose and total cholesterol levels, liver enzyme activities (asat and alat), as well as, lipid peroxidation end products {malondialdhyde (mda) + 4-hydroxynonenal (4- hne)} were elevated significantly in the brain , liver ,kidney and testes tissues when compared with control group. On the other hand, serum triglycerides and tissue (liver, kidney and testes) intracellular antioxidants glutathione (gsh) and superoxide dismutase (sod) and liver glutathione peroxidase (gshpx) activity decreased significantly.	[2]

12	Male mice	Orally	$AlCl_3$ (3.7×10^{-4}g/kg)	30 days	Increases o^{-2} production, mda concentrations and decreased in sod activities.	[23]

Conclusion

The present review analysis provides further evidence for the neurotoxic action of Al in the experimental animal models brain. Administration of Al chloride in the different dose of mice resulted in distinct morphological alterations in the brain and behavioral results indicate cognitive impairment and enhanced anxiety of mice in an unfamiliar environment. Therefore $AlCl_3$ exposure has negative effects on behavioral alternation such as cognitive deficits and other changes, including decreased maze-learning ability, altered general motor activity, impaired motor coordination, which are also evidenced by the histopathological alternation found in specific region of the brain.

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