EFFECTS OF ALUMINIUM CHLORIDE ON THE HISTOLOGY OF THE CEREBRAL CORTEX, BRAIN ALUMINIUM UPTAKE AND BEHAVIOURAL ENDPOINTS OF ADULT WISTAR RATS

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APRIL, 2013.

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NIGERIA.

APRIL, 2013.

DECLARATION

I hereby declare that the work in the dissertation entitled "Effects of Aluminium Chloride Exposure on The Histology of the Cerebral Cortex, Brain Aluminium Uptake and Behavioural Endpoints of Adult Wistar Rats" was performed by me in the Department of Human Anatomy under the guidance and supervision of Professor S.A. Ojo, Professor J.O. Hambolu and Dr. S.S. Adebisi.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation has been previously presented for another degree or diploma at any University.

Buraimoh Adebayo Adekunle 11th April, 2013.

| --- | --- | --- |
| Name of student | Signature | Date |

CERTIFICATION

The dissertation entitled "The Effects of Aluminium Chloride Exposure on The Histology Of The Cerebral Cortex, Brain Aluminium Uptake and Behavioural Endpoints of Adult Wistar Rats" by Buraimoh Adebayo Adekunle meets the requirements and regulations governing the award of the degree of Doctor of Philosophy (Ph.D.) of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This Dissertation is dedicated to God and to my beloved father Late Pa. Abolarinwa, Buraimoh.

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ABSTRACT

Aluminium is presents in many manufactured foods, medicines and is also added to drinking water for purification purposes. Aluminium exposure and neurological impairments demonstrate mixed findings. The cerebral cortex is a sheet of neural tissue that is outer-most to the cerebrum of the mammalian brain and it plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness. Anxiety is a general term for several disorders that cause nervousness, fear, apprehension and worrying. Anxiety affects one-eighth of the total population world-wide. The objectives of this study was to investigate the possible effects that aluminium chloride exposure could have on spatial learning and memory, anxiety-related behaviour, brain aluminium uptake, histology of cerebral cortex, body and brain weights of adult wistar rats. Fifty adult wistar rats were used for this study. The wistar rats were divided into five groups; group I was the control, group II received 475mg/Kg, group III received 950mg/kg, group IV received 1,425mg/kg and group V received 1,900mg/kg via oral intubation for a duration of eight weeks. The LD50 was 3,807kg/kg. Study of spatial learning and memory was done (measured) with the aid of Morris water maze while the elevated plus maze was used to study anxiety-like behaviour. Neutron activation analysis method (NAA) was used to determine the quantity (concentration) of aluminium uptake by the brain, while haematoxylin and eosin, as well as Hirano-Zimmerman stains were used for the histology of the cerebral cortex. The results of this study showed that on the Morris water maze, at the first run of training, the wistar rats in the control and aluminium treated groups got to the platform at different time as they were trying to learn tasks which were not statistically significant at $P<0.05$; But for the second and third runs (testing), we observed that wistar rats in the control group got to the platform faster than the aluminium treated groups which was statistically significant at $P< 0.05$ and this, implies that aluminium chloride impaired the spatial learning and memory of the wistar rats.

Activities on the elevated plus maze showed that there was statistically significant increased rate of anxiety across the groups which were prominent among the aluminium treated groups. There was brain accumulation of aluminium in the aluminium treated rats and the histology of the cerebral cortex showed neuronal vacuolation and necrosis of the cerebral cortex in the aluminium treated groups when compared with the control.We also observed statistically significant decrease in the body weight but the brain weight loss observed was not statistically significant at $P<0.05$. Based on our observations, we therefore conclude that aluminium chloride exposure had negative effects on behavioural end points as manifested in impaired spatial learning and memory, and increase anxiety (affect anxiety-like behaviour); It also resulted into increased brain uptake (accumulation) of aluminium in the treated groups, caused neuronal vacuolation and necrosis of the cerebral cortex and caused weight loss in wistar rats.

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LIST OF ABBREVIATIONS

Abbreviations	English Translation
A.B.U.	Ahmadu Bello University
AD	Alzheimer ’s Disease
$AlCl_3$	Aluminium Chloride
Al	Aluminium
AS	Arsenic
BIS	Bureau of Indian Standard
B/W	Body Weight
CEPA	Canadians Environmental Protection Agency
CERT	Centre for Energy Research and Training
EEC	European Economic Community
E.g.	For example
EPM	Elevated plus Maze
Etc.	And so forth (Etceteras)
Fe	Iron
Fig.	Figure
G	Gram
HCL	Hydrochloric Acid

H&E	Haematoxylin and Eosin
HPGe	High-Purity Germanium
NH_3	Ammonia
HNO3	Nitric Acid
Hr	Hour
Hrs	Hours
"	Inches
ICP-MS	Inductively Coupled Plasma- Mass Spectrometry
ICP- AAS	Inductively Coupled. Plasma-Atomic Absorption Spectrometry
i.e.	That is
Kg	Kilogram
<	Less than
Lab.	Laboratory
LD50	Lethal Dose
L	Litre
MDA	Malondialdhyde
Mg	Magnessium
Mg/kg	Milligram per kilogram

Mmol	Milli-mole
MCA	Multi-channel analyzer
Min	Minutes
MWM	Morris Water Maze
NAA	Neutron Activation Analysis
Ni	Nickel
NIRR-1	Nigeria Research Reactor-1
P	Probability Level
PC	Personal Computer
PD	Parkinson's Disease
Ppm	Part Per Million
%	Percentage
("+")	Plus shaped
S	second
SPSS	Statistical Package for the Social Sciences
Si	Silicon
□	Square root of
Ti	Titanum
USA	United State of America

USEPA	United State Environmental Protection Agency
Vis	Namely
Vs	Versus; against
W.H.O.	World Health Organization
X100	Magnifications at 100
X250	Magnifications at 250
Zr	Zirconium

CHAPTER ONE

1.0. INTRODUCTION:

The nervous system is a vulnerable target for toxicants due to critical voltages which must be maintained in the cells and the all responses when voltages reach threshold levels. It is the major controlling, regulatory, and communicating system in the body and the centre of all mental activity including thought, learning, and memory. It consists of the brain and spinal cord (Brodal, 1992).

Aluminium chloride has its derivation from purified chlorine with molten aluminium. It is composed of aluminium with molecular weight of 133.34 and formula (symbol) of $AlCl_3$. The elemental aluminium does not occur in its pure state but is always combined with other elements such as chloride, hydroxide, silicate, sulphate and phosphate. Due to its reactivity, aluminium in nature is found only in combination with other elements such as oxygen, silicon and fluoride (Jiang et al., 2008; Verstraeten et al., 2008). Other names (synonymous) for this compound include aluminium trichloride, Trichloro aluminium. It is whitish powder with melting point of 194^0C and specific gravity of 2.44 while its boiling point sublimes with vapour density of 4.5. It is not combustible but react violently (decomposes) with water and heat will contribute to instability (Laschkarew, 1930). Aluminium is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere. It is ubiquitous element and the third most prevalent (abundant) element in the earth's crust, comprising approximately 8% of the earth's crust, exceeded only by oxygen (47%) and silicon (28%). The almost ubiquitous presence of this element has so heavily contaminated the environment that exposure to it is virtually inescapable. Daily intake by humans is estimated to be 1-10 mg (0.04 - 0.37 mmols). It is difficult to provide generalized estimates of environmental concentrations because of the complexity of its chemistry which is controlled by pH, mineralogical constituents and the

quantities and indeed quality of associated natural organic matter. Dietary Aluminium is ubiquitous but in such small quantities that it is not a significant source of concern in persons with normal elimination capacity. Urban water supplies may contain a greater concentration because water is usually treated with aluminium before becoming part of the supply. Subsequent purification processes that remove organic compounds take away many of the same compounds that bind the element in its free state, further increasing aluminium concentration (Proudfoot, 2009).

The wide distribution of this element ensures the potential for causing human exposure and harm (Berthon, 1996; Candura *et al*., 1998; Williams, 1992; Zhang and Zhou, 2005). Aluminium has the potential to be neurotoxic in human and animals. It presents in many manufactured foods and medicines and is also added to drinking water for purification purposes (Newairy *et al*., 2009). Aluminium is widely used in antacid drugs, as well as in food additives and tooth paste (Abbasali *et al*., 2005). Environmental pollution with different aluminium containing compounds, especially those in industrial waste expose people to higher than normal levels of Aluminium (Kloppel *et al.*, 1997). Particulate matters distributed by cement producing factories contain, high amount of Aluminium and populations residing in the vicinity are exposed to the pollution (Shehla *et al*., 2001). Evidence for the contribution of Aluminium to Alzheimer's disease (AD), remains contradictory (Flaten, 2001; Gupta et al., 2005). However, epidemiological studies have indicated 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. (Yokel, 2000; Schmidt et al., 2001; Exley, 2005; Buraimoh et al., 2011). It has been shown clearly that aluminium accumulates in various mammalian tissues such as brain, bone, liver and kidney (Wills *et al*., 1993; Sahin *et al.*, 1994) and is accompanied by renal failure (Alfrey, 1980) or associated with age (Gómez *et al*., 1997). Patients on dialysis or on long-term treatment with total parenteral nutrition

have been shown to accumulate this metal in different organs (Alfrey et al., 1976; Yokel and McNamara, 2001; Klein, 1993). Although, aluminium has been implicated in Alzheimer's disease, Parkinsonism, Dementia complex and causes extensive damage to the nervous system, to date the mechanism of aluminium neurotoxicity has not been fully elucidated (Niu *et al.*, 2007). In different researches, aluminium has been reported to accelerate oxidative damage to bio-molecules like lipid, protein and nucleic acids (Jyoti *et al.*, 2007).

Aluminium is released to the environment by natural processes and from various anthropogenic sources. As a light weight metal with a melting point of 649.8°C., it is being used more and more for a number of different and important uses. It is not an overstatement to aver that the modem technological age could not have occurred without the availability of aluminium. It has been suggested that there is a relationship between high levels of aluminium and increased risk of a number of neurodegenerative disorders including dialysis encephalopathy, Alzheimer's disease (AD) and Parkinson's disease (PD) (Becaria et al., 2002; Berthon, 1996; Corain et al. 1996; Hughes, 1989; Yokel, 2000; Yokel, 2002). It has recently become clear that when aluminium is mobilized from soil by acid rain, it poses a hazard to all exposed organisms (Birchall et al., 1989). Aluminium is also thought to be a causal agent in some cases of encephalopathy and osteomalacia observed in patients with chronic renal failure caused by long-term hemodialysis (Tahara, 2004). Although, aluminium toxicity in humans has been implicated in many neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, and parkinsonism-dementia but the mechanism of aluminium-inducted neurotoxicity and identification of effective treatment for such impairments is, therefore, an important public and occupational health priority for industrial and developing nations. (Roberts, 1986; Garruto and Brown, 1994; Solomon et al., 2001)

Aluminium contributes to a variety of cognitive impairments in mice, rabbits, and rat pups (Muller et al., 1990; Yokel, 1985, Bilkei-Gorzo, 1993; Mari et al., 2001). Studies on workers exposed to aluminium dust in industrial environments demonstrate similar effects (Rifat et al., 1991; Bast-pettersen et al., 1994; White et al., 1992, Akila et al., 1999). Many researchers have found elevated aluminium levels to be associated with a decline in visual memory, attention, concentration, frontal lobe function and lower vocabulary scores in hemodialysis patients (Bolla et al., 1992). He et al., (2003), demonstrated that exposure to electrolytic Aluminium fumes adversely affects neurobehavioral performance in workers, including motor coordination, mood, and autonomic nervous function; although other reports on occupational Aluminium exposure and neurological impairments demonstrate mixed findings (Sim et al., 1997). Provan and Yokel (1992) reported that Aluminium levels in the brain were found to disturb G proteins, Ca2+ channel and PKC. Chronic exposure to Aluminium has been shown to reduce the basal activity of guanylate cyclase, and impairs the glutamate—nitric oxide-cyclic GMP pathway in rats in vivo and in cultured neurons (Hermenegildo et al., 1999). It has been stated that the use of Aluminium as an experimental neurotoxicant has recapitulated virtually every feature of the neurodegenerative spiral afflicting Alzheimer's patients (Grant et al., 2002). Additional evidence suggests that Aluminium accumulates in the human brain with aging (Markesbery et al., 1981). Crapper *et al*., (1976) reported that the concentrations of Aluminium in the brains of Alzheimer's disease patients were significantly high. Long-term administration of soluble salt of Aluminium to rats worsens their learning ability together with diminished cholinergic function and the rats become lethargic (Berlyne *et al.,* 1972; Deloncle et al., 1990). Role of Aluminium intoxication in neurodegenerative diseases has been recently emphasized (Exley, 1999; Somova et al., 1997).

Williams, 1992 reported that Aluminium compound have been used for 30 years to control phosphate levels in patients undergoing haemodialysis. The toxic effect arising from the absorption and accumulation of aluminium have well been documented and include a progressive encephalopathy which eventually leads to dementia. Aluminium compounds are also the major cause of damage to fish gill epithelia and plant root membrane in the acidic conditions produced by “acid rain”. Although aluminium is known to be a neurotoxin which affects many neurochemicals reactions, the details of the biochemistry underlying cell damage and death are largely matters of conjecture. Aluminium has also been implicated as a toxic agent in aetiology of Alzheimer's disease and parkinsonism-dementia (Hewitt *et al*., 1990).

Aluminium chloride is an effective antiperspirant that is applied to the skin to control severe excessive sweating (hyperhidrosis) that works by blocking the sweat glands and causes the pressure of fluid within the glands to rise to the point where it shuts off sweat production (www.Net Doctor, 2004). The antiperspirant is applied before going to bed at night and should be washed off in the morning. It should not be reapplied during the day. Overtime, sweating will stop during the day and the number of times you apply the antiperspirant at night can be reduced. Aluminium has a wide variety of uses which include for aircraft, utensils, apparatus and electrical conductors. The coarse powder is used in aluminothermic (thermite process): the fine powder as flashlight in photography, in explosives, fireworks and in aluminium paint; for absorbing gases in manufacturing of steel (www.Net Doctor, 2004).

Behaviour is the manner of acting or controlling yourself. The action or reaction of something under specific circumstances. In psychology, it is the aggregate of responses or reactions or movements made by an organism in any situation. It is also the way a person, or organism, or group responds to a specific set of conditions. (Encarta, 2009) It refers to the actions and mannerisms made by organisms, systems, or artificial entities in conjunction with its environment, which includes the other systems or organisms around as well as the physical environment. In humans, behaviour is believed to be controlled primarily by the endocrine system and the nervous system. It is most commonly believed that complexity in the behavior of an organism is correlated to the complexity of its nervous system. Generally, organisms with more complex nervous systems have a greater capacity to learn new responses and thus adjust their behaviour. Behaviour can be either innate or learned (Dusenbery, 2009).

The cerebral cortex is a sheet of neural tissue that is outer-most to the cerebrum of the mammalian brain. It plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness. It is constituted of up to six horizontal layers, each of which has a different composition in terms of neurons and connectivity. It also integrates higher mental functions, general movement, visceral functions, and behavioral reactions. (Brodal, 1992; Cauller, 1995).

Neurons of the cerebral cortex are grouped into six main layers which include the following: The molecular layer I, The external granular layer II, The external pyramidal layer III, The internal granular layer IV, The internal pyramidal layer V and The multiform layer VI, (Jones, 1998; Gilbert and Sigman 2007; Shipp and Stewart, 2007).

1.1 Significance of the Study

This study becomes important in order to ascertain whether or not the exposure to aluminium chloride yields or results into any damage (impairment) to the cerebral cortex, body weight, learning and memory, anxiety related behaviour of wistar rats.

1.2. Objectives of the Study are:

(a) To study and observe the aggregate of responses, spatial learning and memory (behavioural attribute) of the wistar rats.

(b) To check for the anxiety-related behaviour of wistar rats following the oral administration of aluminium chloride.

(c) To assay the concentrations of aluminium uptake in brain of wistar rats.

(d) To describe any histological changes observed on the cerebral cortex of wistar rats following the administration of aluminium chloride.

(e) To evaluate the effects that aluminium chloride could have on the body and brain weights of wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background Literature Review

Aluminium chloride has its derivation from purified chlorine with molten aluminium. It is composed of aluminium with molecular weight of 133.34 and formula (symbol) of $AlCl_3$. Other names (synonymous) for this compound include Aluminium trichloride, TrichloroAluminium. It is whittish powder with melting point of 194^0C and specific gravity of 2.44 while its boiling point sublimes with vapour density of 4.5. It is not combustible but react violently (decomposes) with water and heat will contribute to instability (Laschkarew, 1930).

Under the sales specification of $AlCl_3$, its assay is 99.3% min with free Aluminium of 100 ppm max and Fe (iron) of 100 ppm max. According to Li Runshen (1981), Aluminium chloride was used as a coagulant in Shenzhen water supply and found to have a coagulating effect as good as 2-3 times of traditional aluminium salt. With its much lower price in comparison with organic coagulant, it has great advantages in improving water quality. It has the following characteristics: Floccus forms rapidly. It needs a short time to react and settle down; With the same dosage, the pH value reduces less to use aluminium chloride than other inorganic coagulant, especially when with the highly turbid water is treated; we may use less lime to adjust the pH value of treated water; and it has a wide range of dosage and it is well suitable for variety of turbidity, alkalinity, organics in the water compared with aluminium

sulphate. It has a better coagulation effect even in lower temperature. –T<10. Li Runshen (1981).

There are some problems in application that coagulation does not affect expectantly. In a given condition of raw water physical and chemical properties, flocculent kind, purification, it is the key factor to get high water output with high quality water and low cost whether coagulant is used correctly. Considering the practice of liquid Aluminium chloride used in Shenzhen, an experiment was carried out with different dosage, diluting times and different settlement time. It is expected that the result of the experiment will give reference for the water works to use aluminium chloride in water treatment. Aluminium is the third most abundant element in the earth's crust after oxygen and silicon. It occurs in nature in combination with other elements such as oxygen, silicon and fluoride. Traditionally, aluminium has been considered as nontoxic to humans. However, in recent years, increased attention is being focussed on possible adverse effects of aluminium on human health. Human exposure to aluminium is from its natural occurrence in the environment i.e. through food, water and air as well as from aluminium deliberately introduced into the environment by man. Aluminium compounds are used in pharmaceuticals (antacids, analgesics, antiperspirants) in water treatment processes (as coagulant) and as metal in consumer products (Proudfoot, 2009).

Aluminium is present in virtually all plants. Foods naturally high in aluminium include potatoes, spinach and tea. Processed dairy products, flour and infant formula may be high in aluminium,if they contain aluminium compounds as food additives (WHO, 1998).Aluminium is present in small amounts in mammalian tissues, yet there is little or scanty research work to support its physical usefulness. However, its neurotoxic effect on living organisms is becoming clear, aluminium being implicated as interfering with a variety of cellular metabolic processes in the nervous system and in other systems. Although molecular

mechanisms by which aluminium exerts its neurotoxicity remain to be established, several pieces of evidence suggest that Aluminium can interfere with cellular metabolism in terms of biological stimulation, inhibition, or metal accumulation and compartmentation (Zatta *et al*., 1991).

Aluminium in natural waters depends on the source. It was observed that ground waters had low aluminium levels as compared to surface waters (Miller, *et al.* 1984). At neutral pH, it may range from 0.01 to 0.05 mg/l, except in turbid waters (WHO, 1998). Higher concentrations can be present in acidic water or in the presence of organic compounds even at neutral pH. Aluminium sulphate (Alum) is routinely used as coagulant in water treatment units. There are several reports on the increase of total aluminium concentration in treated water as compared to raw water (Miller, et al. 1984; Cech and Montera, 2000; Lettermann and Driscoll, 1988).

The average intake of aluminium per day by an adult may range from 5-14 mg from all exposure routes (WHO, 1998; Health Canada, 1997), which comes primarily from food. Drinking water contribution has been estimated to be ranging from 3 to 8% (WHO, 1998, Health Canada, 1997). Persons, who regularly ingest aluminium containing antacids and buffered analgesics may be consuming as high as 5 g aluminium per day. Contribution of air for the total aluminium exposure is generally negligible. In 1989, the FAO/WHO Expert Committee on Food Additives reported that the daily intake of aluminium in children ranges between 2 and 6 mg, while in adults 6-14mg (Gramiccioni et al., 1996; Starska, 1993; Sepe, et al., 2001). According to Drugs Institute guidelines in investigations carried out on animals, a dose of drug applied to the rodents should be 4- to10-fold higher than a human dose. Aluminium was found to be toxic to the nervous system of animals over 100 years ago. Injecting aluminium into the brains of sheep was reported in 1965 to result in changes in the brain that shrink: "a striking resemblance" to Alzheimer Disease (AD) in people (Savory and

Garruto, 1998). Dougall, (2004) stated that in 1973, brains of Alzheimer Disease (AD) patients were found to contain aluminium than people dying without this disease. About the same time, kidney patients on dialysis were found to suffer, sometimes fatal, brain damage (encephalopathy) from aluminium in their antacids (these antacids were used to bind phosphates in their intestines) (Savory and Garruto, 1998).

More than 100 toxic of aluminium have been identified and many are damaging to the human brain. The progressive deterioration in cognitive function associated with Alzheimer had been correlated with loss of cholinergic function and the degeneration of cholinergic neurons (Collerton, 1988; Francis *et al*., 1985; Smith and Swash, 1978; Whitehouse *et al*., 1985). Aluminium is generally a recognized neurotoxin that is believed to be at the root cause of Alzheimer Disease (AD). Aluminium becomes more toxic when combined with a high cholesterol diet. They work together by means that have yet to be fully determined to create the senile plagues and ultimately the mental deterioration known as AD. However, there are at least two recognized synergistic ways that these factors contribute to brain damage. First, an acid-forming diet-one high in meat, poultry, eggs, and cheese-leads to increased senile and brain concentrations of aluminium. Second, aluminium enhances inflammation. The immune enhancing properties of aluminium discovered after immunization with diphtheria and tetanus vaccines in studies performed in the 1940s and 1950s and aluminium is used today to enhance the effectiveness of the inflammatory response of most vaccines given to adults and children. In the brain, aluminium enhances the inflammation that may result from the formation of senile plagues driven by cholesterol build up initial injury-this metal is known toxic to the nervous system-that starts the disease processes, leading to brain cell death, senile plagues neurofibillary tangles. However, according to Dougall (2004), aluminium is present in our water, foods, medications and air. The healthy human body has effective barriers such as skin, lungs, and gastrointestinal tracts, against aluminium. Aluminium is not a nutrient-in

other words and the body has no need for this metal and avoidance has no negative consequences. All foods naturally contain aluminium, but some, such as tea, are partly high in this metal. Fortunately, most of the aluminium in natural plant foods is bound with other substances such as Silicon which prevents absorption of the aluminium into the body. The harmful (unbound, more absorbable) forms of aluminium enter our foods additives such as leaving agent and emulsion. Aluminium is used over the counter as Deodorants, vaginal douches, baby wipes, skin creams, suntan lotions, toothpaste, buffered aspirin, and as some hemorrhoid and diarrhea products. It is also used medicinally as vaccinations, allergy testing, intravenous solutions, allergens, wound and antacid, ulcer treatment. It is used in foods as aluminium cans, foils, containers, baking powder, cake mixes, frozen dough, pancake mixes, self-rising flour, grains and processed cheese (Dougall, 2004).

2.2 Production and Health Risk Associated with Aluminium

Although, Aluminium was first identified in 1808 by Humphrey Davy, it was only in 1825 that Christian Oersted succeeded in preparing it by reduction of aluminium chloride ($AlCl_3$) with potassium. The processes for its production, such as those developed by Friedrich Wohler in 1827, were very complicated and at that stage, aluminium was one of the most expensive metals on earth costing about $US500 per kilogram; even at the start of electrolytic production it cost $US 17 /kg. This is reflected in the fact that it was used as coinage in France for many years. Its cost did not fall to about 50c/kg until the start of the 20th century. In 1886, the real breakthrough came when scientists, working independently in France and the United States of America, discovered the means of making aluminium economically from an alumina powder and electricity: the Héroult and Hall processes (Stobart, 1986). The electrical power required for its production was so great that it was referred to at one time as 'solidified electricity'. It was clear from the outset that as an

engineering metal, it was extremely useful where light weight was more important than cost of production and it is only because of its availability that the aircraft industry developed. Because of the demands of the aircraft industry, the processes of aluminium production and development of aluminium alloys progressed (Grjotheim et al., 1982; Doutre *et al.,* 1985).

Aluminium is produced from ore (primary aluminium) and scrap (recycled aluminium). In 2006, global aluminium production was 50.4 million tones, - 34 million tones metal primary and 16.4 million tones recycled metal. Aluminium ore, most commonly bauxite, is plentiful and occurs mainly in tropical and sub-tropical areas - Africa, West Indies, South America and Australia - with some deposits in Europe. Bauxite is mined then refined into aluminium oxide trihydrate (alumina) using the Bayer Process, which has changed very little since the first plant opened more than 100 years ago. Alumina is then electrolytically reduced via a smelting process into metallic aluminium. Primary aluminium smelting plants are located all over the world; however, because it is an energy-intensive process, they are often in areas where there are abundant supplies of inexpensive energy, such as hydroelectric power. Once aluminium is formed, it is alloyed with other materials to make an array of metals with different properties and it can be processed in any number of ways - rolled, cast, extruded. It is used in countless applications and products and can be recycled repeatedly. Two to three tonnes of bauxite are required to produce one tonne of alumina and two tonnes of alumina are required to produce one tonne of aluminium metal. (Alcan Ltd.; Edwards et al., 1930; Grjotheim et al., 1982; Pawlek, 1998).

Aluminium is historically been considered as nontoxic for healthy individuals. However, there is now enough evidence to show that under certain circumstances, excess aluminium can cause a variety of physiological and biological effects in humans (Alfrey, 1983). Aluminium compounds can have inhibitory effect on gastrointestinal muscle contraction, resulting well-recognized complications of constipation in patients consuming

oral antacid formulations. Pulmonary disease resulting from the inhalation of aluminium and aluminium oxide powder has been reported as an occupational hazard for people working in aluminium industries. Resulting pulmonary fibrosis leads to restrictive and obstructive airway diseases (Alfrey, 1983).

Recent interest in aluminium toxicity is due to its possible role in multiple neurological diseases such as Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer disease (AD). In 1970's, many reports were published on a specific form of progressive dementia (dialysis encephalopathy) characterized by speech and behavioural changes, tremors, convulsions in chronic renal patients, who were on dialysis. This was traced to the presence of aluminium in dialysis fluids. Eliminating aluminium in dialysis fluids eliminated the dementia. These findings proved the neurotoxicity of aluminium. Aluminium has been linked with other forms of dementia and AD in particular. There are several reports, both for and against the hypothesis, that aluminium plays an active role in AD (Alfrey, 1983; WHO, 1998; Alzheimer Society, 2002). A number of theories have been proposed for the onset of AD, which includes genetic factors, abnormal proteins, environmental agents etc. Still complex interactions between aging and genetic predisposition and the series of events ultimately leading to the onset of AD is not known. From the available information, evidence linking aluminium and Alzheimers's disease cannot be made with any certainty (Alfrey, et al., 1976; Alzheimer Society, 2002).

Although large portion (≈ 90%) of aluminium intake by humans comes from food, presently there is much concern on the presence of aluminium in drinking water. It is proposed that residual aluminium in drinking water after treatment with alum may be in a bio available form (Health Canada, 1997). Several epidemiological studies have been carried out

to assess whether aluminium in drinking water is a risk factor for AD (WHO, 1998). Still no conclusion has been drawn on this issue.

2.3 Dietary and other sources of Aluminium Intake

According to Greger *et al*. (1985), aluminium in the food supply comes from natural sources including water, food additives and contamination by aluminium utensils and containers. Oral exposure to aluminium is from food, water and pharmaceutical products. The major route of exposure to aluminium for the general population is through food. Aluminium in drinking water represents another, minor, source of exposure. Additional exposures may arise from the use of aluminium compounds in pharmaceuticals and consumer products. Most unprocessed foods typically contain less than 5 mg aluminium/kg. Higher concentrations (mean levels 5 to 10 mg/kg) were often found in breads, cakes and pastries (with biscuits having the highest levels), some vegetables (with mushrooms, spinach, radish, swiss card, lettuce and corn salad having the highest levels), glacé fruits, dairy products, sausages, offals, shellfish, sugar-rich foods baking mixes, and a majority of farinaceous products and flours. Foods with very high mean concentrations included tea leaves, herbs, cocoa and cocoa products, and spices (Aguilar *et al.,* 2008).

Under normal and typical conditions the contribution of migration from food contact materials would represent only a small fraction of the total dietary intake. However, the Panel noted that in the presence of acids and salts, the use of aluminium-based pans, bowls, and foils for food such as apple puree, rhubarb, tomato puree or salted herring could result in increased aluminium concentrations in such foods. Also, the use of aluminium vessels and trays for convenience and fast food in might moderately increase the aluminium concentrations, especially in foods that contain tomato, different types of pickles, and vinegar. Total dietary exposure to aluminium from all sources has been estimated from

duplicate diet studies (the Netherlands, Hungary, Germany, Sweden, and Italy), and market basket and total diet studies (UK, Finland, and France). Mean dietary exposure from water and food in non-occupational exposed adults showed large variations between the different countries and, within a country, between different surveys. It ranged from 1.6 to 13 mg aluminium per day, corresponding to 0.2 to 1.5 mg/kg body weight (bw) per week in a 60 kg adult. Children generally have higher food intake than adults when expressed on a body weight basis, and therefore represent the group with the highest potential exposure to aluminium per kg body weight. Large individual variations in dietary exposure to aluminium can occur (Aguilar *et al.,* 2008; Pennington and Schoen, 1995).

The use of aluminium in food contact materials fall into two main fields: use of aluminium and its alloys as food contact materials and use of aluminium and aluminium organic and inorganic compounds as additives for food contact materials (Aguilar *et al.,* 2008).

Aluminium metal and its alloys are used to manufacture articles that are destined to be used for processing, packaging, and storage of foods at the industrial, the retail and the domestic level. A variety of industrial applications of aluminium food containers are now available, in which aluminium is the main component (e.g. cans for beverages, fish or meat, small flexible tubes, caps or tear open closures) or is included in a multilayer structure composed of several materials (e.g. beverages cartons, plastic laminates etc). In these applications, aluminium is generally not in direct contact with foods, being coated or coupled with plastic barrier layers. However, uses of aluminium metal exist also in food industry applications in which aluminium is in direct contact with foods, such as industrial pans and utensils to process foods, or foil and trays for long term packaging of chocolate and cakes. Moreover, aluminium is widely used in the food industry as a component of materials and machinery used in food processing (e.g. surfaces, accessories, tanks etc). Typical examples of

domestic use are pans, coffee pots, baking trays, kitchen utensils and accessories, containers for dried spices, sugar and coffee, and wrapping foils for cooking and storage of foods (Aguilar *et al.,* 2008; Giordano and Costantini, 1993).

The major route of exposure to aluminium for the general population is through food, both as a consequence of the natural occurrence of aluminium in food (e.g. fruit, vegetables, cereals, seeds and meat), and the use of aluminium and aluminium compounds in food processing, packaging and storage, and not least the use of aluminium compounds as food additives. Aluminium in drinking water represents a minor source of exposure. The Panel noted that additional exposures may arise from the use of aluminium compounds in pharmaceuticals and consumer products, such as antiperspirants, and through occupational exposure (Giordano and Costantini, 1993).

Table 2.3: Concentration of Aluminium in Foods before and after Cooking in Aluminium and Stainless steel cookware (µg Al/g wet weight)

FOOD	UNCOOKED	ALUMINIUM POT	STAINLESS POT
Apple sauce	0.13	710	0.12
Beef	0.19	0.85	0.16
Cabbage	0.13	3.60	0.20
Tomato sauce	0.10	57.10	0.16

(Greger *et al.*, 1985)

2.3.1 Water as a Source of Aluminium

The amount of aluminium in surface and ground water is variable; concentrations of 0.012 to 2.25 mg/ litre have been reported in North American rivers. When the pH of water is <5, the amount of soluble aluminium in water tends to increase (Jones and Benneta, 1986). Aluminium is one of the most abundant metals in the earth's crust. Human exposure to aluminium has been increasing over the last decades. This element appears mainly in food products and in drinking water derived from both natural sources and treatment methods (Yokel and McNamara, 2001).Drinking water contribution has been estimated to be ranging from 3 to 8% (WHO, 1998; Health Canada, 1997). The average intake of aluminium per day by an adult may range from 5-14 mg from all exposure routes (WHO, 1998; Health Canada, 1997), which comes primarily from food. Drinking water contribution has been estimated to be ranging from 3 to 8% (WHO, 1998; Health Canada, 1997). Persons, who regularly ingest aluminium containing antacids and buffered analgesics may be consuming as high as 5 g aluminium per day. Contribution of air for the total aluminium exposure is generally negligible (WHO, 1998).

Aluminium containing flocculants are used to clarify municipal water supplies. Miller *et al*., (1984) reported that the concentration of aluminium in the finished water at 186 water utilities in the United States ranged from < 0.014 to 2.67 mg Al/mg/ l. They estimated that there was a 40 – 50 % chance that aluminium coagulants increased the Aluminium concentration of finished water above that naturally present in water. However, the median level of aluminium in the finished water samples studied was very low (< 0.017 mg/ l). Thus, individuals consuming two litres of water daily would take in less than 0.04 mg aluminium

from water. Furthermore, the evidence that links aluminium in drinking water to Alzheimer's disease is derived to a large extent from ecological studies that compare rates of the disease in populations that are supplied with water containing different amounts of aluminium. This type of study is notoriously vulnerable to confounding. One possible confounder is silicon. Waters that are high in aluminium tend to be low in silicon, and vice versa (Birchall, 1992).

2.4 Guidelines and Standards for Aluminium in Drinking Water

International Water Quality Standards / Guidelines are given in Table 2.4 Below: Guideline value of 0.2 mg/l as 'total aluminium,' proposed by WHO (1998), is based mainly on aesthetic considerations, as excess aluminium results in discoloration of water.

Table 2.4 Drinking Water Quality Standards for Aluminium

Organization / Government	**Guideline Level (mg/L)**	**Maximum acceptable concentration (mg/L)**
WHO	0.2	-
EEC	0.05	0.2
Sweden	-	0.1
Switzerland	-	0.5
USEPA	0.05	0.2
Finland	-	0.2
Denmark	0.05	0.2
Austria	-	0.2
Canada		
BIS	0.03	0.2
Nigeria	0.2	-

(WHO, 1998; and Nigerian Industrial Standard (NIS), 2007)

In the addendum to the guidelines published in 1998, considering the beneficial effects of aluminium as a coagulant in water treatment as well as health concerns about aluminium, a practical level was derived based on optimization of the coagulation process in drinking water plants using aluminium based coagulants, to minimize aluminium levels in the finished water. Thus 0.1 mg/l (for large treatment facilities) is suggested as the achievable concentration, whereas 0.2 mg/l or less is a practical level for the small facilities.

At a meeting, to plan the revision of guidelines, held by WHO, in Berlin in June 2000, it was concluded that the guideline document for aluminium did not need to be revised but that aluminium is a candidate for future review, when new data that are being generated on health based issues, become available (WHO, 2000). Canada is considering the recommendation of a guideline for aluminium (presently there is no guideline value for maximum acceptable concentration for aluminium in drinking water) based on the free form i.e. soluble unbound form present, not the "total" aluminium that is currently measured and quoted in the reports (Health Canada, 1997).

2.5 Aluminium Toxicology

The health effects of antiperspirants are a matter of dispute regarding their extent. A small percentage of people are allergic to aluminium and may experience contact dermatitis when exposed to aluminium-containing deodorants (Abreo, 2009). Aluminium from use of deodorants caused contact dermatitis to the skin. After using a deodorant containing zirconium the skin may develop an allergic, axillary granuloma response. Deodorant alum crystals are a weak irritant to the skin. Alcohol-free deodorant is available for those with sensitive skin. It is one of the few abundant elements that appear to have no beneficial function to Living cells (non-essential metal) (Exley *et al.,* 2007). Aluminium, present most often in antiperspirants, but not usually present in non-antiperspirant deodorants, has been

established as a neurotoxin in very high Doses. At high doses, Aluminium itself adversely affects the blood-brain barrier, is capable of causing DNA damage, and has adverse epigenetic effects.(Lankoff, 2006) Research has shown that high doses of the Aluminium salts used in antiperspirants have detrimental effects to a number of species such as non-human primates, mice, and dogs. An increased amount of Aluminium is also present in the brains of many Alzheimer's patients (Zubenko and Hanin, 1989; Banks and Kastin, 1989; Sarin, et al., 1997). Experiments with mice applying aqueous solution of Aluminium chloride to the skin resulted in "a significant increase in urine, serum, and whole brain Aluminium" and transplacental passage (Anane, 1995). A study shown that the use of aluminium chlorohydrate, the active ingredient in many antiperspirants, does not lead to a significant (vs. ingestion via diet) increase in aluminium levels in the body with one-time use. The association between exposure to Aluminium and long term use of antiperspirants and Alzheimer's disease is a trend toward a higher risk with increasing frequency of use. The use of aluminium-containing antiperspirants has been linked with the systemic accumulation of aluminium which increases the risk of Alzheimer's disease. (Graves et al., 1990; Anane, 1997).

2.5.1 Neurotoxicity of Aluminium

Aluminium has shown neurotoxicity in patients undergoing dialysis and thereby chronically exposed parenterally to high concentrations of aluminium. It has been suggested that aluminium is implicated in the aetiology of Alzheimer’s disease and associated with other neurodegenerative diseases in humans. However, these hypotheses remain controversial. Based on the the available scientific data, the Panel does not consider exposure to aluminium via food to constitute a risk for developing Alzheimer’s disease**.**

Behavioural impairment has been reported in laboratory animals exposed to soluble aluminium salts (e.g. lactate, chloride) in the diet or drinking-water. Both rats (Connor et al., 1988) and mice (Yen-Koo, 1992) have demonstrated such impairments at doses exceeding 200 mg of aluminium per kg of body weight per day. Although significant alterations in acquisition and retention of learned behaviour were documented, the possible role of organ damage (kidney, liver, immunological) due to aluminium was incompletely evaluated in these studies (WHO, 1997).In studies on brain development in mice and rats, grip strength was impaired in offspring of dams fed 100 mg of aluminium (as aluminium lactate) per kg of body weight per day in the diet, in the absence of maternal toxicity (WHO, 1997).

2.5.2 Effects on humans

There is little indication that aluminium is acutely toxic by oral exposure despite its widespread occurrence in foods, drinking-water, and many antacid preparations (WHO, 1997). In 1988, a population of about 20 000 individuals in Camelford, England, was exposed for at least 5 days to unknown but increased levels of aluminium accidentally distributed to the population from a water supply facility using aluminium sulfate for treatment. Symptoms including nausea, vomiting, diarrhoea, mouth ulcers, skin ulcers, skin rashes, and arthritic pain were noted. It was concluded that the symptoms were mostly mild and short-lived. No lasting effects on health could be attributed to the known exposures from aluminium in the drinking-water (Clayton, 1989).

It has been hypothesized that aluminium exposure is a risk factor for the development or acceleration of onset of Alzheimer disease (AD) in humans. WHO (1997) has evaluated some20 epidemiological studies that have been carried out to test the hypothesis that aluminium in drinking-water is a risk factor for AD. Study designs ranged from ecological to case–control.Six studies on populations in Norway (Flaten, 1990), Canada (Neri and Hewitt,

1991), France, Switzerland, and England were considered of sufficiently high quality to meet the general criteria for exposure and outcome assessment and the adjustment for at least some confounding variables. Of the six studies that examined the relationship between aluminium in drinking water and dementia or AD, three found a positive relationship, but three did not. However, each of the studies had some deficiencies in the study design (e.g. ecological exposure assessment; failure to consider aluminium exposure from all sources and to control for important confounders, such as education, socioeconomic status, and family history; the use of surrogate outcome measures for AD; and selection bias). In general, the relative risks determined were less than 2, with large confidence intervals, when the total aluminium concentration in drinking-water was 0.1 mg/litre or higher. Based on current knowledge of the pathogenesis of AD and the totality of evidence from these epidemiological studies, it was concluded that the present epidemiological evidence does not support a causal association between AD and aluminium in drinking-water (WHO, 1997). In addition to the epidemiological studies that examined the relationship between AD and aluminium in drinking-water, two studies examined cognitive dysfunction in elderly populations in relation to the levels of aluminium in drinking-water. The results were again conflicting. One study of 800 male octogenarians consuming drinking-water with aluminium concentrations up to 98 μg/litre found no relationship. The second study used "any evidence of mental impairment" as an outcome measure and found a relative risk of 1.72 at aluminium drinking-water concentrations above 85 μg/litre in 250 males. Such data are insufficient to show that aluminium is a cause of cognitive impairment in the elderly. The controversy surrounding the relationship between elevated aluminium concentration in drinking water and increased risk of Alzheimer's disease in elderly populations has ignited the debate about setting standards for aluminium in drinking water. At present there are inadequate scientific bases for setting a health based standard for aluminium in drinking water. The Canadian Environmental

Protection Act (CEPA) recently included aluminium chloride, aluminium nitrate and aluminium sulphate on its Priority Substance List, making those possible candidates for review of their toxicity. They were considered toxic if:

· There is an immediate or long term effect on the environment;

· They may constitute a danger to the environment on which human life depends;

· They may constitute a danger in Canada to human life or health.

The World Health Organisation (WHO) commissioned the development of a criteria document for health effects from exposure to environmental aluminium under its International Programme on Chemical Safety (IPCS) in 1995. The international panel of experts concluded that aluminium has not been shown to pose a health risk to healthy non-occupationally exposed populations. (Armstrong *et al.,* 1994; Gibbs, 1995, 1997).

2.6 Anatomy and development of Cerebral Cortex

The cerebral cortex is a sheet of neural tissue that is outer-most to the cerebrum of the mammalian brain. It consists essentially of three regions; neopallium, the paleopallium and archipallium. The neopallium is the newest portion and also the largest of the three, consisting of gyri and sulci (Sisson and Grossman, 1953). Archipallium now called "hippocampus" and the paleopallium of the pyriform lobe become the centre devoted extensively to olfactory functions. The corpus callosum arises to connect the enlarged neopallia of the two halves of the brain (Theodore and Alan, 1979).

The cerebral cortex is the biggest part of the brain. This large and complicated neural circuit is involved in most of the brain's highest functions, such as memory, language and

sight. In man and higher animals, modifications of behaviour are due to cortical activity. (Pavlov, 1927).Pavlov demonstrated that conditional reflex being mediated through the cerebral cortex can be impaired or abolished in the dog by removal of appropriate cortical areas. Meyer and Woolsey (1952) also demonstrated how studies on frequency and intensity of sound can be used to study conditional reflex. Memories are stored in the cerebral cortex (Penfield, 1950). In his observation on temporal cortex in conscious patients, he made an assumption that integration of memories is not dependent on the association system of the cerebral cortex alone but also on central connections in the higher brain stem.

Mettler, in 1935 explained that the two hemispheres of man are not symmetrical in their control of several functions. Speech, reading and writing for example, are vested in the left hemisphere, whereas the analysis of music is in the right (or right handed persons). These cortical areas account for most of the cortex of lower mammals (Marsupials and Insectivores), but for only one-quarter of the cortex of man. In the parietal and occipital regions, there are secondary and tertiary projections areas that code messages, store information, combine inputs and provide spatial orientation (Milton, 1974). If these areas are impaired, a man might see well, yet confuse right and left; might walk well, yet get lost in a familial place. The frontal region of the cerebral cortex relates to programs intentions, orientation to goals and sequence in the performance of activities. The entire cortex of experimental animals becomes relatively heavy if the animals live in a relatively diverse, stimulating and enriched environment (Milton, 1974). George (1973) described the cerebral cortex as the highest center to which sensory impulse can be projected. This impulse, according to him, gives rise in the cortex to sensations of discriminative nature by recognizing minor differences in the temperature, texture or weight of an object held in the hand. It also appears to be one of the locations where past experiences are stored as memory, he explained.

Cerebral Cortex generally plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness. It also integrates higher mental functions, general movement, visceral functions, and behavioural reactions. The cerebral cortex contains motor areas, which control complex motor skills and some involuntary movements; sensory areas, which receive information from the sense organs; and association areas, which are responsible for thought, learning language, and personality (Brodal, 1992; Cauller, 1995).

Staining cross-sections of the cortex to reveal the position of neuronal cell bodies and the intra-cortical axon tracts allowed neuroanatomists in the early 20th century to produce a detailed description of the laminar structure of the cortex in different species. After the work of Korbinia Brodmann (1909), the neurons of the cerebral cortex are grouped into six main layers, from outside (pial surface) to inside (white matter):

1. The molecular layer I, which contains few scattered neurons and consists mainly of extensions of apical dendritic tufts of pyramidal neurons and horizontally-oriented axons, as well as glial cells (Tomer *et al*., 2010; Shipp and Stewart, 2007). Some Cajal-Retzius and spiny stellate neurons can be found here. Inputs to the apical tufts are thought to be crucial for the ‘‘feedback’’ interactions in the cerebral cortex involved in associative learning and attention (Gilbert and Sigman 2007). While it was once thought that the input to layer I came from the cortex itself (Cauller, 1995), it is now realized that layer I across the cerebral cortex mantle receives substantial input from ‘‘matrix’’ or M-type thalamus cells (Rubio-Garrido *et al*., 2009). (in contrast to ‘‘core’’ or C-type that go to layer IV) (Jones 1998).

2. The external granular layer II, which contains small pyramidal neurons and numerous stellate neurons.

3. The external pyramidal layer III, which contains predominantly small and medium-size pyramidal neurons, as well as, non-pyramidal neurons with vertically-oriented intra-cortical axons; layers I through III are the main target of inter-hemispheric corticocortical afferents, and layer III is the principal source of corticocortical efferents.

4. The internal granular layer IV, which contains different types of stellate and pyramidal neurons, and is the main target of thalamocortical afferents from thalamus type C neurons. (Jones 1998) as well as intra-hemispheric corticocortical afferents.

5. The internal pyramidal layer V, which contains large pyramidal neurons (such as the Betz cells in the primary motor cortex); it is the principal source of subcortical efferents.

6. The multiform layer VI, which contains few large pyramidal neurons and many small spindle-like pyramidal and multiform neurons; layer VI sends efferent fibers to the thalamus, establishing a very precise reciprocal interconnection between the cortex and the thalamus**.** These connections are both excitatory and inhibitory. Neurons send excitatory fibers to neurons in the thalamus and also from collateral to each one of them via the thalamic reticular nucleus that inhibit these thalamus neurons or ones adjacent to them (Lam and Sherman, 2010). Since the inhibitory output is reduced by cholinergic input to the cerebral cortex, this provides the brainstem with adjustable "gain control for the relay of lemnsical inputs"(Jones, 1998; Gilbert and Sigman, 2007; Shipp and Stewart 2007). The maps of cortical areas based on the arrangement of cells made by different investigators do not all agree. Finer distinction of cell layers are therefore disputed (Lashley and Clark, 1946).

It is important to note that the cortical layers are not simply stacked one over the other; there exist characteristic connections between different layers and neuronal types, which span all the thickness of the cortex. These cortical microcircuits are grouped into cortical columns and minicolumns, the latter of which have been proposed to be the basic functional units of cortex (Mountcastle, 1997). In 1957, Mountcastle showed that the functional properties of the cortex change abruptly between laterally adjacent points; however, they are continuous in the direction perpendicular to the surface. Later works have provided evidence of the presence of functionally distinct cortical columns in the visual cortex auditory cortex and associative cortex. In preserved brains, it has a gray color, hence the name "gray matter". In contrast to gray matter that is formed from neurons and their unmyelinated fibers, the white matter below them is formed predominantly by myelinated axons interconnecting neurons in different regions of the cerebral cortex with each other and neurons in other parts of the central nervous system ((Hubeld and Wiesel, 1959).

The surface of the cerebral cortex is folded in large mammals, such that more than two-thirds of it in the human brain is buried in the grooves, called "sulci". The Phylogenetically, most recent part of the cerebral cortex, the neocortex (also called isocortex), is differentiated into six horizontal layers; the more ancient part of the cerebral cortex, the hippocampus (also called archicortex), has at most three cellular layers, and is divided into subfields. Neurons in various layers connect vertically to form small microcircuits, called columns. Different neocortical architectonic fields are distinguished upon variations in the thickness of these layers, their predominant cell type and other factors such as neurochemical markers.

2.6.1 Embryology of the Cerebral Cortex

The cerebral cortex develops from the most anterior part of the neural plate, a specialized part of the embryonic ectoderm. The neural plate folds and closes to form the neural tube. From the cavity inside the neural tube develops the ventricular system, and, from the epithelial cells of its walls, the neurons and glia of the nervous system. The most anterior (frontal) part of the neural tube, the telencephalon, gives rise to the cerebral hemispheres and cortex (Cauller, 1995).

Cortical neurons are generated within the ventricular zone, next to the ventricles. At first, this zone contains "progenitor" cells, which divide to produce glial and neuronal cells (Stephen *et al*., 2001). The glial fibers produced in the first divisions of the progenitor cells are radially oriented, spanning the thickness of the cortex from the ventricular zone to the outer, pial surface, and provide scaffolding for the migration of neurons outwards from the ventricular zone. The first divisions of the progenitor cells are symmetric, which duplicates the total number of progenitor cells at each mitotic cycle. Then, some progenitor cells begin to divide asymmetrically, producing one post-mitotic cell that migrates along the radial glial fibers, leaving the ventricular zone, and one progenitor cell, which continues to divide until the end of development, when it differentiates into a glial cell or an ependymal cell. The migrating daughter cells become the pyramidal neurons of the cerebral cortex (Rakic, 1988).

The layered structure of the mature cerebral cortex is formed during development. The first pyramidal neurons generated migrate out of the ventricular zone and subventricular zone, together with Cajal-Retzius cells from the preplate. Next, a cohort of neurons migrating into the middle of the preplate divides this transient layer into the superficial marginal zone,

which will become layer one of the mature neocortex, and the subplate, forming a middle layer called the cortical plate. These cells will form the deep layers of the mature cortex, layers five and six. Later born neurons migrate radially into the cortical plate past the deep layer neurons, and become the upper layers (two to four). Thus, the layers of the cortex are created in an inside-out order. The only exception to this inside-out sequence of neurogenesis occurs in the layer I of primates, in which, contrary to rodents, neurogenesis continues throughout the entire period of corticogenesis. (Zecevic and Rakic, 2001).

2.7 Neutron Activation Analysis (NAA)

The application of Neutron Activation Analysis (NAA) to investigate archaeological problems began in the mid-1950s, when scientists at Brookhaven National Laboratory (Sayre and Dodson 1957) recognized its potential for relating artifacts to source materials through their chemical signatures. The advantages of NAA over other analytical techniques were quickly recognized, including: (1) ease of sample preparation; (2) high precision; (3) simultaneous measurement of multiple elements; (4) outstanding replicability; and (5) excellent inter-laboratory comparability. Interest in NAA expanded throughout the 1960s, 1970s, and 1980s as a result of an increased curiosity in archaeological questions by physical scientists (e.g., Perlman and Asaro 1969; Harbottle 1976, 1982) and direct involvement of archaeologists (e.g., Bieber *et al*. 1976; Bishop 1975) in the application of nuclear analytical procedures.

Neutron Activation Analysis (NAA) is a sensitive analytical technique useful for performing both qualitative and quantitative multi-element analysis of major, minor, and trace elements in samples from almost every conceivable field of scientific or technical interest. For many elements and applications, NAA offers sensitivities that are superior to those attainable by other methods, on the order of parts per billion or better. In addition,

because of its accuracy and reliability, NAA is generally recognized as the "referee method" of choice when new procedures are being developed or when other methods yield results that do not agree (Harbottle, 1982).

Neutron Activation Analysis (NAA) is a sensitive quantitative and qualitative method of high efficiency for the precise determination of a number of main-components and trace elements in different types of samples. NAA, based on the nuclear reaction between neutrons and target nuclei, is a useful method for the simultaneous determination of about 25-30 major, minor and trace elements of geological, environmental and biological samples in Part per million (ppm) range without or with chemical separation (Vértes *et al*., 1998).

In NAA, samples are activated by neutrons. During irradiation the naturally occurring stable isotopes of most elements that constitute the rock or mineral samples, biological materials are transformed into radioactive isotopes by neutron capture. Then the activated nucleus decays according to a characteristic half-life; some nuclides emit particles only, but most nuclides emit gamma-quanta, too, with specific energies. The quantity of radioactive nuclides is determined by measuring the intensity of the characteristic gamma-ray lines in the spectra. For these measurements a gamma-ray detector and special electronic equipment are necessary. As the irradiated samples contain radionuclides of different half-lives different isotopes can be determined at various time intervals (Ehmann and Vance, 1991; Erdtmann and Soyka, 1979; Friedlander *et al.,* 1981; Glascock, 1996; Keisch, 1972; Kruger, 1971; Parry, 1991; Soete *et al*., 1972; Vértes *et al*., 1998). Although, the development of analytical techniques has led to the expansion of new methods (ICP-AAS, ICP-MS, etc.), which can also be widely applied in analytical chemistry, NAA is still competitive in many areas. The indisputable advantage of the method is its sensitivity and accuracy especially in respect of some trace elements (Das *et al*., 1989).

The method is of a multi-element character, i.e. it enables the simultaneous determination of many elements without chemical separation. In the case of instrumental determination, the preparation of samples involves only the preparation of representative samples, i.e. pulverization or homogenization in most cases, and this reduces the danger of contamination to a minimum and accelerates the whole analytical process. Due to the emitted fast neutrons, in NAA the neutron generators are used for the determination of elements of high cross section in this energy region. Examples of elements of geochemical interest determined by the fast neutrons of generators are: Magnesium: 26Mg (n,) 23Ne T½ = 37.6 s, Aluminium: 27Al (n,p)27Mg T½ = 9.5 min, Silicon: 28Si (n,p)28Al T½ = 2.3 min, Titanium: 46Ti (n,p)46mSc T½ = 18.7 s, Iron: 56Fe (n,p)56Mn T½= 2.58 h, Zirconium: 90Zr (n,2n)89mZr T½ = 4.2 min and Nickel: 60Ni (n,p)60mCo T½ = 10.5 min, (Alfassi,1990; Alfassi, 1994; Alfassi,1998; Al-Mugrabi and Spirou,1987;Das *et al*., 1989).

2.8 Morris Water Maze

The Morris Water Maze Task is a behavioral task, that is, the dependent measure is the animal's behaviour. Behavioural tests measure the responses of whole animals, allowing experimental manipulations to affect in vivo systems operating in harmony to provide a clear understanding of the outcome. Because there are so many factors involved between experimental manipulations and behaviour, the effects can sometimes be unpredictable, and it can be difficult to ascertain whether the behaviour was indeed a main effect of the experiment. Still behavioural experiments offer great insight into the effects of experimental manipulations on whole organisms (Hebb, 1949; Hull, 1943; Kandel *et al.,* 2000).

Rats are natural swimmers. They want to get out of the water, but swimming does not distress them. Two advantages of Morris water maze over others mazes are:

1. The rat wants to get out, so it searches - it does not wait about.

2. In water there are no local cues (e.g. scent trails) (Morris, 1981).

The essential feature of the technique is as follows: rats are placed into a large circular pool of water from which they can escape onto a hidden platform. The platform is hidden, first by arranging that its top surface is just beneath the water surface, and second by rendering the water opaque so that the platform is invisible.

Thus, the platform offers *no* local cues to guide escape behaviour. In principle, rats could escape from the wafer by swimming randomly or in unsystematic search paths throughout the pool; but in practice, normal rats very quickly learn to swim directly towards the platform from any starting position at the circumference of the pool. The accurate directionality of their escape behaviour (and other measures of Performance) provides evidence that the rats escape by learning the spatial position of the platform relative to distal cues. Comparison of the performance of normal and brain-damaged rats (Morris *et al*., 1982; Sutherland *et al*., 1983), and of rats given drugs (Sutherland *et al*., 1982) or cerebral neurotoxins (Hagan *et al*., 1983) offers a new way of examining the neurobiology of spatial learning.The technique of using escape from water to motivate learning has been used for many years (Glaser, 1910; Wever, 1932; Waller *et al*., 1960).

Rats or mice are trained to use extra-maze visual cues to locate an escape platform hidden just below the surface of the opaque water (Morris, 1984). The hidden platform version of the Morris Water Maze is a test of spatial memory which is sensitive to hippocampal damage, while the visible platform version of the Morris Water Maze is a non-hippocampal task, which is disrupted by dorsal striatum lesions (McDonald and White, 1994). While both rats and mice can be tested in the Morris Water Maze, they may show different learning strategies (Frick *et al*., 2000). There are also concerns that anxiety or fear

may be induced in mice in the water maze, suggesting involvement of the amygdala as well as the hippocampus (Akirav *et al*., 2001). Although extensive use has been made of the Morris Water Maze as a test of spatial learning, it is not without its critics (Wolfer *et al*., 1998). The use of the Morris Water Maze in the study of learning and memory has been extensively reviewed by D'Hooge and De Deyn (2001).

The Morris Water Maze has been used extensively to study strain differences in spatial learning in mice (Upchurch and Wehner, 1988) and the effects of maze parameters on strain differences in spatial learning (Klapdor and van der staay, 1996; Chapillon and Debouzie, 2000). Considerable use has been made of the Morris Water Maze for studying spatial learning in transgenic and mutant mice (Owen *et al*., 1997; Pardee *et al*., 1999; Chen *et al*., 2000), the effects of brain injury on spatial learning (Fox *et al*., 1998) and the effects of drugs on spatial learning (Von Lubitz *et al*., 1993; Fordyce *et al*., 1995).

2.9 Elevated plus Maze

Anxiety is a general term for several disorders that cause nervousness, fear, apprehension, and worrying. These disorders affect how we feel and behave, and they can manifest real physical symptoms. Mild anxiety is vague and unsettling, while severe anxiety can be extremely debilitating, having a serious impact on daily life. Anxiety affects one-eighth of the total population world-wide and has become an important area of research interest in psychopharmacology during this decade. Anxiety is considered a problem when symptoms interfere with a person's ability to sleep or otherwise function. Generally speaking, anxiety occurs when a reaction is out of proportion with what might be normally expected in a situation. Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety. However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in

the hope that other anxiolytic drugs will have less undesirable effects(Grundman, et al., 2007). Basic research of complex behaviour has led to a dramatic increase in the use of rodent behaviour tests (Crawley, 2007). The elevated plus maze is a widely used behavioural assay for anxiety behaviour of rodents or rats. It is easy to use, can be fully automated and valid results can be obtained in a short time, 5-minutes testing period. The patterns of results obtained using these tasks are replicable across other species (Pellow, *et al*., 1985). The elevated plus maze test is based on the creation of a conflict between the exploratory drive of a rat and its innate fear of open, exposed and elevated areas. Reduced open-arm exploration serves as an index of increased anxiety (Shepherd, *et al*., 1994).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The materials used for this experiment were; wistar rats, chloroform, steel cages, water rubbers, elevated plus maze, Morris water maze, wistar rats, grower's mash, cotton wool, Tap water, distil water, syringes, needles, forceps, knife, sterile surgical blades, table top, mortar, pestle, hand gloves, face mask, Formaline (10% formal saline), aluminium chloride, Absolute Alcohol, 95% alcohol, 70% alcohol, Toluene paraffin, Canada basam, Xylene, beakers, wax, egg albumin, weighing scale (digital), pencil, rotary microtome, slides, cover slips, oven, Nigerian Research Reactor-1 (NIRR-1), Uranium (235U), light water, Beryllium, Nitric acid, gamma-ray data acquisition system, A Multi-Purpose Gamma-Ray Spectrum Analysis Software, Multi-channel analyzer (MCA) card, desiccators, PC-based gamma-ray spectrometry, Lab. coat, water bath, automatic tissue processor (Histokinette Bench model) light microscope, memory card, Digital camera, air tight sampling bottles, Bouin's fluid, aqueous potassium permanganate, aqueous potassium metabisulphite, aqueous ferric ammonium sulphate (iron alom), Haematoxylin and Eosin stain and Hirano-Zimmerman stain.

3.2. Experimental Animals

Fifty (50) Adult wistar rats of both sexes (male and female, 25each) were used for this experiment. The wistar rats were housed in steel cages maintained at conducive environmental conditions with sufficient food, water and under good ventilation. The wistar rats were kept for two weeks (14days) before commencement of administration. This was to enable the animals to acclimatise to the environmental conditions. The body weights of the wistar rats were taken with the aid of weighing balance (Mettler Instrument AG., CH-8606 Greifensee-Zurich; Made in Western Germany; 110-2400, 50-60cls, No.522667, Type: P3), before the commencement of the experiment and after the experiment. The brain weight was also measured after eight weeks of oral administration of aluminium chloride.

3.3. Source of Aluminium

The aluminium chloride was manufactured from Fischer's Scientific Company, manufacturing Chemist-Fairlawn,N.J., U.S.A.,with Cat.No-574, Lat.No.755582,F.W.241.447 Fischer Certified Reagent.

Certificate of Analysis

--- Insoluble matter 0.001%

--- Free acid (HCL) 0.0097%

--- Alkalies and Earths 0.003%

--- Sulfate ($S0_4$) 0.17%

--- Ammonia (NH_3) 0.003%

--- Arsenic (AS) 0.00001%

--- Heavy metal 0.0001%

--- Iron (Fe) 0.0003%

3.4 Acute toxicity test

Lorke's method (1983) was employed to verify acute toxicity of aluminium chloride; although, the manufacturer gave a lethal dose of 3,800mg/kg for rat through oral route. The study (toxicity test) was carried out in two phases. In the first phase, the animals were randomly divided into three groups of three rats each. Their weights were taken and the first group received 10mg/kg body weight of aluminium chloride; the second and the third groups received 100mg/kg and 1000mg/kg of the aluminium chloride respectively in order to establish the range of doses producing toxic effects and the rats were observed for signs of toxicity and death.. In this first phase, there was no mortality.

For the second phase, further doses of 1,600mg/kg, 2,900mg/kg and 5,000mg/kg body weight were given to three groups of one rat each based on the results of the first phase. The rats were observed for twenty four hours (24hrs) for signs of toxicity which include limited movement and low feed intake. LD50=square root of lowest dose multiply by highest dose of toxicity. There was mortality at 5,000mg/kg. Therefore, Lethal dose (LD50) was calculated thus: □2, 900x5, 000= 3,807mg/kg.

3.5 Experimental design

The wistar rats were divided into five groups: The control group was given distil water only while the four aluminium exposed groups were given various concentrations of aluminium chloride. The LD50 was 3,807mg/kg and the route of administration was through oral intubation. The duration of administration was eight weeks.

Group I was the control that received distil water only,

Group II received 475mg/Kg,

Group III received 950mg/kg,

Group IV received 1,425mg/kg,

And group V received 1,900mg/kg.

3.6 The Study of Spatial Learning and Memory



Fig. 3.6 Morris Water Maze with wistar rat on the platform

The study spatial learning and memory was studied with the aid of the modified Morris water maze by Paylor *et al*., (1996). Thirty wistar rats were randomly selected for the spatial learning and memory test.The Morris water maze consists of a round tank (pool) of tap water, which was made opaque with the addition of non-toxic white liquid tempura paint (Schola, Marieville) and there was an escape platform. Water temperature for wistar rats was 26^{o}C ±2 deg. Cold encourages activity, but can cause hypothermia, which impairs learning. During the training phase, the platform was set at 1/2" (half inches) above the water and the side painted black. This training enabled the rats to know that there was a platform and that was how to get out of the water. The wistar rats were dropped into the water, supported in the

hand and gently lowered into the water with the tail-end lower, so the head does not go under water. This was because dropping them in head-first stresses them, which could impair learning. The wistar rats were put in with the head pointing towards the pool-side, to minimize bias using the methods of Mark *et al*., (1996) and Cornwell *et al*., (2008). The pool (Morris Water Maze) is a delicate balance of motivation provided by stress/reward, and a difficult task. Time on platform was sufficient for them to adjust to the feel and location, and to see where they were. Each wistar rats was given sixty seconds(60s) to get to the platform and any rats that could not get to the platform within the stipulated time was considered to have failed the test. The pool was sufficiently high-sided in order to stop them leaping out into the room. The high-side requirement is a major determinant of water level and location of cues.

At first, the wistar rats swan around the side. After a while, they understood that there was no way out by doing that; then, they searched more to the centre. If the wistar rat did not find the platform after approximately one minute (60s), it was lifted out and dried. However, if it really did not get the point, it was gently guided to the platform. It got on the platform and usually reared and looked around. It learned that there was an escape platform. The rats were left for 15 seconds, to orientate on the platform. When they failed to find the platform, they were guided to it so that they could climb on and stay on for 10 seconds. If the rats immediately took to the water and kept swimming, they were guided back. They were to learn that rescue is dependent on staying on the platform. The training of the rats to study spatial learning and memory on the maze was done before the commencement of administrations aluminium chloride (first run) and the study of spatial learning and memory was conducted on Morris water maze after completions of administrations of aluminium chloride (second and third runs).

3.7 The Study of Anxiety-related behaviour



Fig. 3.7 Elevated plus maze with wistar rat on the open arm

The elevated plus maze (EPM) is a piece of equipment and protocol used to measure anxiety levels in laboratory rodents. Different-sized mazes can be used for either rats or mice, but the same principles apply to each experiment. Ten adult wistar rats were randomly selected for the test of anxiety.The EPM apparatus used for this experiment 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 two opposite arms, 50 x50 cm, crossed with two enclosed arms of the same dimension but having 40cm high walls. The two closed arms have sides, so providing some shelter and a more secure feeling for the rats and the other two arms are open. The arms were connected with a central square, 10x10cm, giving the apparatus shape of a plus sign. The maze was kept in a room and elevated 50cm above the floor.

The maze was thoroughly cleaned with water containing a mild detergent (liquid soap) before the introduction of each wistar rat. At the beginning of each test, the wistar rat was placed onto the central area of the maze, facing a closed arm. Monitoring of behaviour, which commenced immediately and lasted for five (5min) minutes was via a video camera mounted above the platform. An arm entry was defined when all four paws of the wistar rats were in the arm and an observer scored the performance for the five minutes.

The Following were noted:

1. The number of entries into open and closed arms (an entry was counted when both forepaws of the rats were placed into the respective arm).

2. The time spent in both types of arms (open and close arms).

3. The number of faecal boli; the significant of the faecal boli is that more anxious animal tend to defecate quickly and more frequently as sign of nervousness or fear.

The premise behind the elevated plus maze was that more anxious rats will spend less time in the uncovered, exposed arms of the maze and less anxious rats will explore the open arms more freely (Shepherd, *et al*.,1994).

3.8 Tissues preparations for Histological Analysis

At the end of eight weeks of administrations of aluminium chloride, ten wistar rats were randomly selected (2per group) and were humanely sacrificed by anaesthetising (rendered physically insensible) with chloroform, in a closed box. The brains were removed according the method already described by Harper and Maser (1975) and immediately fixed in Bouin's fluid.

Tissue preparation method for histological analysis was differentiated into the following stages; fixation, tissue processing, sectioning, staining and photomicrography. This tissue preparation was done using standard techniques in accordance with Gurr, (1962), Culling (1963) and Bradbury (1977). Haematoxylin and Eosin (H&E) staining was used to demonstrate general structure of tissues while Hirano-Zimman method was used for nerve cells and fibre. (Hirano and Zimmerman, 1962; Luna, 1968). The slides obtained were viewed under the light microscope fitted with the laptop and digital camera for photomicrographs.

3.9 Tissues Preparations for Quantification (Concentrations) of Aluminium Uptake by the Brain of Wistar Rats.

Aluminium concentration in the cerebral cortex was assay by Neutron Activation Analysis (NAA).Neutron Activation Analysis (NAA) is a sensitive quantitative and qualitative method of high efficiency for the precise determination of a number of main-components and trace elements in different types of samples. NAA, based on the nuclear reaction between neutrons and target nuclei, is a useful method for the simultaneous determination of about 25-30 major, minor and trace elements of geological, environmental, biological samples in ppm range without or with chemical separation(Jonah *et al.,* 2005).

At the end of eight weeks of aluminium chloride administration, the wistar rats were humanely sacrificed and the brain tissues was immediately removed and dried in an oven for two weeks before taken to Centre For Energy Research and Training (CERT), Ahmadu Bello University, Zaria for determination of quantity of aluminium uptake by Neutron Activation Analysis (NAA) with sample reference of number NIRR-1/JC/11/021.The samples were placed in desiccators and the brain weight of each rat were maintained at 1g.

Ten brain samples (Ref. No.NIRR-1/JC/11/021) were prepared for irradiation without further treatment at the sample preparation laboratory of Centre for Energy Research and Training, CERT, Ahmadu Bello University, Zaria. The samples were placed in a high density polythene vials, and weighted using a Mettler Toledo balance model AE 240. These measured samples were double heat sealed in small pieces of cleaned polythene sheets using heat from a modern drier.

3.9.1 Irradiation and Counting

The irradiation facility is the Nigeria Research Reactor-1 (NIRR-1) at the Centre for Energy Research and Training (CERT), Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Like all Miniature Neutron Source Reactor (MNSR) facilities, NIRR-1, is specifically designed for neutron activation analysis (NAA), therefore it has the capabilities for the analysis of trace, minor and major elements in different sample matrices (Jonah *et al.,* 2005). For analysis of short-lived nuclides requiring shorter irradiation times, the samples were placed in the 7cm3 rabbit capsules and sent to the reactor through a pneumatic transport system into the outer irradiation channel B4, where the neutron spectrum is 'soft in order to eliminate corrections due to nuclear interferences caused by threshold reactions (Jonah *et al.,*2005). The irradiation took 2 minutes and after the cooling time of 2-15 minutes, the first round of counting was performed for 600 seconds. The second rounds of counting were carried out after a waiting period of 3-4 hours and a cooling for another 600 seconds.

The counting of the samples and certified reference sample was carried out using the gamma-ray data acquisition system which consists of a horizontal dip-stick High-Purity Germanium (HPGe) detector with a relative efficiency of 10% at 1332.5keV gamma-ray line, the MAESTRO emulation software compatible with the ADCAM® multi-channel analyzer (MCA) card, associated electronic modules all made by EG & G ORTEC and a personal

computer (Jonah *et al*., 2005). The gamma-ray spectrum analysis software *WINSPAN* 2004 (Liyu, 2004) software developed at CIAE, Beijing, China, was used for the identification of gamma-ray of product radionuclides through their energies and quantitative analysis of their concentration.

Samples were counted on a plexi-glass plated denoted H1 which refers to source detector geometry of 1cm. The PC-based gamma-ray spectrometry set-up performed the radioactivity of the irradiated sample after counting was completed. There are warning light signals that automatically comes on together in whenever the reactor is operation or turned on. There are also the safety precautions taken by the scientists and engineers by wearing Lab coats, compulsory and strict restriction of pregnant women into the vicinity. The results obtained were expressed at standard error of mean (SEM) and the results were said to be significant when $P < 0.05$.

3.9.2 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) for Windows Version 10.0 was used to perform one-way analysis of variance (ANOVA), Results were expressed as standard error of mean (SEM), student t- test, paired sample test and Post-Hoc Test was used to compare the results of treated with the control and to check the differences within groups. Bar charts were used to illustrate and interpret results obtained. The probability level was set at $P<0.05$ (95% confidence interval).

CHAPTER FOUR

4.0 RESULTS:

4.1 Spatial Learning and Memory of wistar rats during the first run of training

The mean values of time taken by the rats to locate the platform on the Morris water maze for both the control and the treated groups varies and was not consistent during the first run of training showing that the rats were trying to learn the task of locating the platform as a way of escape. The mean value of time taken by the control group to locate the platform was 32.00±3.37 while wistar rats of groups II, III, IV and V located the platform at 24.00±5.58s, 47.00±0.00s, 21.00±9.10s and 22.40±1.96s respectively. This results indicated that the wistar rats located the platform at different times and differences in times of location of the platform was not statistically significant at $P<0.05$ (Fig.4.1).

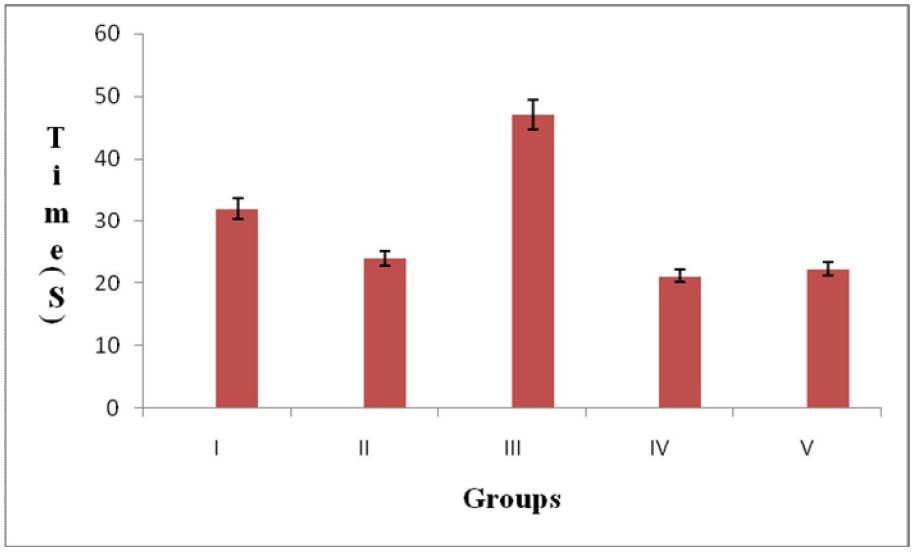


Fig. 4.1: Activities of wistar rats on the Morris water maze during the first run of training.

4.1.1 Effects of Aluminium Chloride on Spartial Learning and Memory of Wistar Rats of Second Run of Testing

The mean value of time taken for wistar rats in the control the locate the platform was 4.17±4.13s while wistar rats in groups II, III, IV and V located the platform at 4.40±2.00s,6.61±0.68s, 11.00±1.57s and 17.50±2.51s respectively(Fig.4.1.1). These results showed that the wistar rats in the control group located the platform faster than the aluminium treated groups which indicated that learning and memory was impaired in the aluminium treated groups when compared with the control. The differences in the time was statistically significant at $P<0.01$.

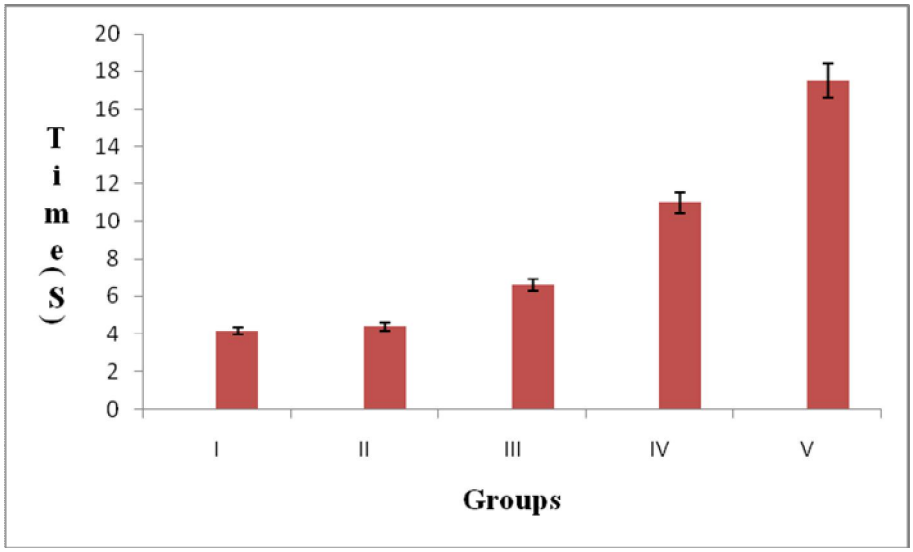


Fig. 4.1.1: Effects of aluminium chloride on spartial learning and memory of wistar rats of second run of testing.

4.1.2 Effects of Aluminium Chloride on Spartial Learning and Memory of Wistar Rats of Third Run of Testing

The mean value of the time taken to locate the platform by the control group was 3.17±0.48, while that of groups II, III, IV and V were 5.67±1.59s, 7.00±1.82s, 7.20±1.11 and 9.69±1.69s respectively. This showed that there were increase in time taken to get to the platform from groups I to IV and this indicated that rats in the control group performed the task better and faster than the aluminium treated groups; pointing to the fact that learning and memory of the aluminium treated groups were impaired when compared with the control group (Fig. 4.1.2.). The differences observed in the time were statistically significant at $P<0.05$

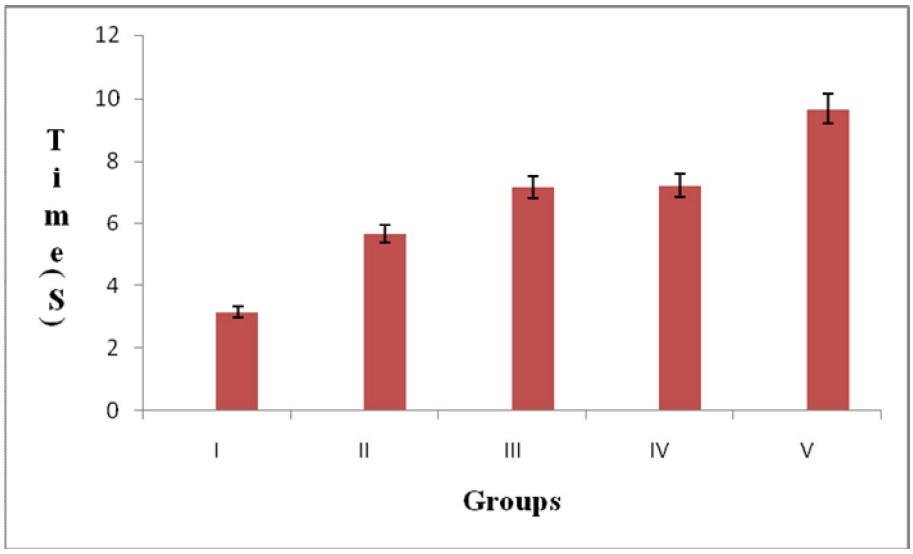


Fig. 4.1.2: Effects of aluminium chloride on spartial learning and memory wistar rats of third run of testing

4.2 Effects of Aluminium Chloride on Anxiety-Related Behaviour of Wistar Rats (Time Spent on Open Arms of the Elevated Plus Maze)

The activities of wistar rats on the open arms of the elevated plus maze showed that there was significant decreased time spent on the open arms from groups I to V ($P<0.001$). Group I was had mean value of 285±5.00s seconds in exploration of the open arms while the aluminium treated groups II, III, IV and V had mean values of 265±5.00s, 195±5.00s, 170±10.00s and 130±10.00s respectively, showing decrease in exploration of open arms across the group which indicated that the aluminium treated groups had increased anxiety when compared with the control (Fig. 4.2.0.).

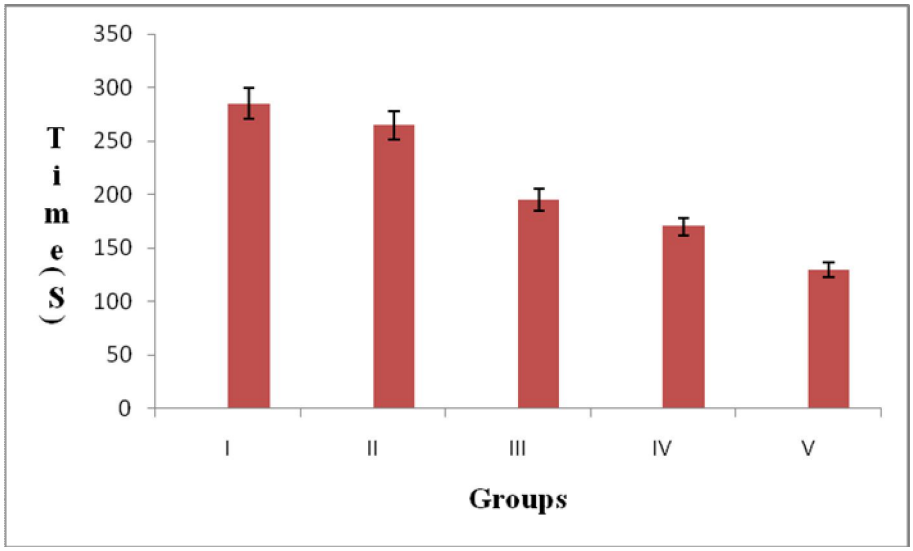


Fig.4.2: Effects of aluminium chloride on anxiety-related behaviour of wistar rats (Time spent on open arms of the elevated plus maze)

4.2.1. Effects of Aluminium Chloride on Anxiety-Related Behaviour of Wistar Rats (Time Spent on Closed Arms of the Elevated Plus Maze)

The activities of the wistar rats on the closed arms of the elevated plus maze revealed that there was statistical increase in time spent on the closed arms from groups I to V. Group I had mean time of 15±5.00s, while groups II, III, IV and V had mean values of 35±5.00s, 105±5.00s, 130±10.00s and 170±10.00s respectively (Fig.4.2.1.). The preference for the wistar rats in aluminium treated groups to spend more time in the closed arms of the elevated plus maze than the control group indicated that the aluminium treated rats had increase anxiety than the control and the increase in exploration of the closed arms was statistically significant at $P< 0.001$.

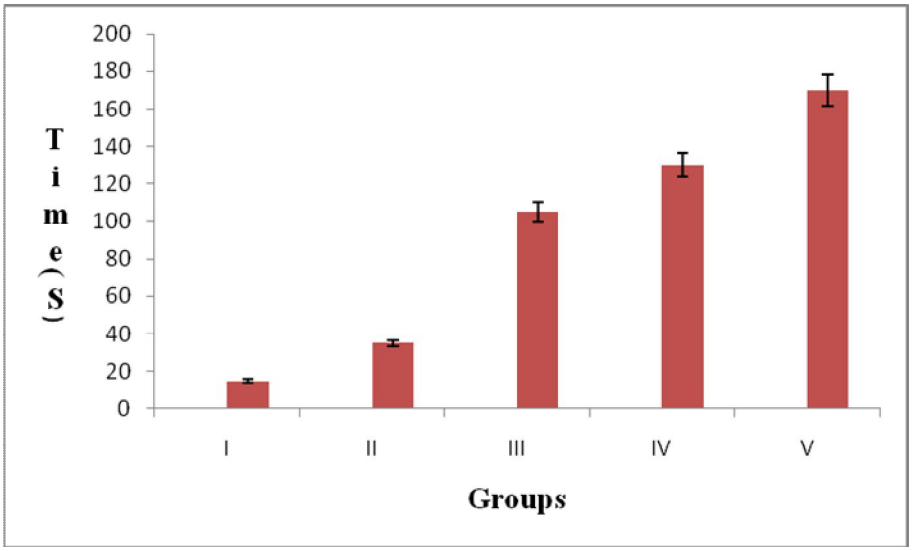


Fig.4.2.1: Effects of aluminium chloride on anxiety-related behaviour of wistar rats (Time spent on closed arms of the elevated plus maze)

4.2.2 Effects of Aluminium Chloride on Anxiety-Related Behaviour of Wistar Rats (Number of Faecal boli)

There was increased in the number of faecal boli across the groups. The mean value of faecal boli of wistar rats in group was 0.00±0.00, while aluminium treated groups II, III, IV, and V had mean values of 1.50±0.50, 2.00±0.00, 3.00±0.00 and 3.50±0.50 respectively, and increased observed was statistically significant at $P<0.01$(Fig. 4.2.2).The increase of faecal boli in the aluminium treated groups indicated that the rats had increased anxiety when compared with the control.

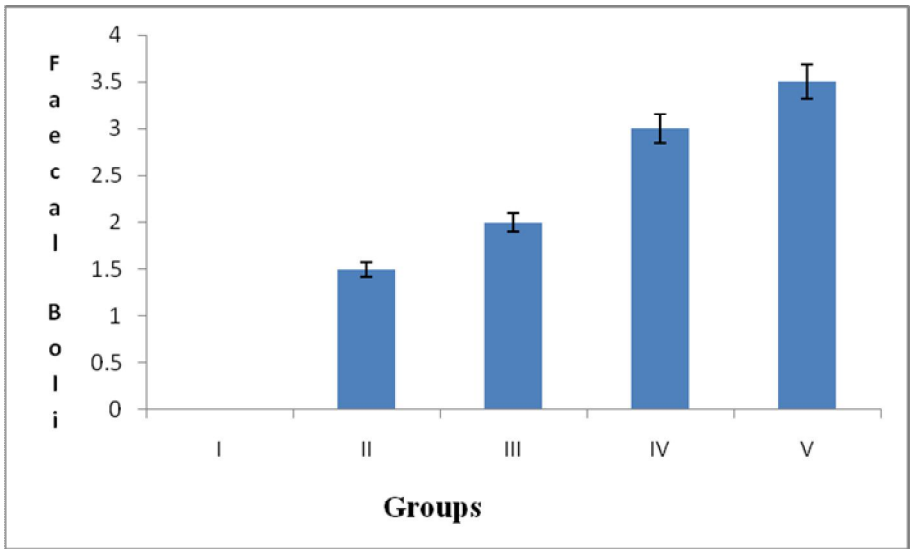


Fig. 4.2.2: Effects of aluminium chloride on anxiety-related behaviour of wistar rats (Number of faecal boli)

4.2.3 Effects of aluminium chloride on the number of time on close arm entries of elevated plus maze.

There was statistically significant increase in the number of close arms entries of the elevated plus maze. Rats in group I had mean entries of 4±0.00 times to the close arms while the aluminium treated groups II, III, IV and V had 6.50±0.05times, 8.50±0.05times, 9.50±0.50times and 11.00±0.00 times of entries into the closed arms of the elevated plus maze (Fig. 4.2.3.).

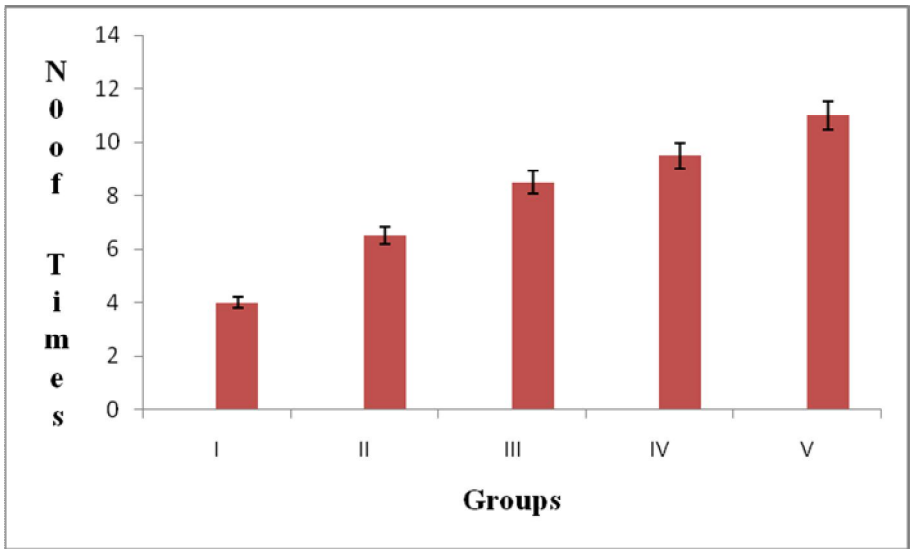


Fig. 4.2.3: Effects of aluminium chloride on the number of time on close arm entries of elevated plus maze.

4.2.4 Effects of aluminium chloride on the number of time on open arms entries of the elevated plus maze.

There was statistical increase in the numbers of time on open arms entries by the wistar rats of the aluminium treated groups when compared with the control (Fig. 4.2.4.). The wistar rats in control group had entry time of 2.00±0.00 into the open arms of the elevated plus maze while wistar rats in groups II, III, IV and V had entry times of 4.00±0.00, 5.50±0.50, 8.00±0.00 and 9.00±0.00 respectively into the open arms of the elevated plus maze.

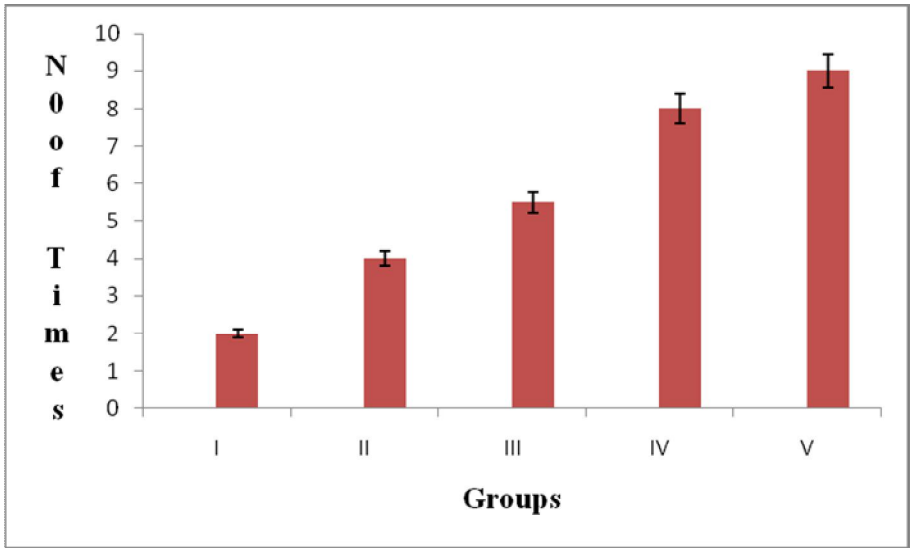


Fig. 4.2.4: Effects of aluminium chloride on the number of time on open arms entries of the elevated plus maze.

4.3 Effects of oral administration of aluminium chloride on brain aluminium uptake

The results of NAA have indicated the presence of aluminium in varying concentrations on the brain tissues.There was gradual increase (accumulation) of aluminium on the brain tissues of wistar rats (Fig. 4.3.). Group I had 26.00±1.00Ppm of aluminium uptake while groups II, III, IV and V had 328.5±8.50Ppm, 782.50±2.50Ppm, 903.50±3.50Ppm and 4249.50±9.50Ppm uptake respectively. The gradual increase (accumulation) of aluminium uptake in the brain tissues of wistar rats across the groups was statistically significant at $P<0.001$.

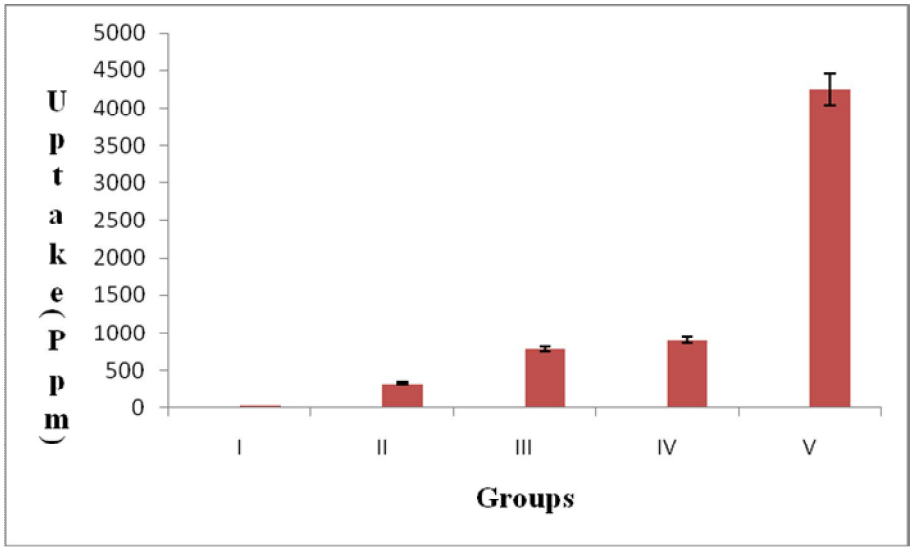


Fig. 4.3: Effects of oral administration of aluminium chloride on brain aluminium uptake

4.4 Effects of aluminium chloride on brain weights of wistar rats

There was decrease in the brain weights of wistar rats in the aluminium treated groups II, III, IV and V, when compared with the control group (Fig. 4.4.). Group I had 1.66±0.05g, while the aluminium treated groups II, III, IV and V had 1.59±0.02g, 1.63±0.03g, 1.65±0.03g and 1.65±0.10±0.01g respectively; but the decrease observed was not statistically significant at $P<0.05$.

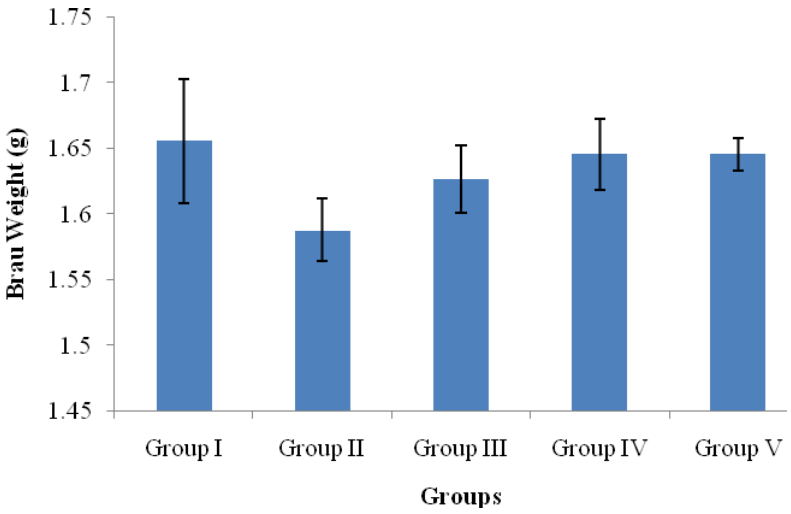


Fig. 4.4: Effects of aluminium chloride on brain weights of wistar rats

4.5 Effects of Aluminium Chloride on the Body Weights of Both Sexes (Male and Female) of Wistar Rats

There was statistically significant increase in the body weight of wistar rats in the control group (Fig.4.5.); with the mean weight of 164.30±7.74g before the commencement of the experiment and increased mean weights of 190.80±6.05g after the experiment ($P<0.001$). The aluminium treated groups had statistical decrease in weights across the groups. Wistar rats in group II had mean weight of 179.30±12.94g before the administration of aluminium chloride and later decrease to 172.20±12.46g after eight weeks of aluminium chloride administration; and the observed decrease in weight of group II was statistically significant at $P<0.001$. Group III had mean weight of 157.00±12.32g before administration of aluminium chloride but later decreased to 151±10.10g after eight weeks of aluminium chloride administration and the observed decrease was statistically significant at $P<0.05$. The wistar rats in group IV had mean weight of 174.11±11.39g before administration of aluminium chloride but later decrease to 160.78±10.65g after eight weeks of administration of aluminium chloride and the decrease observed was statistically significant at $P<0.001$. Group V mean weight was 176.56±13.39g before aluminium chloride administration but later decrease to 162.56±11.94g which statistically significant at $P<0.001$ (Fig.4.5.).

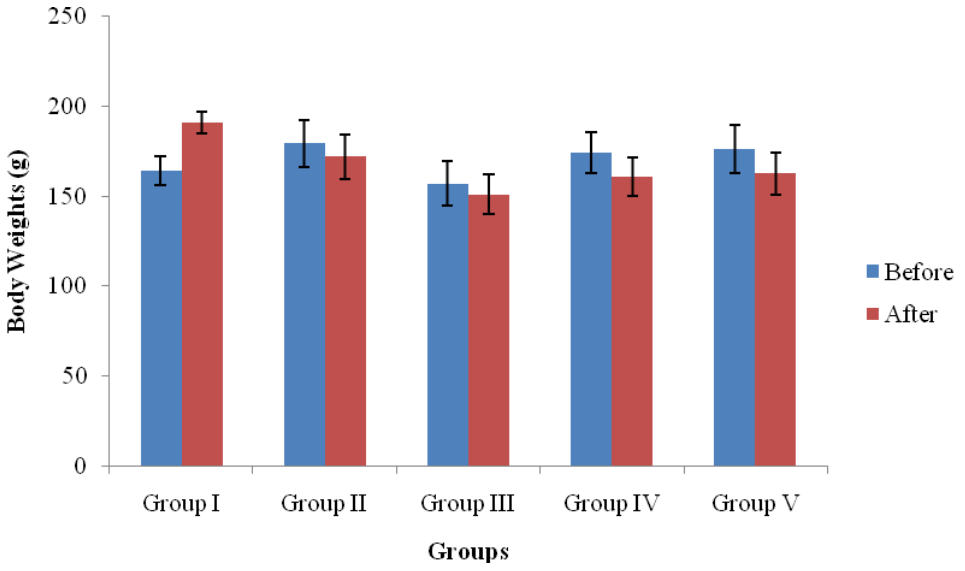


Fig.4.5 Effects of aluminium chloride on the body weights of both sexes (Male and Female) of wistar rats

4.6 Effects of aluminium chloride on the body weight of male wistar rats

There was statistically significant increase in the weight of the control group with 177.00±10.85g before the experiment and 197.80±8.66g after the experiment ($P<0.01$). There was statistically significant decrease in the mean body weights of the aluminium treated groups (Fig. 4.6) with group II having mean weight of 200.80±20.93g before administration of aluminium chloride and later decreased to 191.00±21.00g after eight weeks of aluminium chloride administration($P<0.01$).The mean weight of wistar rats in group III was 167.60± 20.03g before commencement of administration but later decreased to160.20±18.73g after eight weeks of aluminium chloride administration. Group IV mean weight was 199.40± 7.34g before aluminium administration but later decrease to 185.20±4.95g after eight weeks of administration($P<0.05$).The mean weight of wistar rats in group V was 208.00±9.06g before administration and later decreased to 190±9.08g after eight weeks of administration ($P<0.01$.

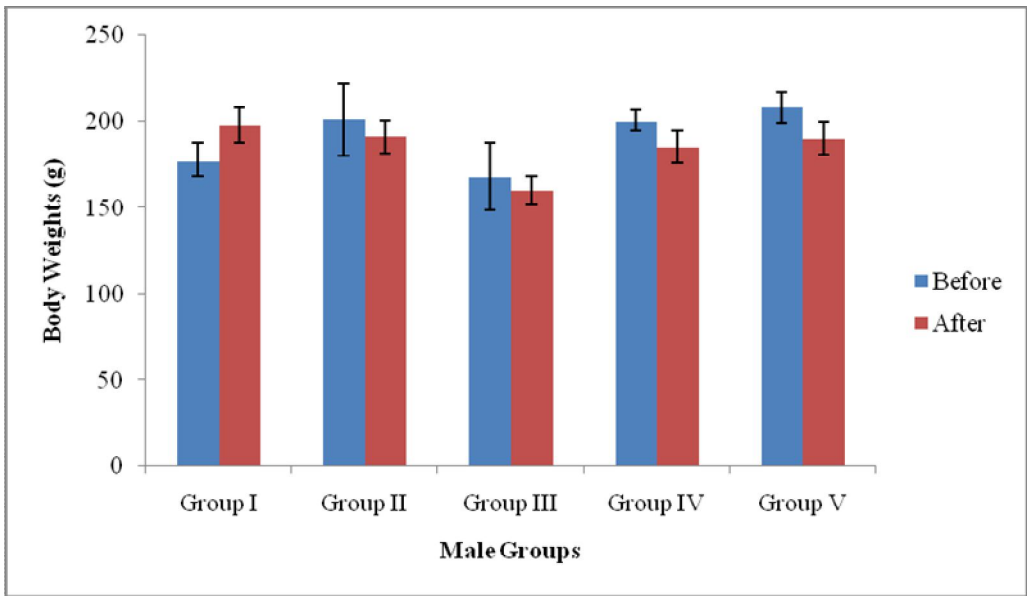


Fig.4.6: Effects of aluminium chloride on the body weight of male wistar rats

4.7 Effects of aluminium chloride on the body weight of female wistar rats

The mean value of the control group showed that there was statistically significant increase in body weight of the group having mean weight of 151.60±8.43g before the experiment which later became 183.80±8.22g after the experiment($P<0.001$). Mean weight of wistar rats in group II was 157.80±9.21g before administration of aluminium chloride and later decrease to 153.40±9.00g after eight weeks administration but decrease observed was not statistically significant at $P<0.05$. The mean weight of wistar rats in group III was 146.40±15.02g before administration of aluminium chloride and later decrease to 141.80±12.29g after eight weeks of administration of aluminium chloride but the decrease observed was not statistically significant at $P<0.05$. For group IV, there was statistically significant decrease in weight with the mean weight of 142.50±9.46g before aluminium chloride administration and 130.25± 8.99g after eight weeks of administration ($P<005$). The wistar rats in group V had mean weight of 137.25±3.42g which latter decrease to 128.25±3.40g after eight weeks of aluminium administration but the decreased observed in this group was not statistically significant at $P<0.05$ (Fig. 4.7)

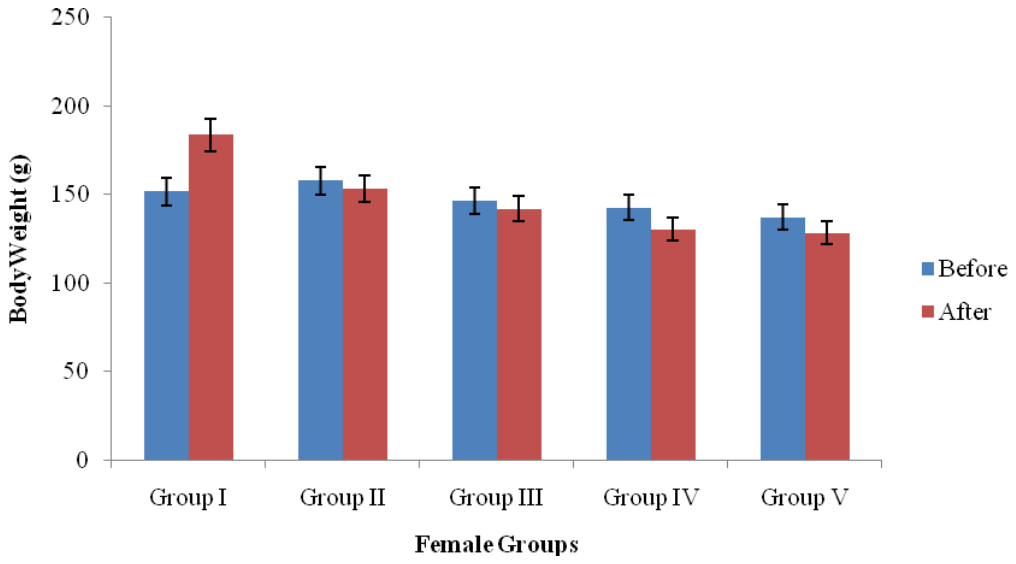


Fig.4.7: Effects of aluminium chloride on the body weight of female wistar rats

4.8 Microscopic Examinations of the Cerebral Cortex (Haematoxylin and Eosin Stain)

The microscopic examinations of the histology of the cerebral cortex showed normal structure in the control groups that received distil water only at magnifications of 100 and 250 respectively (Plates I and II). Histology of the cerebral cortex for group II, III, IV and V that received 475mg/kg, 950mg/kg and 1425mg/kg respectively showed slight neuronal vacuolation and necrosis (plates III, IV, V, VI, VII and VIII). The histology of the cerebral cortex of wistar rats in group V that received the highest dose showed extensive neuronal vacuolation and necrosis, as most of the cells were lost(Plates IX and X).

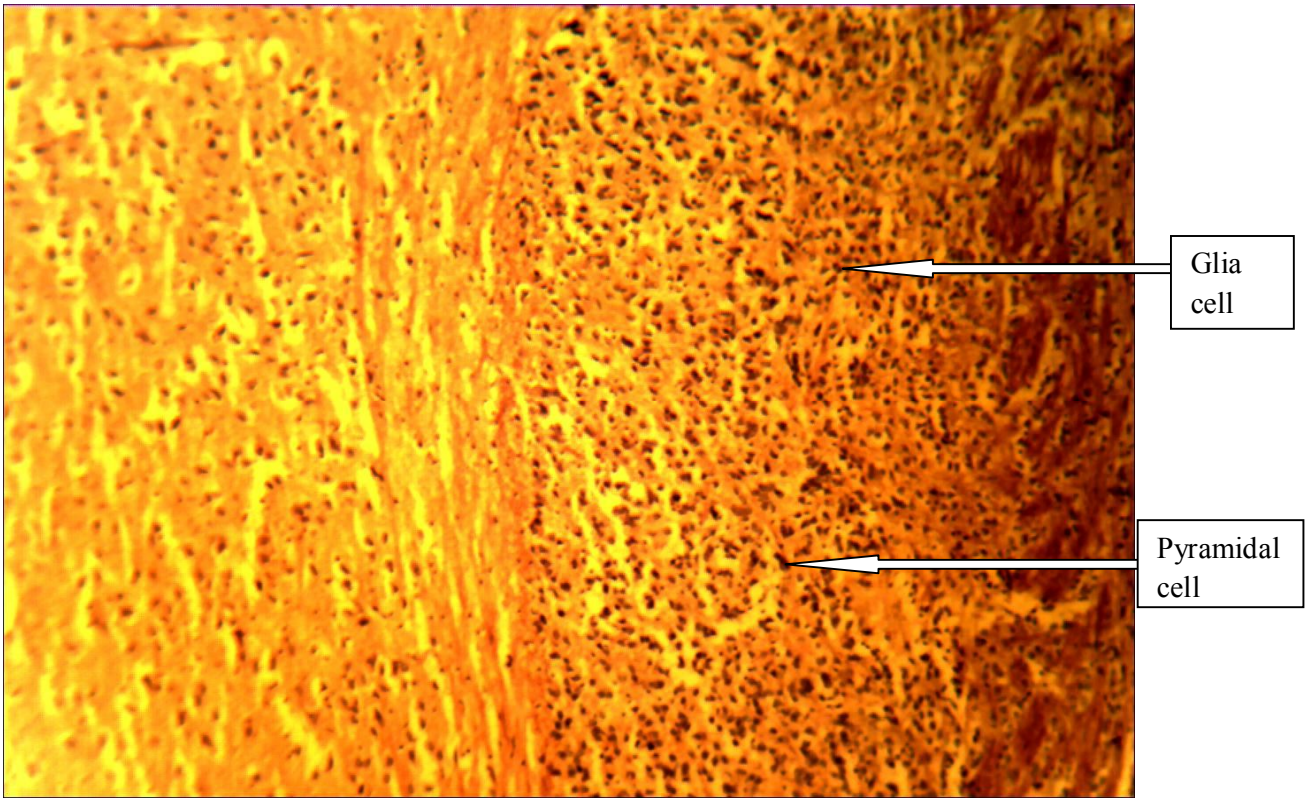


Plate I: Longitudinal section of the normal cerebral cortex of wistar rats of the control group that received distil water only, stained with H&E. X100

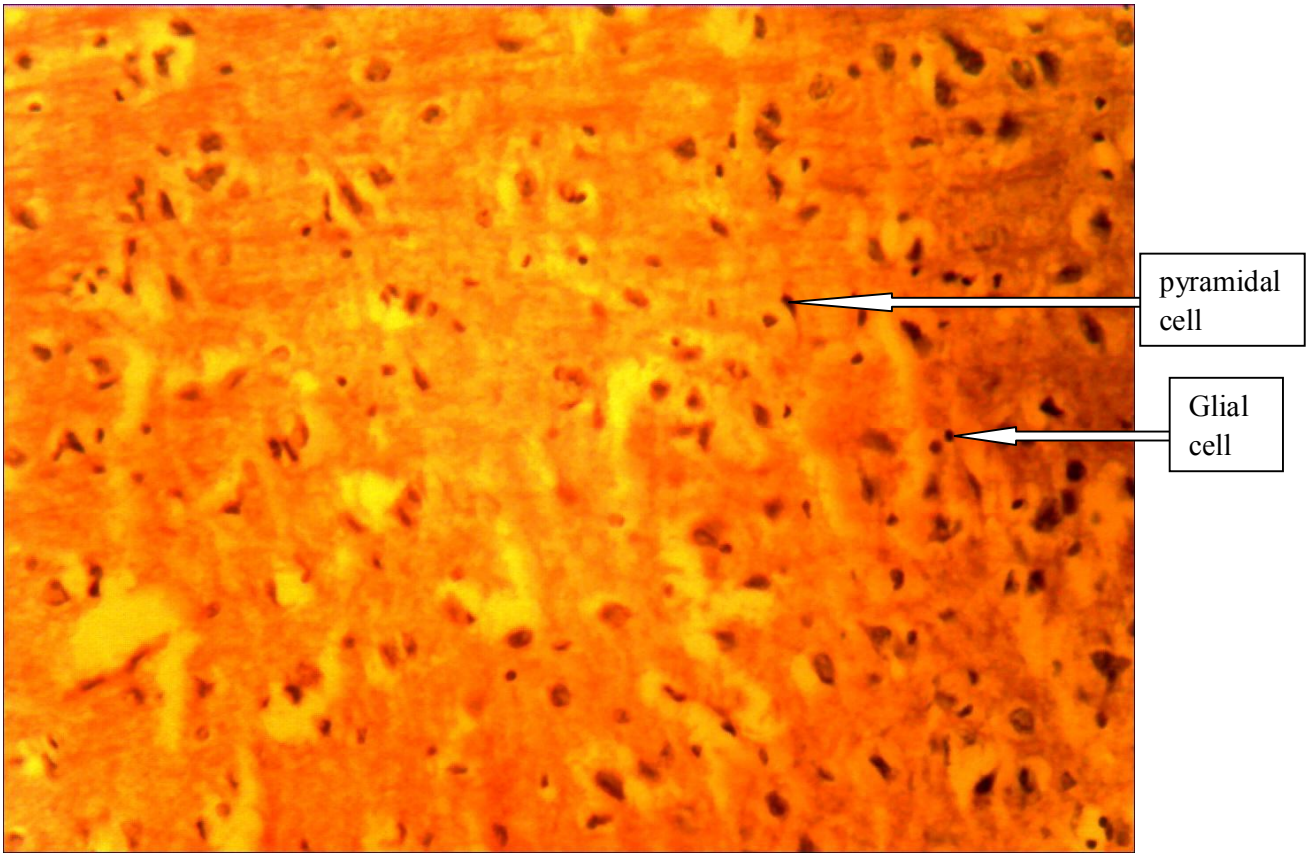


Plate II: Longitudinal section of the normal cerebral cortex of wistar rats of control group that received distil water only, stained with H&E. X250

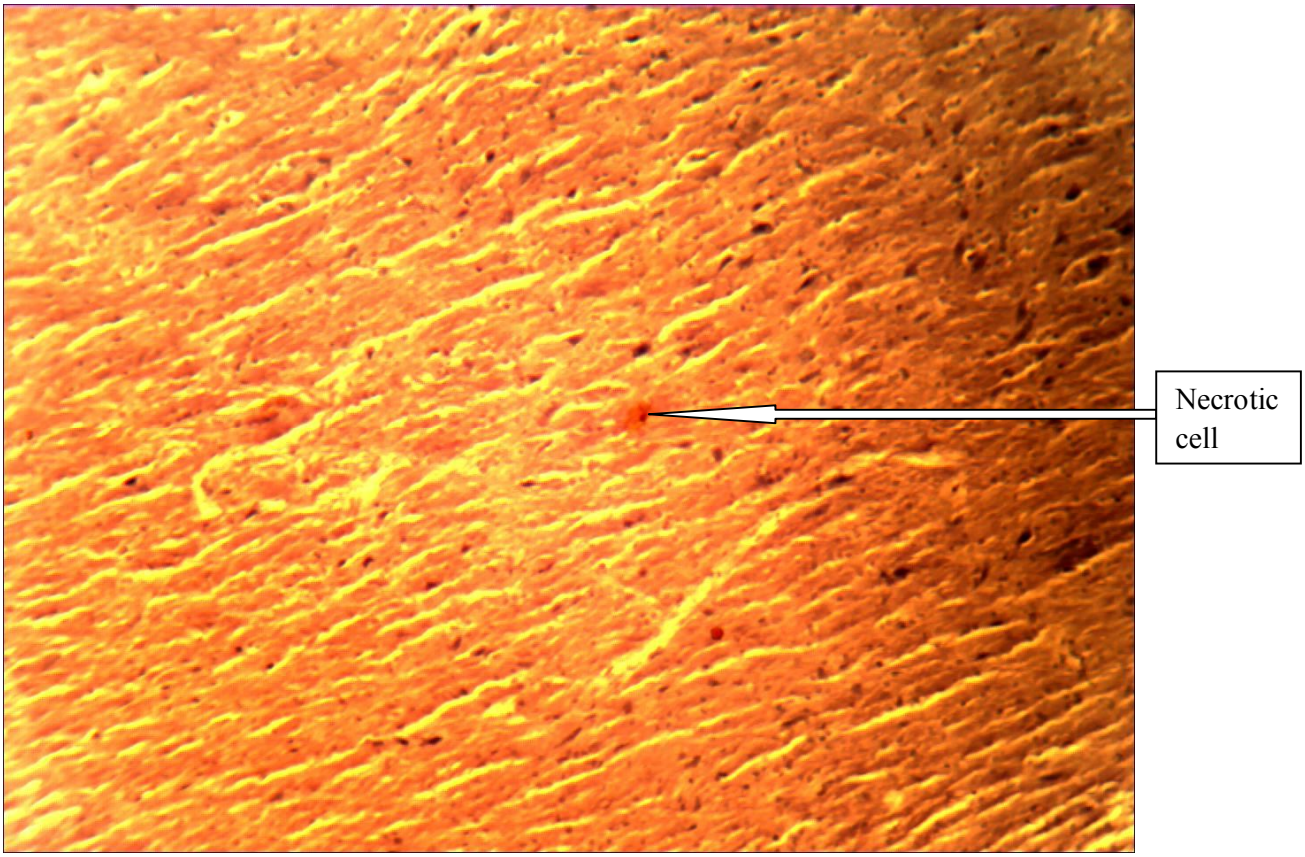


Plate III: Longitudinal section of the cerebral cortex of wistar rats of group II that received 475mg/kg of $AlCl_3$, showing slight neuronal vacuolation and necrosis, stained with H&E.X100

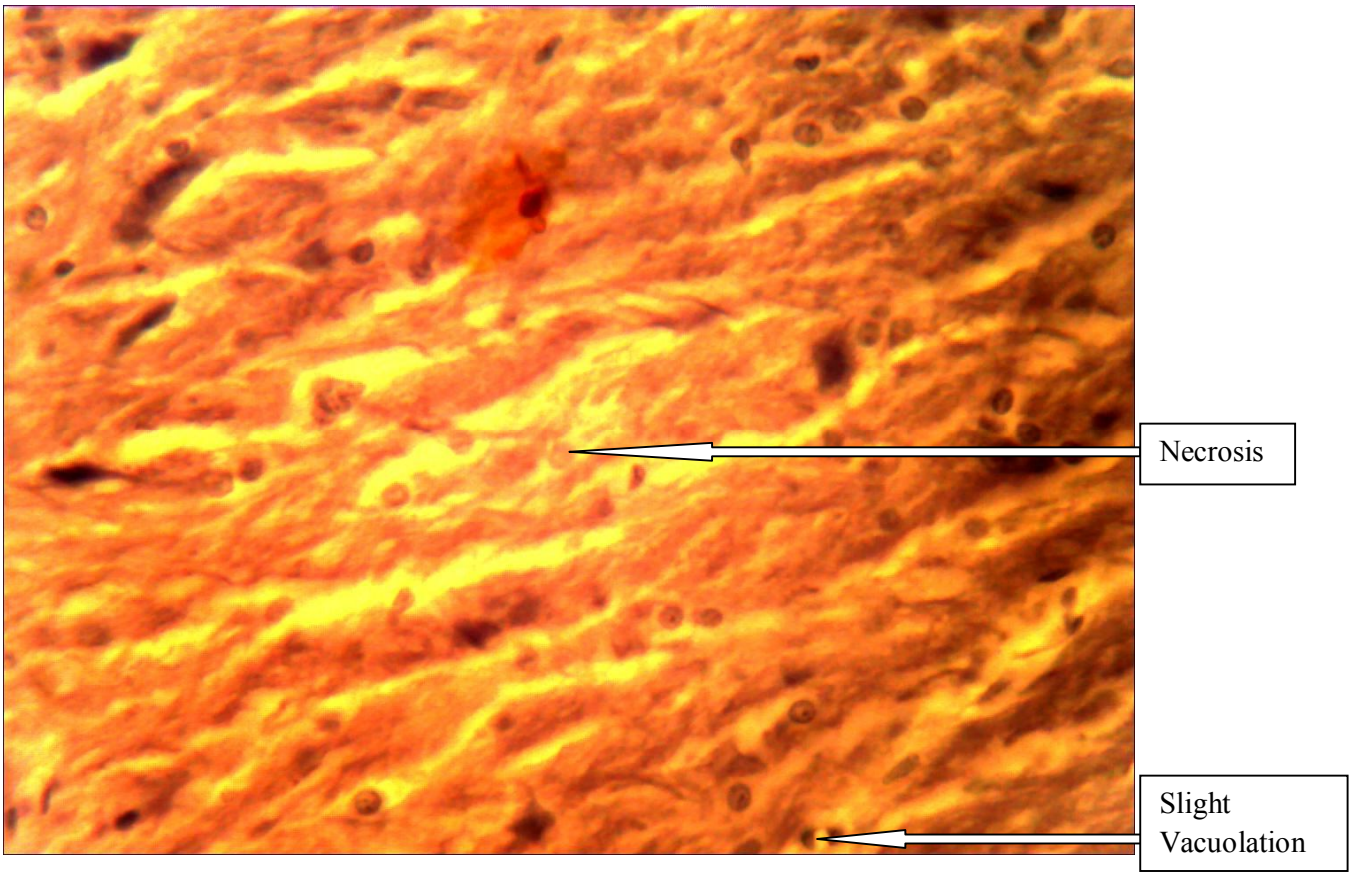


Plate IV: Longitudinal section of the cerebral cortex of wistar rats of group II that received 475mg/kg of $AlCl_3$, showing slight neuronal vacuolation and necrosis, stained with H&E.X250

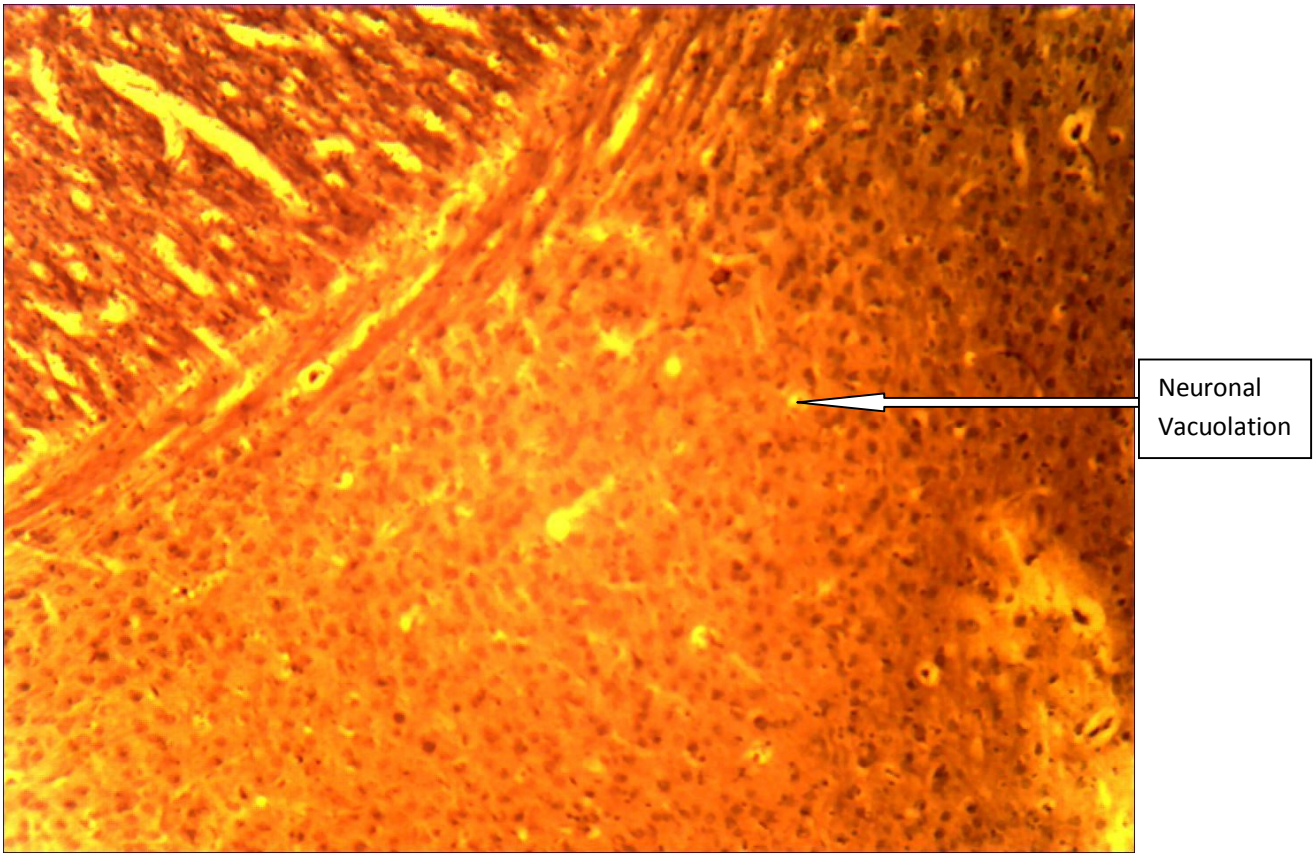


Plate V: Longitudinal section of the cerebral cortex of wistar rats of group III, that received 950mg/kg of $AlCl_3$, showing slight neuronal vacuolation and necrosis, stained with H&E.X100

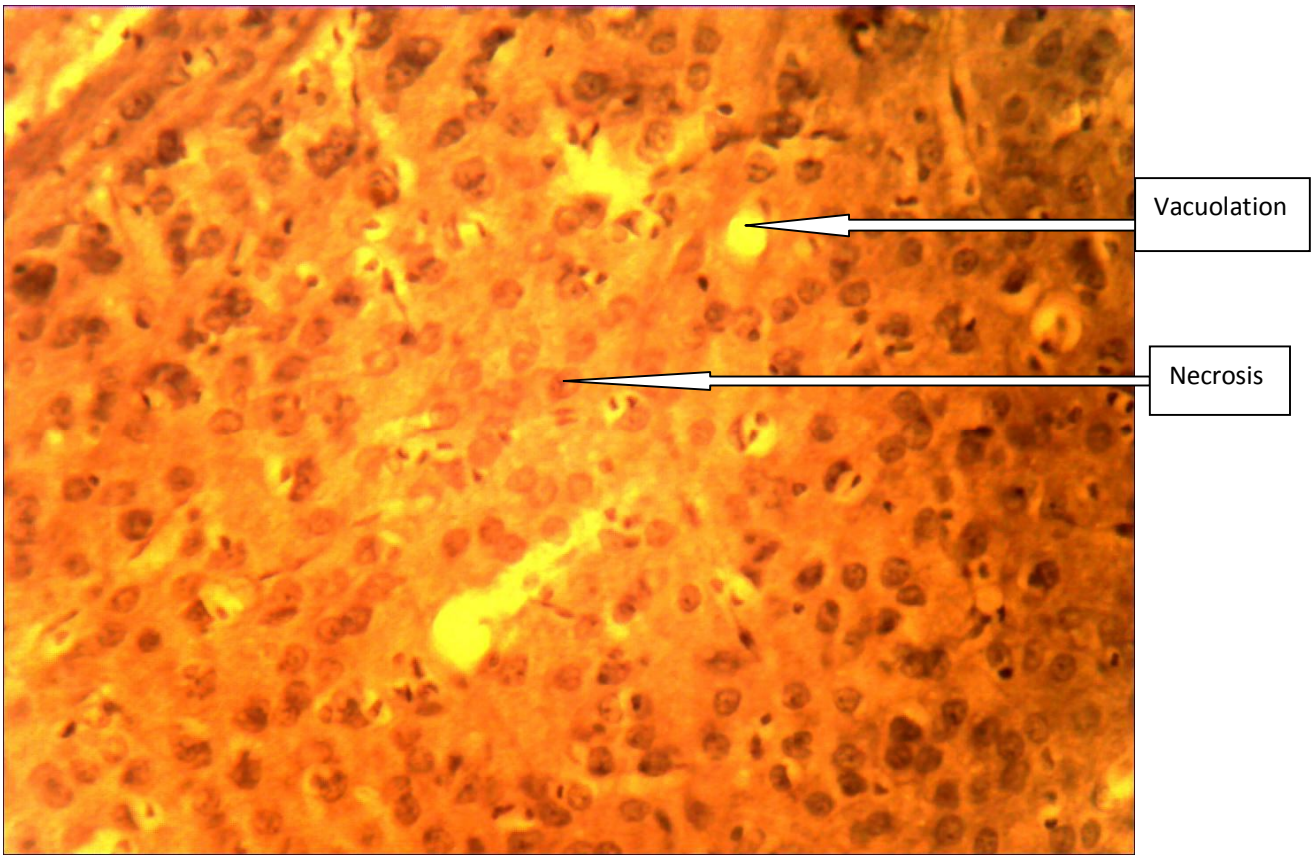


Plate VI: Longitudinal section of the cerebral cortex of wistar rats of group III that received 950mg/kg of $AlCl_3$, showing slight neuronal vacuolation and necrosis, stained with H&E.X250

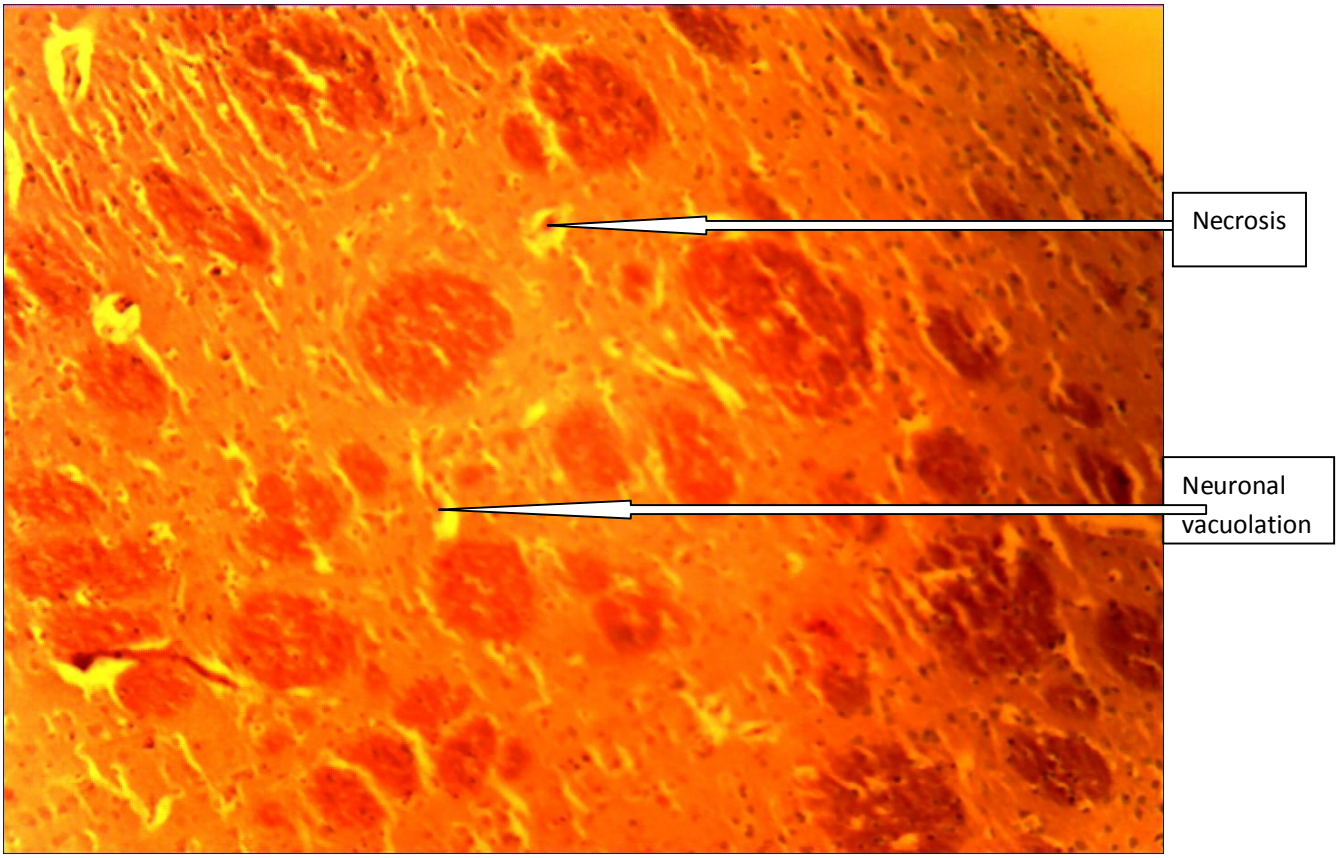


Plate VII: Longitudinal section of the cerebral cortex of wistar rats of group IV that received 1425mg/kg of $AlCl_3$, showing slight neuronal vacuolation and necrosis, stained with H&E X100

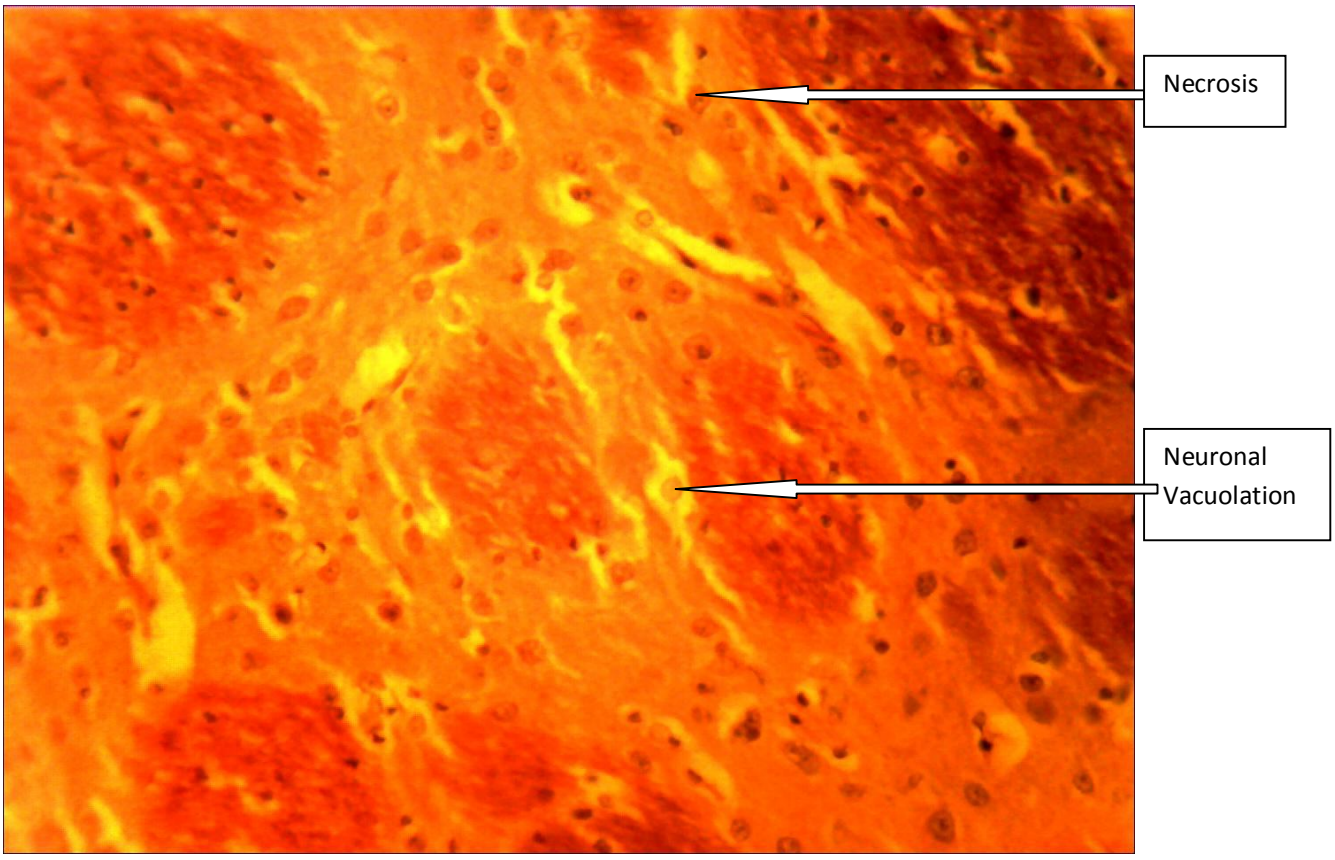


Plate VIII: Longitudinal section of the cerebral cortex of wistar rats of group IV that received 1425mg/kg of $AlCl_3$, showing slight neuronal vacuolation and necrosis, stained with H&E X250

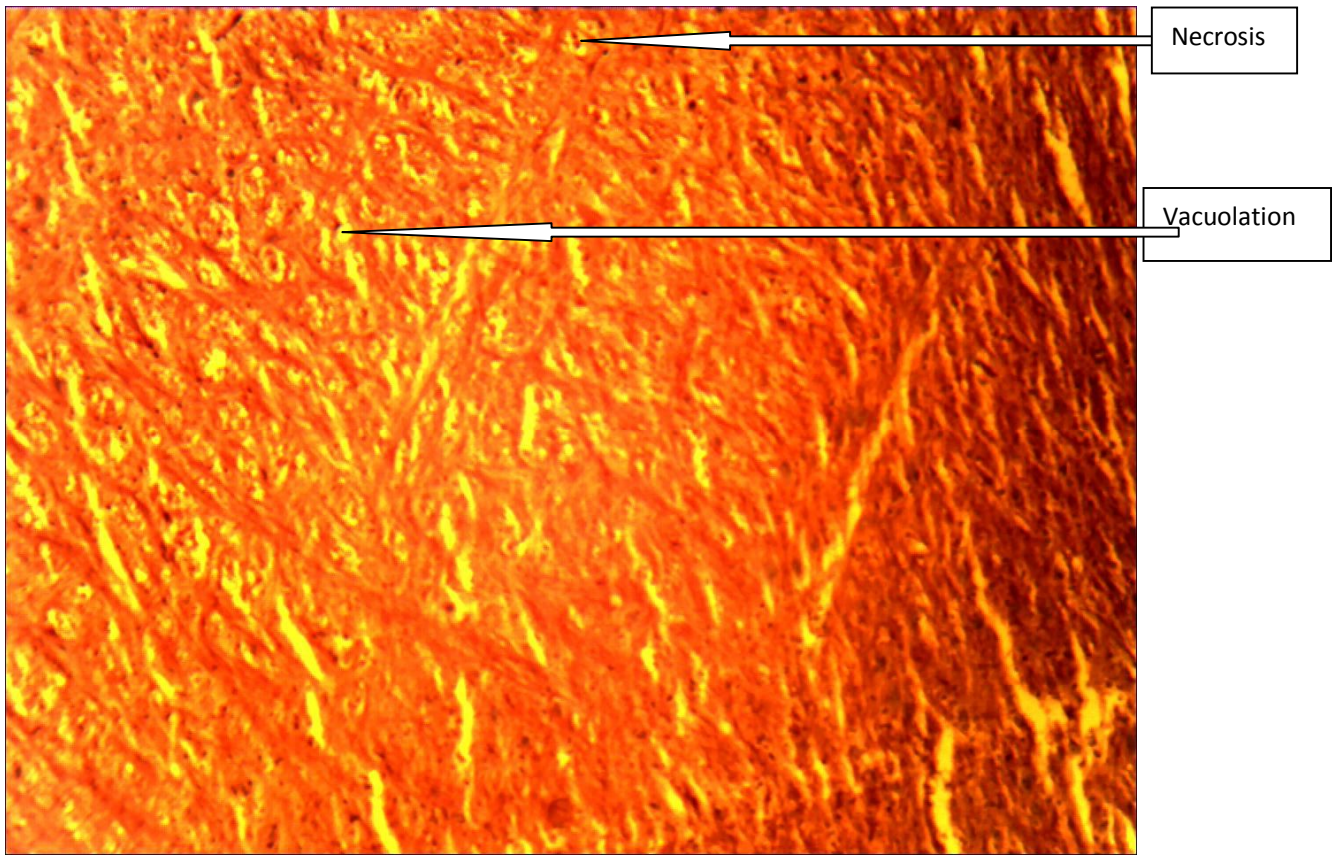


Plate IX: Longitudinal section of the cerebral cortex of wistar rats of group V that received 1900mg/kg of $AlCl_3$, showing extensive neuronal vacuolation and necrosis, stained with H&E X100

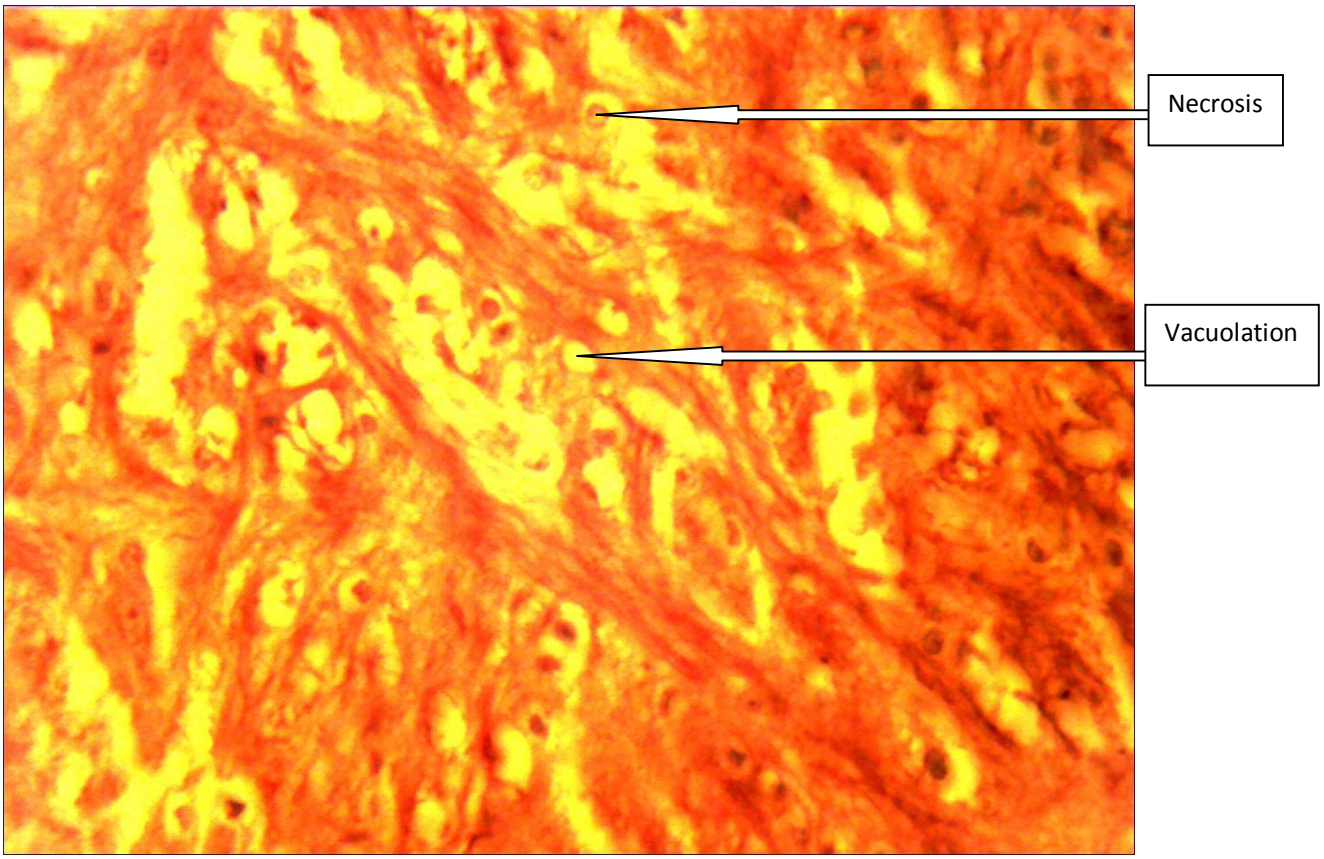


Plate X: Longitudinal section of the cerebral cortex of wistar rats of group V that received 1900mg/kg of $AlCl_3$, showing extensive neuronal vacuolation and necrosis, stained with H&E X250

4.9 Microscopic Examinations of the Cerebral Cortex (Hirano-Zimmerman method)

The microscopic examinations of the cerebral cortex stained with Hirano-Zimmerman stain revealed that the wistar rats of the control group showed normal histological appearance of the nerve cells (Plates XI and XII) with some projections of the axons especially at 250 magnifications (See Plate XII).The cerebral cortex of group II showed slight neuronal vacuolation and necrosis at magnifications of 100 and 250 (Plates XIII and XIV). The histology of cerebral cortex of wistar rats in group III showed neuronal vacuolation and necrosis at magnifications of 100 and 250 (Plates XV and XVI). There was also neuronal vacuolation and necrosis of the cerebral cortex of wistar rats in group IV (Plates XVII and XVIII). The cerebral cortex of the wistar rats in group V showed extensive neuronal vacuolation and necrosis and a number of the nerve cells were lost (Plates XIX and XX).

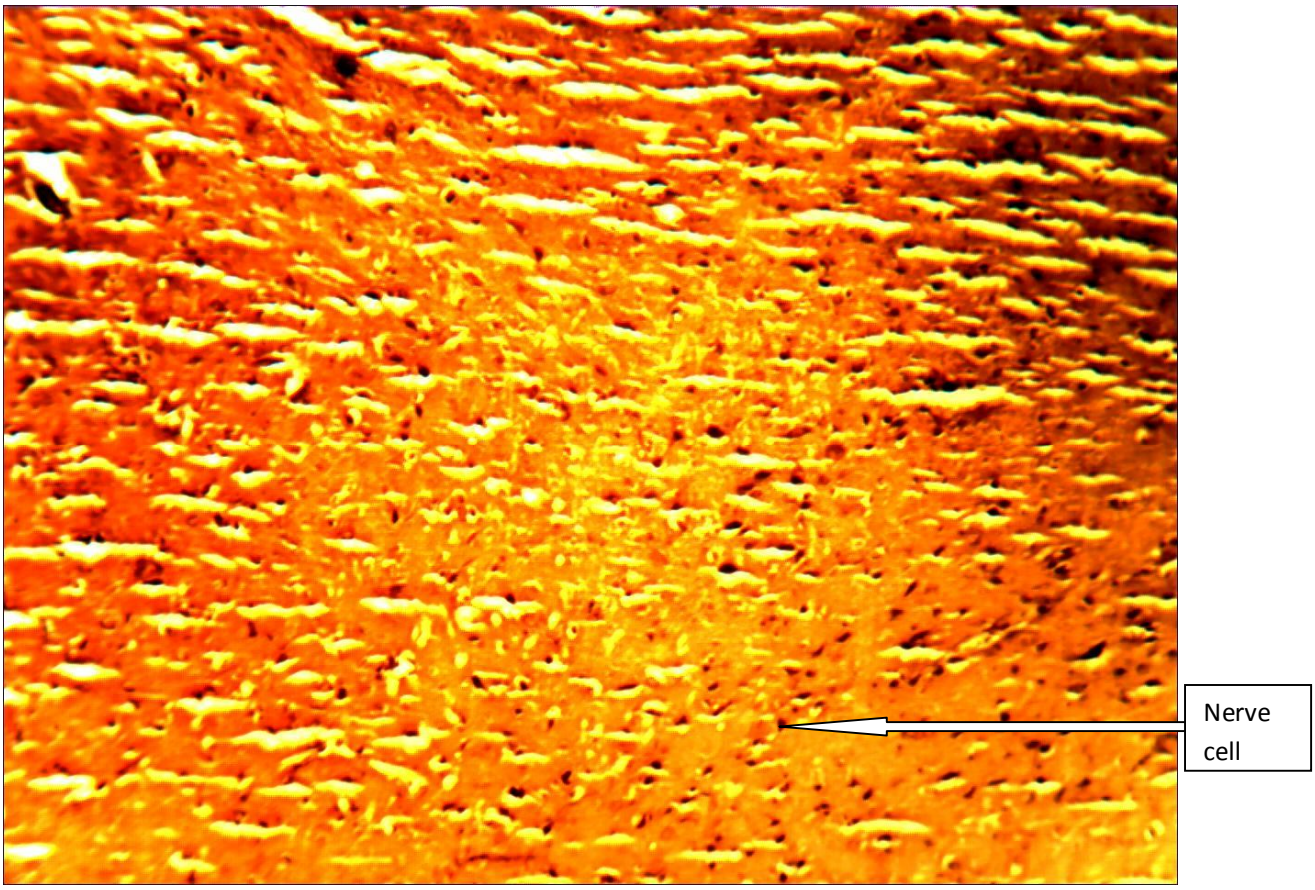


Plate XI: Longitudinal section of normal histology of the cerebral cortex of wistar rats of group I that received distil water only. Hirano-Zimmerman stain, X100

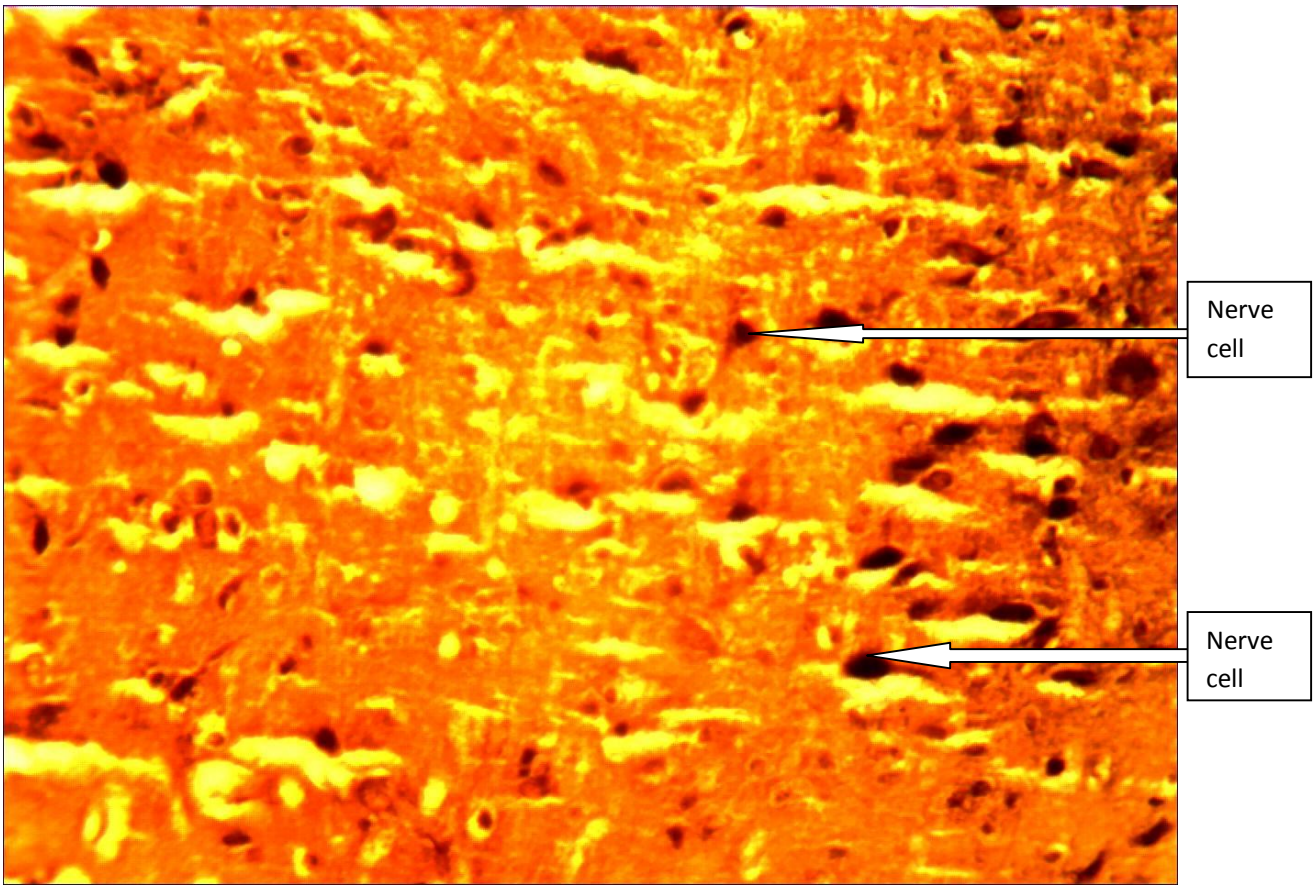


Plate XII: Longitudinal section of normal histology of the cerebral cortex of wistar rats of group I that received distil water only. Hirano-Zimmerman stain, X250

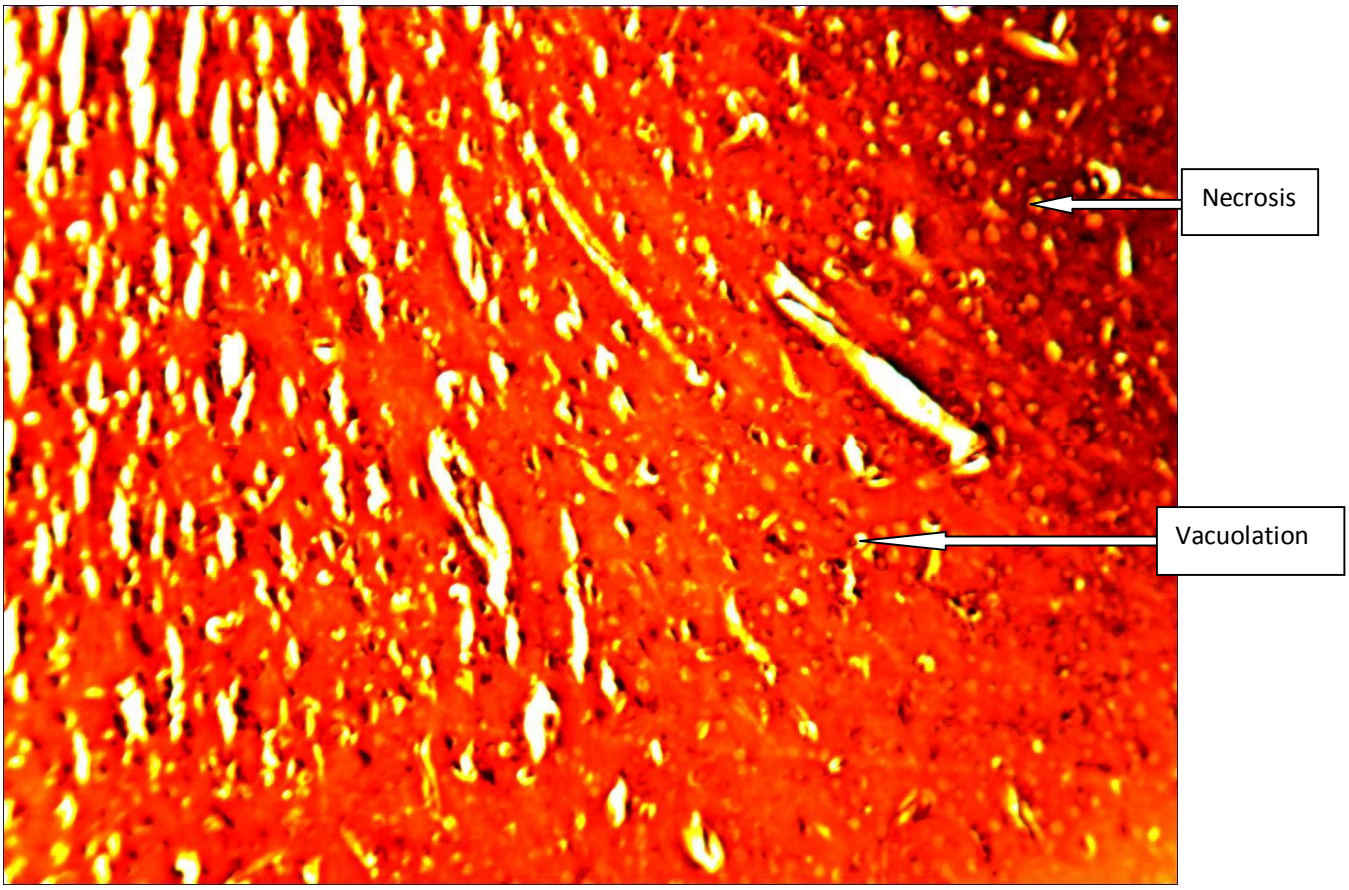


Plate XIII: Longitudinal section of the cerebral cortex of wistar rats of group II that received 475mg/kg of $AlCl_3$, showing neuronal vacuolation and necrosis.Hirano-Zimmerman stainX100

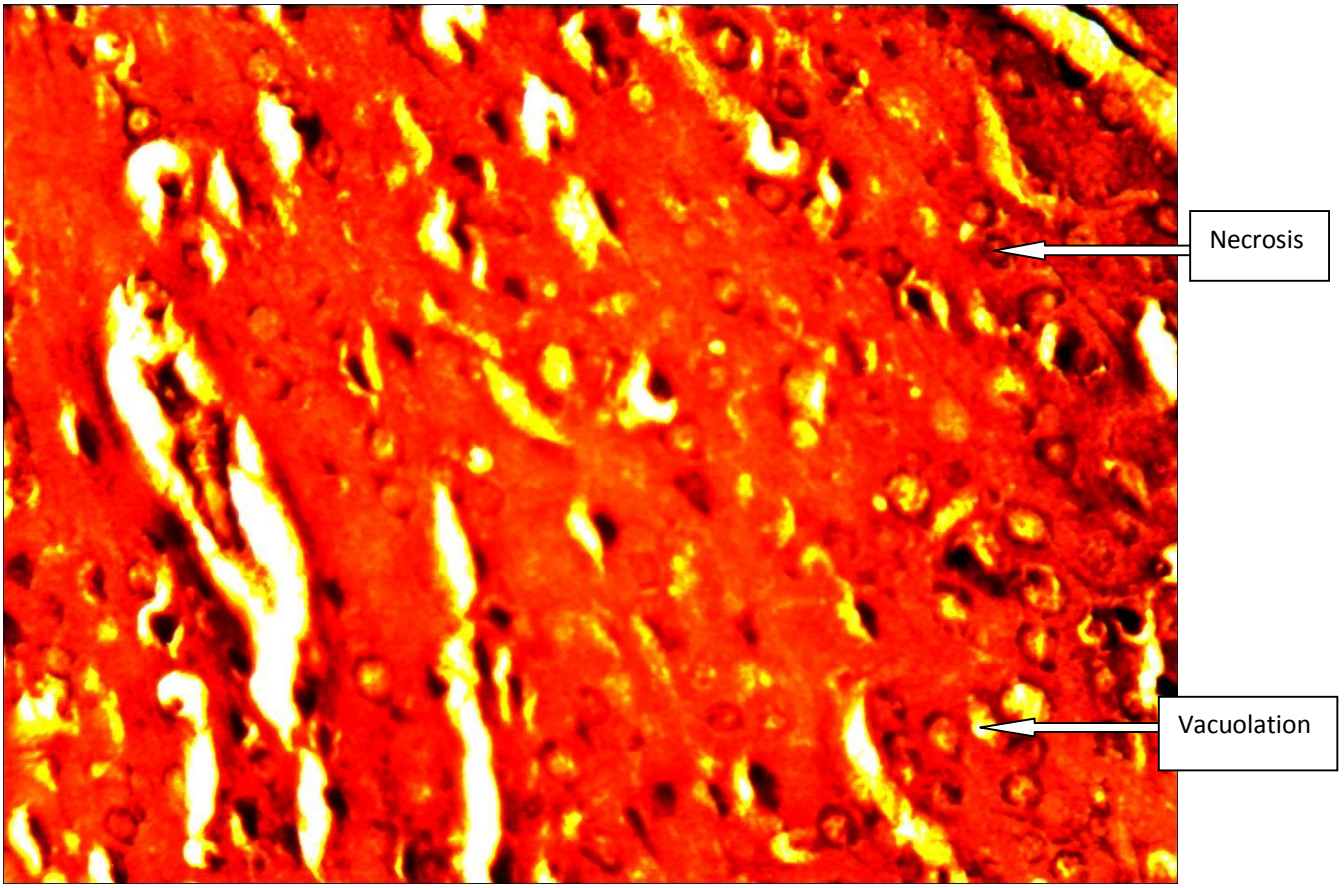


Plate XIV: Longitudinal section of the cerebral cortex of wistar rats of group II that received 475mg/kg of $AlCl_3$, showing neuronal vacuolation and necrosis.Hirano-Zimmerman stainX250

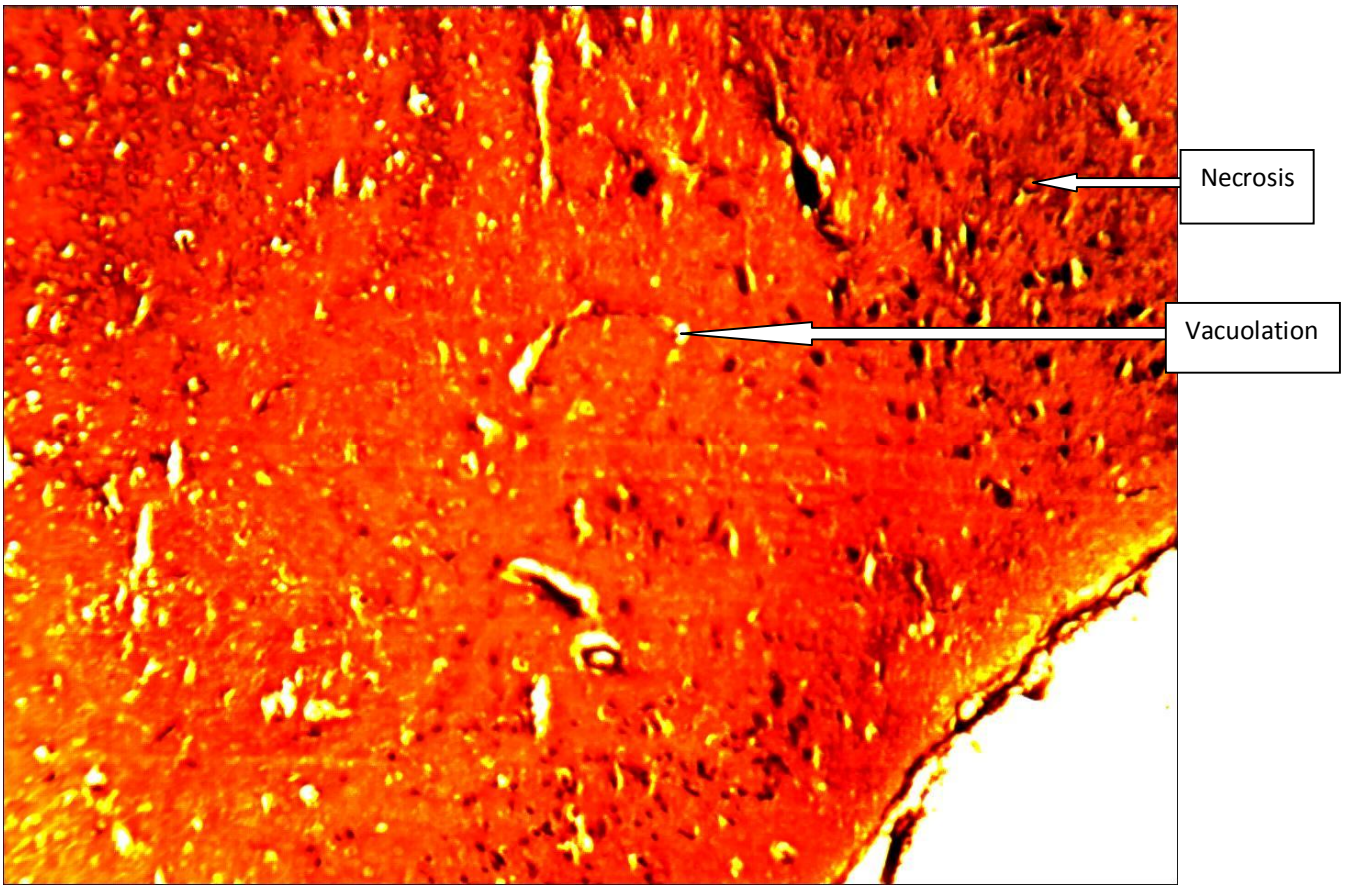


Plate XV: Longitudinal section of the cerebral cortex of wistar rats of group III that received 950mg/kg of $AlCl_3$, showing neuronal vacuolation and necrosis.Hirano-Zimmerman stainX100

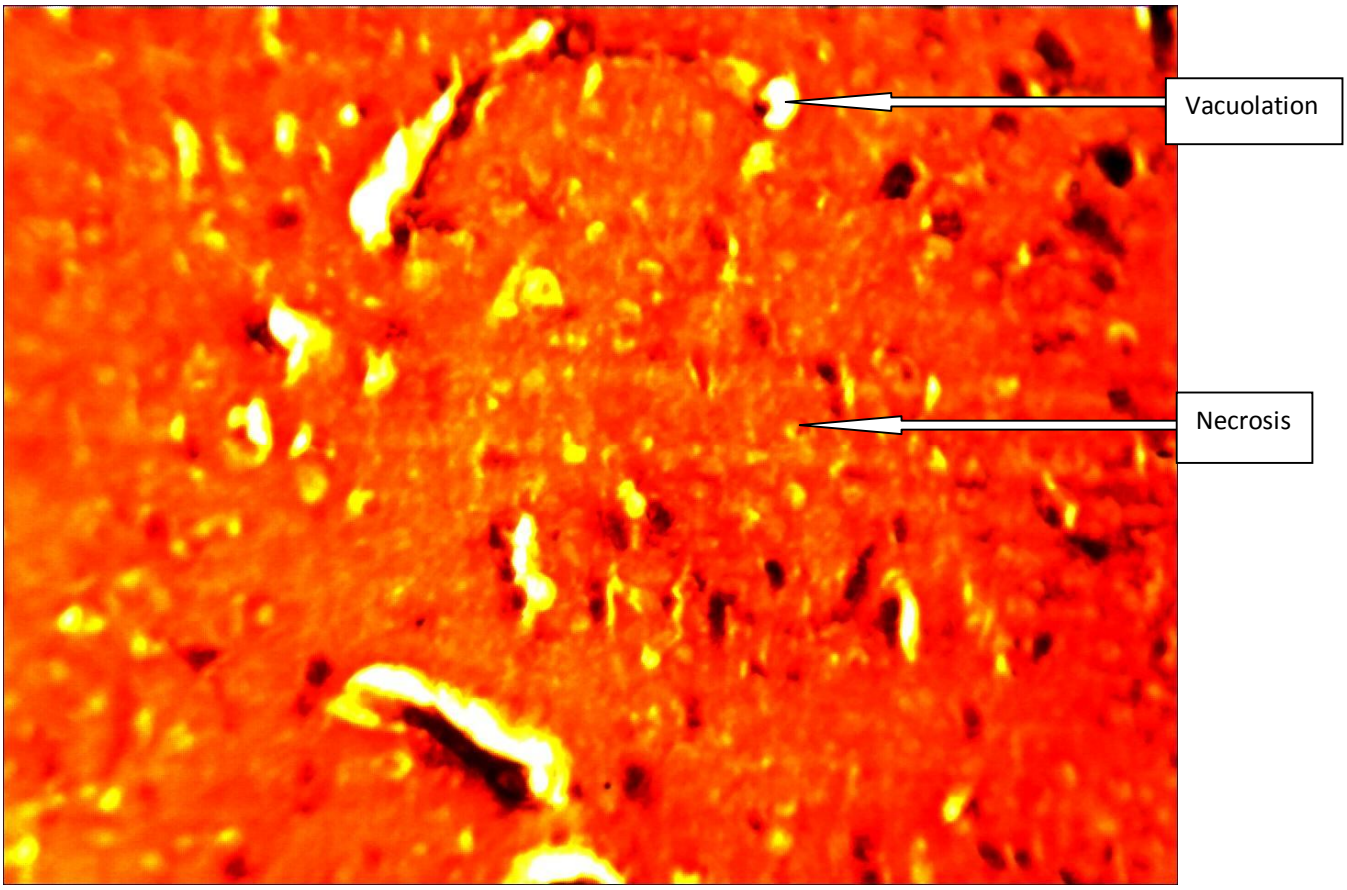


Plate XVI: Longitudinal section of the cerebral cortex of wistar rats of group III that received 950mg/kg of $AlCl_3$, showing neuronal vacuolation and necrosis.Hirano-Zimmerman stainX250

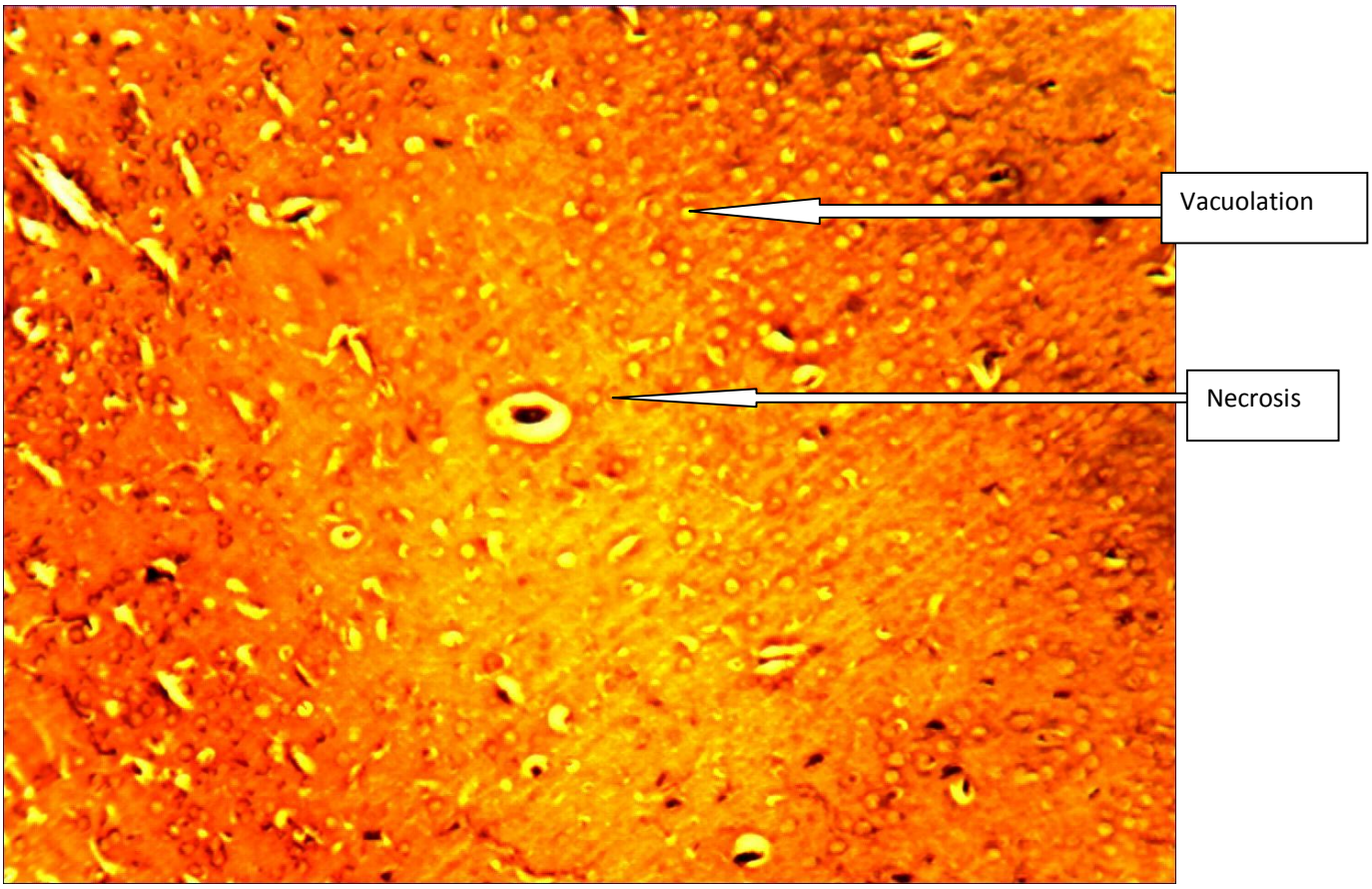


Plate XVII: Longitudinal section of the cerebral cortex of wistar rats of group IV that received 1425mg/kg of $AlCl_3$, showing neuronal vacuolation and necrosis.Hirano-Zimmerman stain.X100

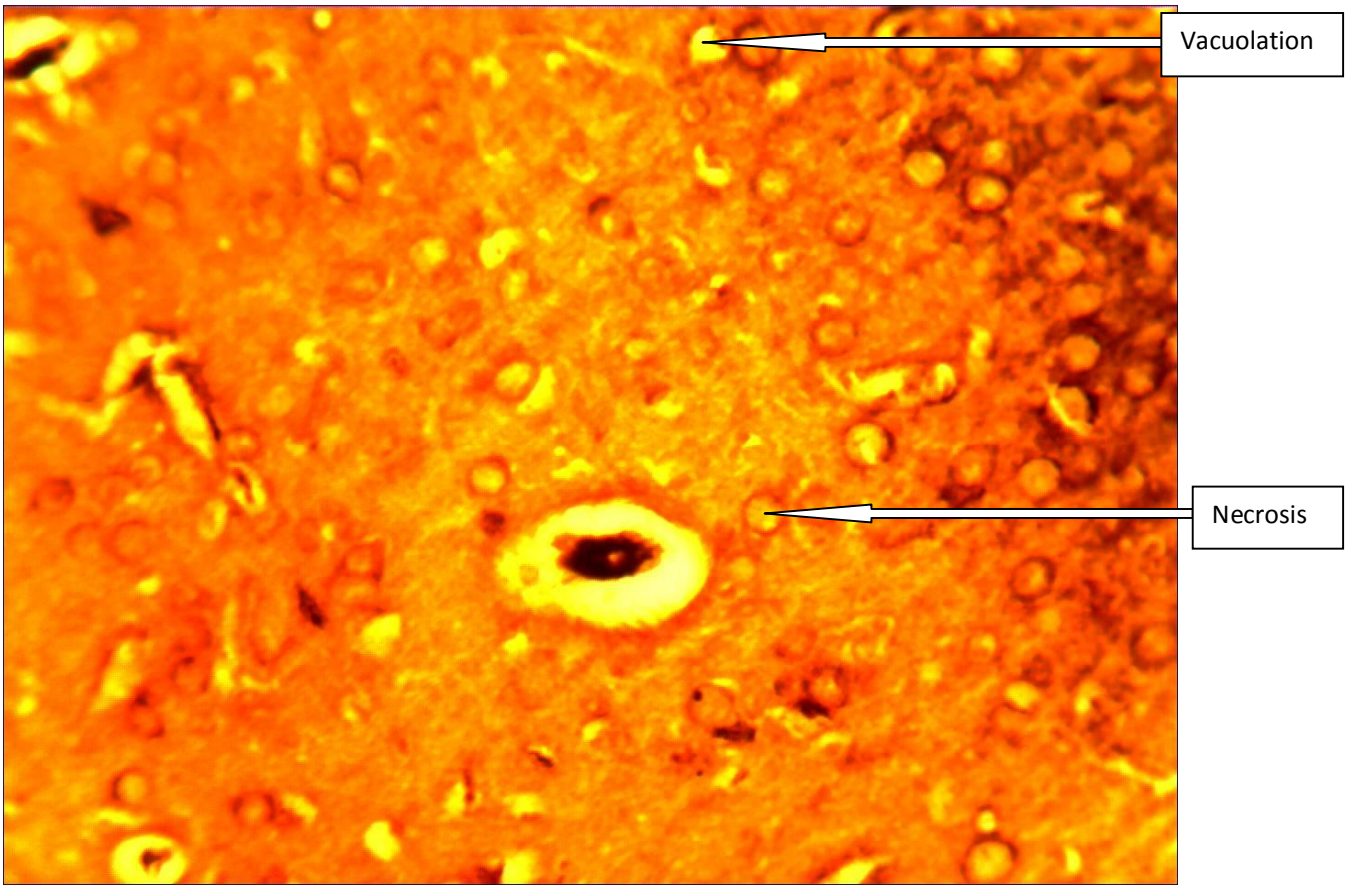


Plate XVIII: Longitudinal section of the cerebral cortex of wistar rats of group IV that received 1425mg/kg of $AlCl_3$, showing neuronal vacuolation and necrosis.Hirano-Zimmerman stain. X250

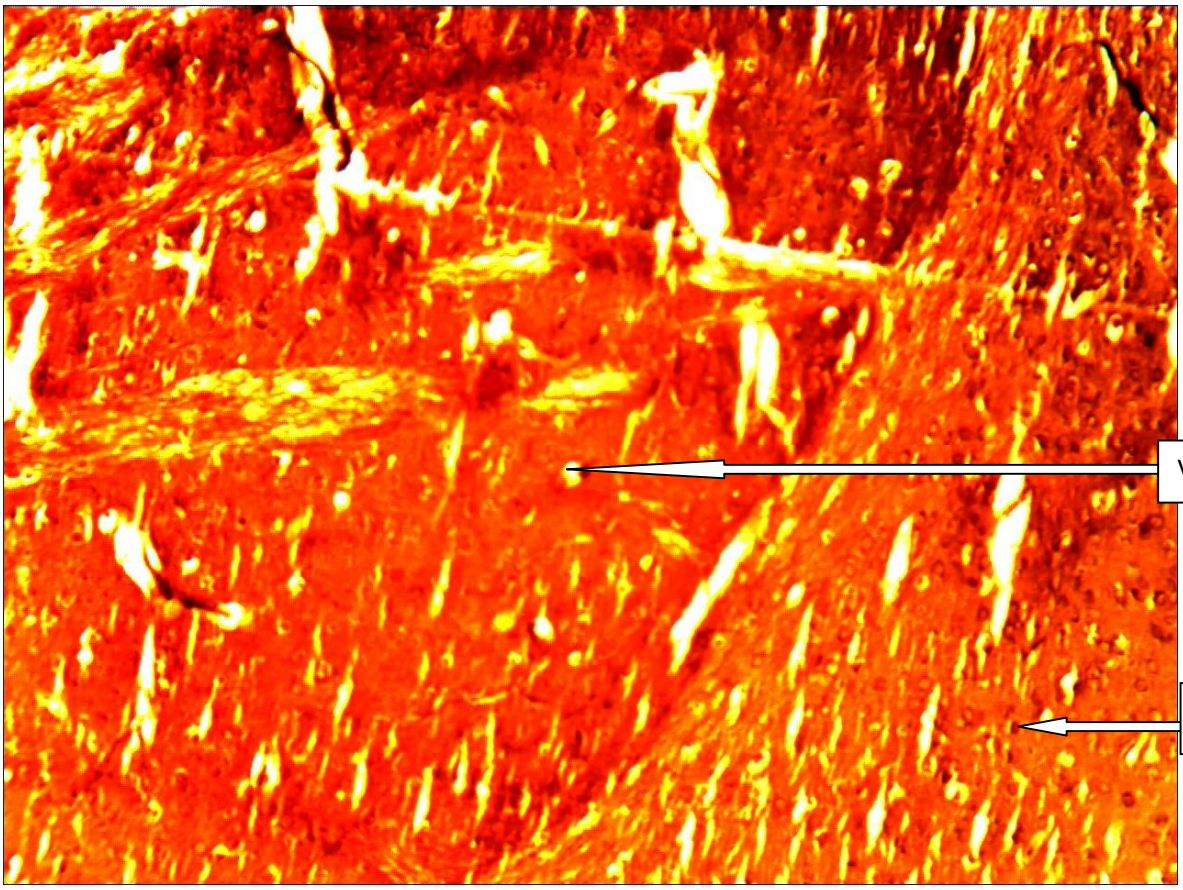


Plate XIX: Longitudinal section of the cerebral cortex of wistar rats of group V that received 1900mg/kg of $AlCl_3$, showing extensive neuronal vacuolation and necrosisHirano-Zimmerman stain.X100

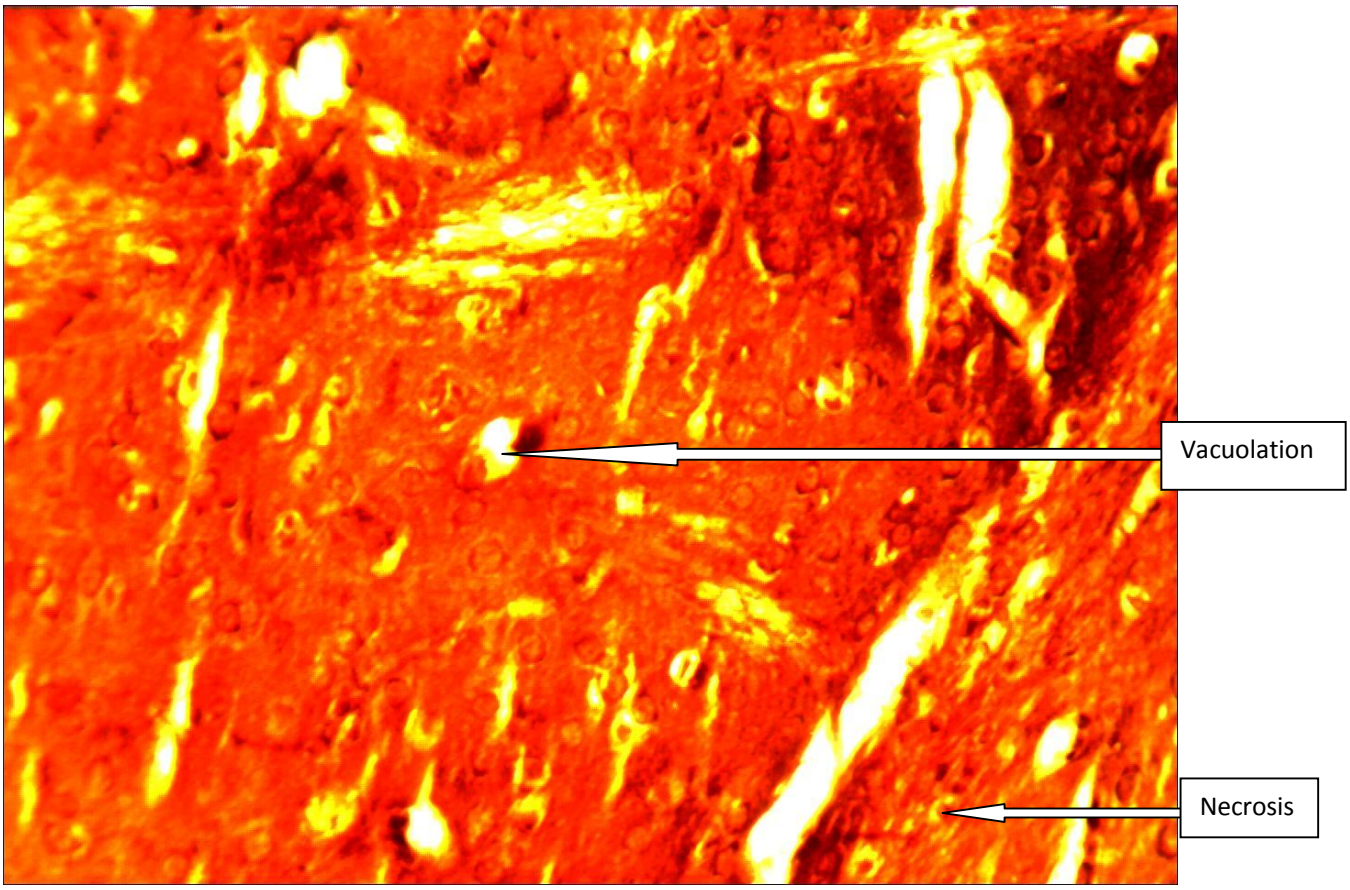


Plate XX: Longitudinal section of the cerebral cortex of wistar rats of group V that received 1900mg/kg of $AlCl_3$, showing extensive neuronal vacuolation and necrosisHirano-Zimmerman stain .X250

CHAPTER FIVE

5.0 DISCUSSION

The cerebral cortex is the key structures of memory formation. It also integrates higher mental functions, general movement, visceral functions, and behavioral reactions. (Brodal, 1992; Cauller, 1995).

Aluminium is historically been considered as nontoxic for healthy individuals. However, there is now evidence to show that under certain circumstances, excess aluminium can cause a variety of physiological and biological effects in humans (Alfrey, 1983). Aluminium has been shown to contribute to a variety of cognitive impairments in mice, rabbits, and rat pups (Muller *et al*., 1990; Yokel, 1985, Bilkei-Gorzo, 1993; Mari *et al*., 2001). Studies on workers exposed to aluminium dust in industrial environments demonstrate similar effects (Rifat, *et al*., 1991; Bast-pettersen, *et al*., 1994; White *et al*., 1992; Akila *et al*., 1999).

Our study revealed that aluminium chloride exposure affected spatial learning and memory of the wistar rats (Figures 4.1.1 and 4.1.2). At the first run of training of rats on the Morris Water Maze (MWM), the wistar rats were trying to learn task and the difference observed was not statistically significant at $P< 0.05$ (Fig. 4.1). The second and third runs of testing for memory showed that, although, the wistar rats had learnt the task by getting to the platform for escape within the stipulated time (60s), the control group that received distil water only performed the task better than the aluminium treated groups (i.e. got to the platform faster than the treated) and the differences observed was statistically significant at $P<0.05$ (Figures 4.1.1 and 4.1.2). It was therefore inferred from this experiment that aluminium chloride exposure impaired spatial learning and memory.

This was similar with the works of Muller *et al*., 1990; Yokel, 1985, Bilkei-Gorzo, 1993 and Mari *et al*., 2001, who stated that aluminium has been shown to contribute to a variety of cognitive impairments in mice, rabbits, and rat pups but contrary to the finding of WHO,1997, who stated that there is little indication that aluminium is acutely toxic.

Many researchers have found elevated aluminium levels to be associated with a decline in visual memory, attention, concentration, frontal lobe function and lower vocabulary scores in haemodialysis patients (Bolla *et al*., 1992). In our study, there was statistically significant increased in brain aluminium uptake (i.e. accumulation of aluminium in the brain) in the aluminium treated groups when compared with the control and the results indicated that the more exposure the wistar rats were to aluminium, the higher the brain uptake of aluminium (Fig. 4.3).

Our findings were in agreement with the work of He *et al*., (2003) that demonstrated that exposure to electrolytic aluminium fumes adversely affected neurobehavioral performance in workers, including motor coordination, mood, and autonomic nervous function. Although, other reports on occupational aluminium exposure and neurological impairments demonstrate mixed (conflicting) findings (Sim *et al*., 1997). Despite strong experimental and clinical evidence for aluminium neurotoxicity, the mechanism of aluminium effects on the nervous system is still not completely clear. We observed in our study that as the wistar rats learned its position in the Morris water maze, the latencies to locate the platform were reduced for those in the control group while reversed was the case for the wistar rats in the aluminium treated groups(figures 4.1.1 and 4.1.2). After some training to swims, the wistar rats were seen going directly to the platform; this could be because they have spatial map in their

cerebral cortex with exception to the aluminium treated groups (Figures 4.1, 4.1.1 and 4.1.2).

These findings were similar to the work of Berlyne, *et al*., (1972) and Deloncle *et al*., (1990): that stated that long-term administration of soluble salt of aluminium to rats worsens their learning ability together with diminished cholinergic function and the rats become lethargic.The role of aluminium intoxication in different organs in neurodegenerative diseases has been recently emphasized (Somova, *et al*., 1997; Exley, 1999).

Anxiety affects one-eighth of the total population world-wide and has become an important area of research interest in psychopharmacology. It occurs when a reaction is out of proportion with what might be normally expected in a situation (Grundman, et al., 2007). In this study the effects of aluminium chloride on anxiety-like behaviour in wistar rats were shown in Figures 4.2, 4.2.1, 4.2.2, 4.2.3 and 4.2.4. The locomotor activity of wistar rats was assessed by the number of crossing squares noted as scores per time of five minutes (5min) for each wistar rats. The numbers of times of entries of wistar rats into both opened and closed arms of the elevated plus maze significantly increased across the treated groups (Figures 4.2.3 and 4.2.4); there was statistically significant increase in defecation which may be due to heightened autonomic activity in the treated groups when compared with the control group (Figures 4.2.2). The preference for being in open arms over closed arms was calculated to measure anxiety-like behaviour and an increase in open arm activity (time spent on open arms) observed in the control group reflected anti-anxiety behaviour exhibited by the control groups (Fig. 4.2). This was evident in the gradual significant decrease in open arm activity (time spent in open arms) of the elevated plus maze from groups I to V. (Fig. 4.2), as the wistar rats in the control group exhibited anti-anxiety behaviour (Increased open arms activity) when compared with the treated groups II to V (decreased open arms activity). The

preference of wistar rats for staying in closed arms over open arms observed with the treated groups was used as a measure of anxiety-like behaviour and hence aluminium chloride in this study was implicated in anxiety-like behaviour (increase anxiety) unlike the control that showed preference for more exploration of open arms, shown by statistically significant increased time spent in open arms and less exploration of closed arms in control group when compared with the treated groups (Figures 4.2 and 4.2.1).

We also observed that the wistar rats treated with aluminium chloride had increased faecal boli across group and spent lesser time in the open arms of the elevated plus maze than the control group which was statistically significant at $p<0.05$ (Figures 4.2 and 4.2.2). This in turn indicated that the aluminium treated groups were more anxious than the control groups in exploration of their activities on the maze (Figures 4.2, 4.2.1, 4.2.2, 4.2.3 and 4.2.4).

According to WHO, 1997, there is little indication that aluminium is acutely toxic by oral exposure despite its widespread occurrence in foods, drinking-water, and many antacid preparations. In 1988, a population of about 20 000 individuals in Camelford, England, was exposed for at least 5 days to unknown but increased levels of aluminium accidentally distributed to the population from a water supply facility using aluminium sulfate for treatment. Symptoms including nausea, vomiting, diarrhoea, mouth ulcers, skin ulcers, skin rashes, and arthritic pain were noted. It was concluded that the symptoms were mostly mild and short-lived. No lasting effects on health could be attributed to the known exposures from aluminium in the drinking-water (Clayton, 1989).The average intake of aluminium per day by an adult may range from 5-14 mg from all exposure routes (WHO, 1998; Health Canada, 1997), which comes primarily from food. Drinking water contribution has been estimated to be ranging from 3 to 8% (WHO, 1998; Health Canada, 1997). Persons, who regularly ingest aluminium containing antacids and buffered analgesics may be consuming as high as 5 g

aluminium per day. Contribution of air for the total aluminium exposure is generally negligible.

Jabaudon *et al*. (2000) revealed that when extracellular glutamate concentration increase, it become excitotoxic and reaching levels that activate N-methyle-D-aspartate type of glutamate receptor (NMDAR) thereby causing neuronal death. This result was also similar to our histological observations on the cerebral cortex. The control group that received distil water only showed normal histology of cerebral cortex of wistar rats (Plates I and II; Plates XI and XII). Neuronal vacuolation and necrosis of the cerebral cortex of wistar rats was observed in groups II-IV (Plates III-VIII; Plates XIII-XVIII) while extensive neuronal vacuolation and necrosis of the cerebral cortex was observed in group V (Plates IX and X; Plates XIX and XX). The cerebral cortex of the aluminium treated wistar rats revealed neuronal vacuolation and necrosis, which are form of neuro-degeneration (Plates III-X; Plates XIII-XX). Wenk and Stemmer (1981, 1982) stated that altered content of amino acid neurotransmitters might be one of the important mechanisms of aluminium neurotoxicity. Exposure to aluminium may 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 cerebral cortex. Our histological observation was in line with the findings recorded by Bihaqi *et al*. (2009), who found that aluminium chloride caused histopathological lesions in cerebral cortex including neuronal degeneration as cytoplasmic vacuolization haemorrhage, ghost cell and gliosis. Our findings were also correlated with those of Matyja (2000) who noticed that exposure of animals to aluminium causes marked histopathological alteration in the cerebral cortex including neuronal degeneration, precellualr odema and gliosis (Plates III-X; and XIII-XX).

Walton *et al*., (1995) used an ultra-sensitive technique, accelerator mass spectrometry plus 26 aluminium (26Al), to track the movement of physiological amounts of aluminium

from drinking water into the brains of rats. Two weeks after the rats were given 25mg/L 26Al in water, they were overdosed with anaesthetic and a saline solution was used to flush the blood from their brains. The results showed that trace levels of aluminium entered the brain from the equivalent of a single glass of water. Transferrin receptors are found in capillaries of the brain but not of other tissues (Jefferies *et al*., 1984). These receptors facilitate the uptake of transferrin-bound metals across the blood-brain barrier into the brain tissue. Tissue culture experiments show that brain cells are able to concentrate high levels of aluminium available to them. In a 25 mmol aluminium solution, 60-70% of the aluminium was taken up by the cells (Shi and Haug, 1990). In another experiment, in one week of incubation, intracellular levels reached 10-20 mmol aluminium while extracellular levels were held at 100 mmol with an aluminium-EDTA complex (Guy *et al*., 1991). In our study, we estimated the concentration of brain aluminium uptake by the use of Neutron Activation Analysis Technique (NAA) and found that the control group had 26 PPM, groups II, III, IV, and V had 328.5, 782.5, 903.5, and 4249.5 PPM respectively (See Fig. 4.3) These results were found to be statistically significant at $P< 0.05$ and it indicated that the higher the exposure of wistar rats to aluminium, the higher the brain uptake of aluminium. We therefore observed that the concentration of aluminium uptake by the brain was higher across the aluminium treated groups when compared with the control (Fig.4.3). This was because the aluminium treated groups were exposed to various concentrations of aluminium chloride orally, which were readily absorbed (taken up) by the brain of the treated wistar rats unlike the control group that received distil water only (Fig. 4.3). This indicated the there was increased accumulation of aluminium in the brain of aluminium treated rats (Fig.4.3) and our results showed that trace levels of aluminium entered the brain from the equivalent of a single glass of water as estimated by Walton *et al*., (1995).

Since pregnant women may be exposed to untoward levels of aluminium compounds under certain conditions (Clayton *et al*., 1992), therefore aluminium toxicity may be of particular concern to the developing foetus. Effects of oral administration of different doses of aluminium during pregnancy on intra uterine foetal development in mice suggests that excessive ingestion of aluminium may be one of the risk factors contributing to congenital neural tube defects (Lin et al., 1997). It was demonstrated that there was behavioural and neurochemical alterations in the offspring of mice exposed to aluminium during gestation and suggested the persistence of these behavioural deficits in adulthood (Rankin *et al*., 1993).There was also a review on the neurobehavioural alteration in rodents following developmental exposure to aluminium. According to them, the presented data showed behavioural and neurochemical changes in the offspring of aluminium-exposed mouse dams during gestation, which include alterations in the pattern of ultrasonic vocalizations and a marked reduction in central nervous system choline acetyltransferase activity (Alleva *et al*., 1998). This finding correlated to those who noticed that exposure to aluminium causes marked histopathological alteration in the cerebral cortex including neuronal degeneration, precellualr odema and gliosis (Matyja, 2000).Very few studies on humans are available in this regard. However, a number of studies on animal model are presenting conflicting observations (Colomina *et al*., 2002). Our results showed neurodegeneration of the cerebral cortex as shown by the presence of extensive neuronal vacuolation and necrosis (Plate IX, X, XIX and XX).

Weight is the amount or quantity of heaviness or mass; it is system of units for expressing heaviness or mass: measure of the heaviness of an object; the amount anything weighs (Christine, 1997). In science and engineering, the weight of an object is usually taken to be the force on the object due to gravity (Richard, 1999; Igal, 2001).

There was statistically significant increase in the body weight of both sexes (male and female) of wistar rats combine in the control group with mean weight of 164.30±7.74g before the commencement of the experiment and increased to 190.80±6.05g after the experiment ($P<0.001$) (Fig. 4.5). The aluminium treated groups, on the other hand, had statistically significant decrease in body weights across the groups. Wistar rats in group II had mean weight of 179.30±12.94g before the administration of aluminium chloride and later decrease to 172.20±12.46g after eight weeks of aluminium chloride administration; the observed decrease in weight of group II was statistically significant at $P<0.001$. Group III had mean weight of 157.00±12.32g before administration of aluminium chloride but later decreased to 151±10.10g after eight weeks of aluminium chloride administration and the observed decrease was statistically significant at $P<0.05$.The wistar rats in group IV had mean weight of 174.11±11.39g before administration of aluminium chloride but later decrease to 160.78±10.65g after eight weeks of administration of aluminium chloride and the decrease observed was statistically significant at $P<0.001$. Group V mean weight was 176.56±13.39g before aluminium chloride administration but later decrease to 162.56±11.94g which was statistically significant at $P<0.001$ (Fig.4.5).

There was statistically significant decrease in the mean body weights of the aluminium treated groups of male rats only(Fig.4.6) with group II having mean weight of 200.80±20.93g before administration of aluminium chloride and later decreased to 191.00±21.00g after eight weeks of aluminium chloride administration($P<0.01$).The mean weight of male wistar rats in group III was 167.60± 20.03g before commencement of administration but later decreased to160.20±18.73g after eight weeks of aluminium chloride administration. Group IV mean weight was 199.40± 7.34g before aluminium administration but later decrease to 185.20±4.95g after eight weeks of administration($P<0.05$).The mean weight for male wistar

rats in group V was 208.00±9.06g before administration and later decreased to 190±9.08g after eight weeks of administration ($P<0.01$) (See Fig. 4.6).

For the female wistar rats only, there was decreased in the body weight in the aluminium treated groups after eight week of administration of aluminium chloride and decreased observed was only significant for group IV ((Fig. 4.7).

We also observed decrease in the brain weights of wistar rats in the aluminium treated groups II, III, IV, and V when compared with the control group (Fig. 4.4). Wistar rats in group I had brain weight of 1.66±0.05g, while the aluminium treated groups II, III, IV and V had 1.59±0.02g, 1.63±0.03g, 1.65±0.03g and 1.65±0.10±0.01g respectively; but the decreased observed on the brain weight was not statistically significant at $P<0.05$ (Fig. 4.4).

CHAPTER SIX:

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary and Conclusion

Morris Water Maze (MWM) was used in this work to test for the behaviour (spatial learning and memory) of the wistar rats due to exposure to aluminium chloride. At the first run of Morris Water Maze (MWM), the wistar rats were trying to learn task and the differences observed were not statistically significant at $P< 0.05$ (Fig. 4.1).The second and third runs showed that although the wistar rats have learnt the task by getting to the platform for escape within the stipulated time (60s), the control group performed the task better than the treated (i.e. got to the platform faster than the treated) and the differences observed was statistically significant at $P<0.05$(Figures 4.1.1 and 4.1.2).This result implies that aluminium chloride exposure affected(impaired) spatial learning and memory(Figures 4.1, 4.1.1 and 4.1.2).

Activities on the elevated plus maze revealed that the numbers of time of entries of wistar rats into both open and close arms significantly increased across the aluminium treated groups (Figures 4.2.3 and 4.2.4).There was statistically significant increased in the number of faecal boli which may be due to heightened autonomic activity in the aluminium treated groups when compared with the control (Figures 4.2.2). There was statistically significant increased time spent in close arm across the groups while we observed decreased time (lesser time in open arms) spent in the open arms of the elevated plus maze when compared with the control (Figures 4.2 and 4.2.1).The preference for being in open arms over closed arms was calculated to measure anxiety-like behaviour and our study indicated that aluminium treated groups were more anxious (increased anxiety) than the control group in exploration of their activities on the maze (Figures 4.2, 4.2.1, 4.2.2, 4.2.3 and 4.2.4).

The brain aluminium uptake results indicated that the more exposure the wistar rats are to aluminium, the higher the brain uptake of aluminium (Fig. 4.3) i.e. accumulation of aluminium in the brain of wistar rats. There was statistically significant weight loss in the aluminium treated wistar rats (Figures 4.4, 4.5, 4.6, and 4.7).

Our histological observations revealed neuronal vacuolation and necrosis of the cerebral cortex of wistar rats in the aluminium treated groups (Plates III-X; and Plates XIII-XX).

Based on our observations, we therefore draw the following conclusion:

1. Aluminium chloride exposure impaired spatial learning and memory, as wistar rats in the control group located the platform on the Morris water maze faster than the aluminium treated groups (Figures 4.1.1 and 4.1.2).

2. Aluminium chloride exposure increased the rate of anxiety, as it had negative effects on anxiety-like behaviour of wistar rats as indicated by increased rate of anxiety in aluminium treated groups when compared with the control. (Figures 4.2, 4.2.1, 4.2.2, 4.2.3 and 4.2.4).

3. Oral administrations of aluminium chloride resulted into accumulation of aluminium in brain tissue, as evident in graded increased in brain aluminium uptake of wistar rats across groups, as the group that received the highest dose had highest brain aluminium uptake (Fig. 4.3).

4. Aluminium chloride exposure had neurodegenerative effects on the histology of cerebral cortex as evident in the manifestation of neuronal vacuolation and necrosis of wistar rats of the aluminium treated groups. (Plates III-X; and Plates XIII-XX).

5. Aluminium chloride exposure resulted into body and brain weight loss of wistar rats but the brain weight loss was not statistically significant at $P<0.05$.

6.2 Recommendations

- Having found that aluminium is toxic to brain and water is one of the sources, the label of processed drinking water should state clearly the concentration of aluminium in them.

- Further research should be conducted to enable us to fully understand the health risk of aluminium on other organs of the body.

REFERENCES

Abbasali, K.M., Zhila, T. and Farshad, N. (2005). Developmental Toxicity of aluminium from High Doses of AlCl3 in Mice. *The Journal of Applied Research*, 5: 575-579.

Abreo, V. (2009). "The Dangers of Aluminium Toxicihttp:// www.bellaonline. Com/articles/ art7739.asp.

Aguilar, F., Autrup, H., Barlow, S., Castle, L., Crebelli, R., Dekant, W., Engel, K.H., Gontard, N., Gott, D., Grilli, S., Gürtler, R., Larsen, J.C., Leclercq, C., Leblanc, J.C., Malcata, F.X., Mennes, W., Milana, M.R., Pratt, I., Rietjens, I., Tobback, P. and Toldrá, F. (2008).“Safety of aluminium from dietary intake”: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC) (Question Nos EFSA-Q-2006-168 and EFSA-Q-2008-254) Adopted on 22 May 2008. *The European food Safety Association Journal,* 754, 1-34.

Akila, R., Stollery, B.T. and Riihimaki, V. (1999). Decrements in cognitive performance in metal inert gas welders exposed to Aluminium. *Occupational and Environmental Medicine,* 56:632–639.

Akirav, I., Sandi, C. and Richter-Levin, G. (2001). Differential activation of hippocampus and amygdala following spatial learning under stress. *European Journal of Neuroscience, 14,* 719-725.

Alcan Ltd. Website: http://www.alcan.com.

Alfassi, Z.B. (1990). *Activation Analysis*, Volume I and II, CRC Press, Boca Raton,FL.

Alfassi, Z.B. (1994). *Chemical Analysis by Nuclear Methods*. John Wiley and Sons: New York, NY. . ed. / Z.B. Alfassi. Chichester, Pg. 143-162.

Alfassi, Z.B. (1998). *Instrumental Multi-Element Chemical Analysis*. Kluwer Academic Publishers: Dordrecht, the Netherlands. 1; Pg. 520, ISBN-10: 0751404276 | ISBN-13.

Alfrey, A.C. (1980). Aluminium metabolism in uremia. *Neurotoxicology* 1:43–53.

Alfrey, A.C. (1983). Aluminium Toxicity. *Advances in Clinical Chemistry*, 23, 69-71.

Alfrey, A.C., LeGendre, G.R. and Kaehny, W.D. (1976).The dialysis encephalopathy syndrome. Possible Aluminium intoxication. *The New England Journal of Medicine*, 294: 184-188.

Alleva, E., Rankin, J. and Santucci, D. (1998). Neurobehavioral alteration in rodents following developmental exposure to aluminum. *Toxicology and Industrial Health*,14: 209-221.

Al-Mugrabi, M. and Spirou, N. (1987). Neutron Activation Analysis (NAA), based on the nuclear reaction between neutrons and target nuclei, is a useful. *Journal of Radioanalytical and Nuclear Chemistry,* 110, 67. Published /Hosted by Springer. ISSN (printed): 0236-5731.

Alzheimer.Society, (2002): Alzheimer disease and aluminium.www.alzheimer.ca/English disease/causes.alumi.htm.

Anane, R., Bonini, M., Grafeille, J.M. and Creppy, E.E. (1995). "Bioaccumulation of water soluble aluminium chloride in the hippocampus after transdermal uptake in mice". *Archives of Toxicology,* 69 (8): 568–71. PMID 8534202 (http:// www.ncbi .nlm.nih.gov/ pubmed/ 8534202).

Anane, R., Bonini, M. and Creppy, E.E. (1997). "Transplacental passage of Aluminium from pregnant mice to fetus organs after maternal transcutaneous exposure". *Human and Experimental Toxicology* 16 (9): 501–4. PMID 9306136 (http://www.ncbi. nlm.nih. gov /pubmed/ 9306136).

Armstrong, B., Tremblay, C., Baris, D. and Thériault, G. (1994). Lung cancer mortality and polynuclear aromatic hydrocarbons: a case-cohort study of aluminum production workers in Arvida, Quebec, Canada. *American Journal of Epidemiology*, 139(3):250–262.

Banks, W.A. and Kastin, A.J. (1989). "Aluminium-induced neurotoxicity: alterations in membrane function at the blood-brain barrier". *Neuroscience and Biobehavioral Reviews,* 13 (1): 47–53. doi:10.1016/S0149-7634(89)80051-X(http: //dx.doi. org/10. 1016%2FS0149-7634% 2889%2980051-X).

Bast-pettersen, R., Drablos, P.A., Goffeng, L.O., Thomassen, Y. and Torres, C.G. (1994). Neuropsychological deficit among elderly workers in Aluminium production. *American Journal of Industrial Medicine*, 25:649–646.

Becaria, A., Campbell, A. and Bondy, S.C. (2002). Aluminium as a toxicant. *Toxicology and Industrial Health,* 18:309–320.

Berlyne, G.M., Yagil, R., Ari, J.B., Weinberger, G., Knopf, E. and Danovitch, G.M. (1972). Aluminium toxicity in rats. *Lancet,* 7750: 564-568.

Berthon, G. (1996). Chemical speciation studies in relation to Aluminium Metabolism and toxicity. *Coordination Chemistry Reviews*, 149:241–280.

Bieber, A.M., Jr., Brooks, D.W., Harbottle, G. and Sayre, E.V. (1976). Application of multivariate techniques to analytical data on Aegean ceramics. *Archaeometry,* 18: 59–74.

Bihaqi, S.W., Sharma, M., Singh, A.P. and Tiwari, M. (2009). Neuroprotective role of Convolvulus pluricaulis on aluminium induced neurotoxicity in rat brain. *Journal of Ethnopharmacology*, 124(3):409-15.

Bilkei-Gorzo, A. (1993). Neurotoxic effect of enteral Aluminium. *Food and Chemical Toxicology*, 31:357–361.

Birchall, J.D., Chappell, J.S. and Philipps, M.J. (1989).Acute toxicity of Aluminium to fish eliminated in silicon-rich waters. *Nature*; 361:31–39.

Birchall, J. D. (1992). The interrelationship between silicon and Aluminium in the biological effects of Aluminium. *In Aluminium in Biology and Medicine*. Willey, Chichester (ciba found. Symp. 169; p 50-68).

Bishop, R.L. (1975).*Western Lowland Maya Ceramic Trade: An Application of Nuclear Chemical and Geological Data Analysis.* Ph.D. dissertation, Southern Illinois University (unpublished).

Bolla, K.I., Briefel, G., Spector, D., Schwartz, B.S., Wieler, L., Herron, J. and Gimenez, L. (1992). Neurocognitive effects of Aluminium. *Archives of Neurology*, 49:1021–1026.

Bradbury, P. (1977). *Histological Methods*. In: Hewer's Textbook of Histology for Medical Students 9th Ed. ELBS, pp 431-450, OCLC 6565 Kilgour Place, Dublin OH USA 43017.

Brodal, S. (1992). *The Central Nervous system*: Structure and Function. Oxford University Press. ISBN 0-19-505518-7, pg. 464. John Wiley and Sons, Inc.

Brodmann, K. (1909). cortical cytoarchitectonics in *"Vergleichende Lokalisationslehre der Großhirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues"* (Comparative Localization Studies in the Brain Cortex, its Fundamentals Represented on the Basis of its Cellular Architecture).Beitrage Zurhistologischen. Lokalisatinde Grosshinrinde VI, Die Cortexgliedesung de menschen. *Journal of Psychologie and Neurologie,* 10:231-246. Published by Leipzig Verlag Johann Ambrosius Barth.

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. and Adebisi, S.S. (2011). Effects of Oral Administration of Aluminium Chloride on the Histology of the Hippocampus of Wistar Rats. *Current Research Journal of Biological Sciences*, 3(5): 509-515. ISSN: 2041-0778.

Candura, S.M., Manzo, L. and Costa, L.G. (1998). Role of occupational neurotoxicants in psychiatric and neurodegenerative disorders.In: Costa LG, Manzo L (eds) *Occupational neurotoxicology*. CRC Press, Boca Raton, pp 131–167.

Cauller, L. (1995). Layer I of primary sensory neocortex: where top-down converges upon bottom-up. *Behavioural Brain Research*, 71(1-2):163-70.

Cech, I. and Montera, J. (2000). Spatial Variation in total aluminium concentrations in drinking Water Supplies. *Water Research*, 34, 2703.

Chapillon, P. and Debouzie, A. (2000). BALB/c mice are not so bad in the Morris water maze. *Behavioral Brain Research, 117*, 115-118.

Chen, G., Chen, K.S., Knox, J., Inglis, J., Bernard, A., Martin, S.J, Justice, A., McConlogue, L., Games, D., Freedman, S.B. and Morris, R. (2000). A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature, 408*, 975-979.

Christine A. (1997). The American Heritage Dictionary of Idioms. Published by Houghton Mifflin,1997.

Clayton, D.B. (1989). Water pollution at Lowermoore North Cornwall: Report of the Lowermoore incident health advisory committee. Truro, Cornwall District Health Authority, *Occupational,* pp.22.

Clayton, R.M., Sedowofia, S.K., Rankin, J.M. and Manning, A. (1992). Long-term effects of aluminium on the fetal mouse brain. *Life Science*, 51: 1921-1928.

Collerton, D. (1988). Cholinergic function and intellectual decline in Alzheimer's disease. *Neuroscience*, 19, 1-28.

Colomina, M., Teresa, R., Jose, L., Sanchez, D.J. and Domingo, J.L. (2002). Influence of aged on aluminium- induced Neurobehavioral Effects and Morphological Changes in Rat Brain. *Neurotoxicity*. 2002; 23: Pg.775-781.

Connor, D.J., Jope, R.S. and Harrell, L.E. (1988).Chronic, oral Aluminium administration to rats:Cognition and cholinergic parameters. *Pharmacology, biochemistry, and behaviour,* 31:467-474.

Corain, B., Bombi, G.G., Tapparo, A., Perazzolo, M. and Zatta, P. (1996).Aluminium toxicity and metal speciation: established data and open questions.*Coordination Chemistry Reviews*, 149:11-22.

Cornwell, B.R., Johnson, L.L. and Holroyd, T. (2008). Human hippocampal and parahippocampal theta during goal-directed spatial navigation predicts performance on a virtual Morris water maze. *Journal of Neuroscience*, 28(23):5983-90.

Crapper, D.R., Krishnan, S.S. and Quittkat, S. (1976). Aluminium, neurofibrillary degeneration and Alzheimer's disease. *Brain,* 99: 67-80.

Crawley, J.N. (2007). *What's Wrong with my Mouse?* Behavioural Phenotyping of Transgenic and Knockout Mice. 2nd Edn., Wiley-Interscience, New York, ISBN-10: 9780471471929, pp: 523.

Culling, C. F. A. (1963). *Handbook of Histopathological technique* 2nd Ed Butterworth's, London.

Das, H., Faanhof, A. and Van Der Sloot, H. (1989). Developments in Geochemistry. *Radioanalysis in Geochemistry*, Elsevier, Amsterdam. Elsevier Science Ltd pg.484.

D'Hooge, R. and De Deyn, P.P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Research Reviews, 36*, 60 – 90.

Deloncle, G.R., Deloncle, and Guillard O. (1990). Mechanism of Alzheimer's disease: arguments for a neurotransmitter- Aluminium complex implication. *Neurochemical Research*. Publisher Springer Verlag, 15: 1239-1245.

Dougall, M. C. (2004). The Newsletter; Alzheimer's disease can be safely prevented and treated,now.Vol.3,www.drmcdougall.com.http://www.nealhendrickson.com/mcdougall/2004/040600pualzheimer.htm.

Doutre, D., Gariepy, B., Martin, J.P. and Dube, G. (1985) "Aluminum Cleanliness Monitoring: Methods and Applications in Process Development and Quality Control, Light Metals, pg. 1179-1196.

Dusenbery, D.B. (2009). *Living at Micro Scale*. Harvard University Press, Cambridge Mass, pp: 124, ISBN: 978-0-674-03116-6.

Edwards, J. D., Frary, F.C. and Jeffries, Z. (1930). Aluminium and its Production, Volume 1 of the Aluminium Industry, Aluminium Company of America, McGraw-Hill book Company, the University of California.

Ehmann, W.D. and Vance, D.E. (1991). *Radiochemistry and Nuclear Methods of Analysis*. Volume 116, Pg.302-302, John Wiley and Sons: New York, NY.Wiley-Interscience, New York, pp. 302–303 (Chapter 9.6.4).

Encarta, (2009). *Digital multimedia encyclopaedia*. Published by Microsoft Corporation. Complete English version.

Erdtmann, G. and Soyka, W. (1979). The Gamma Rays of the Radionuclides: Tables for Applied Gamma Ray Spectrometry. Angewandte Chemie international Edition, *Verlag Chemie: Weinheim*, 18: 703.Germany.

Exley, C.A. (1999). Molecular mechanism of Aluminium induced Alzheimer's disease. *Journal of Inorganic Biochemistry*, 76: 133-140.

Exley, C. (2005). The aluminium-amyloid cascade hypothesis and Alzheimer's disease aluminium and β-amyloid. *Alzheimer's disease*, 38:225-234. DOI: 10.1007/0-387-23226511.

Exley, C., Charles, L.M., Barr, L., Martin, C., Polwart, A. and Darbre, P.D. (2007). "Aluminium in human breast tissue". *Journal of Inorganic Biochemistry*101 (9): 1344–6.doi:10.1016/j.jin org bio. 2007.06.005 (http://dx.doi.org/10.1016%2Fj.jin or gb\io .2007.06.005) .PMID 17629949.

Flaten, T. P. (1990). Geographical Associations between Aluminium in drinking water and death rates with dementia (including Alzheimer's disease), parking son's disease and amyotrophic lateral sclerosis in Norway. *Environmental Geochemistry and Health*, 12: 152-167.

Flaten, T. (2001). Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. *Brain Research Bulletin*, 55: 187-196.

Fordyce, D., Clark, V., Paylor, R. and Wehner, J. (1995). Enhancement of hippocampally-mediated learning and protein kinase C activity by oxiracetam in learning-impaired DBA/2 mice. *Brain Research, 672,* 170-176.

Fox , G., LeVasseur, R. and Faden, A. (1998). Behavioral responses of C57BL/6, FVB/N, and 129/SvEMS Mouse strains to traumatic brain injury: implications for gene targeting approaches to neurotrauma. *Journal of Neurotrauma, 16,* 377-389.

Francis, P. T., Palmer, A. M., Sims, N. R., Bowen, D. M., Davison, A. N., Esiri, M. M., Neary, D., Snowden, J. S. and Wilcock, G. K. (1985). Neurochemical studies of early onset Alzheimer's disease. *New England Journal of Medicine,* 313g,7-11.

Frick, K.M., Stillner, E.T. and Berger-Sweeney, J. (2000). Mice are not little rats: species differences in a one-day water maze task. *Neuroreport, 11,* 3461-3465.

Friedlander, G., Kennedy, J.W., Macias, E.S. and Miller, J.M. (1981). *Nuclear and Radiochemistry*. John Wiley and Sons: New York, NY. Pg359.ISBN 047186255X, 9780471862550.

Garruto, R.M. and Brown, P. (1994) .Tau protein, Aluminium, and Alzheimer's disease. *Lancet,* 8904:989–993.

George, C.K. (1973). *Comparative Anatomy of Vertebrates*. 7th edition.Pp.375-412. William C. Brown Publication, ISBN: 0801662370.

Gibbs, G.W. (1995). Mortality of aluminium reduction plant workers, 1950 through 1977. *Journal of Occupational Medicine*, 1995; 27: 761 - 770.

Gibbs, G.W. (1997). Estimating residual polycyclic aromatic hydrocarbon (PAH) related lung cancer risks, using occupational data. *Annals of Occupational Hygiene*, 41:49-53.

Gilbert, C.D. and Sigman, M. (2007). Brain states: top-down influences in sensory processing. *Neuron.* 54(5):677-96.

Giordano, R. and Costantini, S., (1993). "Some aspects related to the presence of aluminium in waters". *Sanità, Annali dell'Istituto Superiore di Sanità(Ann. Ist. Super),* 29 (2), 305-311. *ISSN 0021-2571*

Glascock, M.D. (1996). *Tables for Neutron Activation Analysis.* The University of Missouri Research Reactor Center: Columbia, MO. 4th edition. pg. 36-42.

Glaser, O.C. (1910). The formation of habits at high speed. *The Journal of Comparative Neurology*, 20: 165-184.

Gómez, M., Sánchez, D.J., Llobet, J.M., Corbella, J. and Domingo, J.L. (1997).The effect of age on aluminium retention in rats. *Toxicology,* 116:1–8.

Gramiccioni, L., Ingrao, G., Milana, M.R., Santaroni, P. and Tomassi, G. (1996). Aluminium levels in Italian diets and in selected foods from aluminium utensils. *Food Additives and Contaminant***,** 13 (7), 767.

Grant, W.B., Campbell, A., Itzhaki, R.F. and Savory, J. (2002). The significance of environmental factors in the etiology of Alzheimer's disease. *Journal of Alzheimer's Disease*, 4: 179-189.

Graves, A.B., White, E., Koepsell, T.D., Reifler, B.V., van Belle, G. and Larson, E.B. (1990). "The association between Aluminium-containing products and Alzheimer's disease". *Journal of Clinical Epidemiology* 43 (1): 35–44. Doi: 10.1016/0895-4356(90)90053.

Greger, J. L., Goetz, W. and Sullivan, D. (1985). Aluminium levels in foods cooked and stored in aluminium pan, trays and foil. *Journal of Food Protection*, 48: 772-777.

Grjotheim, K., Krohn, C., Malinovsky, M., Matiasovsky, K. and Thonstad, J. (1982). *Aluminium Electrolysis- Fundamentals of the Hall-Heroult Process*. 2nd Edition, Aluminium-Verlag, Dusseldorf, 319-61.

Grundman, O., Nakajima, J., Seo, S. and Butterweck, V. (2007). Anti-anxiety effects of *Apocynum venetum* L. in the elevated plus maze test. *Journal of Ethnopharmacology*, 110, 406–411.

Gupta, V.B., Anitha, G., Hegda, M.L., Zecca, L., Garruto, R.M., Ravid, R., Shankar, S.K., Stein, R., Hanmugavelu, P. and Jagannatha Rao, K.S. (2005). Aluminium in Alzheimer's disease: are we still at a crossroad? *Cellular Molecular Life Science*, 62: 143-158.

Gurr, E. (1962). *Staining Animal Tissues, Practical and Theoretical*, Leoned Hill (book) Ltd, London. 579-593. Dewey number; 591.8240724, 578.64.

Guy, S.P., Jones, D., Mann, D.M.A. and Itzhaki, R.F. (1991). Human neuroblastoma cells treated with aluminium express an epitope associated with Alzheimer's disease neurofibrillary tangles. *Neuroscence Letter,* 121:166-168.

Hagan, J.J., Alpert, J.E., Morris, R.G.M. and Iversen, S.D. (1983). The effects of central catecholaminergic depletion on spatial learning in rats. *Behavioural Brain Research*, 9: 83-104.

Harbottle, G. (1976). Activation analysis in archaeology. In *Radiochemistry: A Specialist Periodical Report,* vol. 3, edited by G.W.A. Newton, pp. 33–72. The Chemical Society: London.

Harbottle, G. (1982). Provenance studies using neutron activation analysis: the role of standardization. In *Archaeological Ceramics,* edited by J.S.Olin and A.D. Franklin, pp. 67-77. Smithsonian Institution Press: Washington, D.C.

Harper, J.C. and Maser, J.D. (1975). A microscopic study of the brain of Bison, the American Plain Buffalo. *Anatomical records*, 18: 187-202.

He, S., Niu, Q. and Wang, S. (2003). Neurobehavioral, autonomic nervous function and Lymphocyte subsets among Aluminium electrolytic workers. *International Journal of Immunopathology and Pharmacology*,16:139–144.

Health Canada, (1997). Aluminium in drinking Water WHO (2000) Guidelines for Aluminium) www.hc- sc.gc.ca/ehp/ehd/catalogue/general/iyh/alwater.htm.

Hebb, D.O. (1949). The Organization of Behavior: *A Neuropsychological Theory*. New York: John Wiley. ISBN-10: 0805843000, pg 378.

Hermenegildo, C., Saez, R., Minoia, C., Manzo, L. and Felipo, V. (1999). Chronic exposure to Aluminium impairs the glutamate-nitric oxide-cyclic GMP pathway in the rat in vivo. *Neurochemistry International*, 34:245–253.

Hewitt, C. D., Savory, J. and Wills, M. R. (1990). Aspects of aluminium toxicity. *Clinical Laboratory Medical Journal*. Vol. 10 (2): 403-22.

Hirano, A. and Zimmerman, H.M. (1962). Alzheimer's neurofibrillary changes: A topographic study. *Archives of Neurology*, 6:114-122.

Hubeld, H. and Wiesel, T.N. (1959). "Receptive fields of single neurones in the cat's striate cortex". *Journal of Physiology. (Lond.)* 148: 574–91. PMID 14403679. PMC1363130.

Hughes, J.T. (1989). Aluminium encephalopathy and Alzhemer's diseases. *Lancet,* 1(8636):490-491.

Hull, C.L. (1943). Principles of Behaviour: An Introduction to Behaviour Theory. *Psychoanalytic Review*, 32:368 New York: Appleton-Century-Crofts. Pg422.

Igal, G. (2001). "Weight versus gravitational force: historical and educational perspectives". *International Journal of Science Education*; 23: 1073. Bibcode 1999.doi: 10.1080/09500690110038585.

Jabaudon, D., Scanziani, M., Ga¨ hwiler, B. and Gerber, U. (2000). Acute decrease in net glutamate uptake during energy deprivation. *Proceedings of the National Academy of Sciences*, vol. 97 (10): 5610–5615.

Jefferies, W.A., Brandon, M.R., Hunt, S.V., Williams, A.F., Gatter, K.C. and Mason, D.Y. (1984). Transferrin receptor on endothelium of brain capillaries. *Science* 312: 162-163.

Jiang, H.X., Chen, L.S., Zheng, J.G., Han, S., Tang, N. and Smith, B.R. (2008). Aluminium-induced effects on Photosystem II photochemistry in citrus leaves assessed by the chlorophyll a fluorescence transient. *Tree of Physiology*, 28 (12):1863-71.

Jonah, S.A., Umar, I.M., Oladipo, M.O.A., Balogun, G.I., Adeyemo. D.J. 2005. Standardization of NIRR-1 irradiation and counting facilities for instrumental neutron activation analysis. Centre for Energy and Training. Ahmadu Bello University, Zaria Nigeria. *Applied Radiation and Isotopes* 64:818-822.

Jones, E.G. (1998). Viewpoint: the core and matrix of thalamic organization. *Neuroscience*. 85(2):331-45. PMID 9622234.

Jones, K. C. and Bennett, B. C. (1986). Exposure of man to environmental Aluminium – an exposure commitment assessment. *Science Total Environment*, 52: 65-82.

Jyoti, A., Sethi, P. and Sharma, D. (2007). Bacopa monniera prevents fromaluminium neurotoxicity in the cerebral cortex of rat brain. *Journal of Ethnopharmacology,* 111, 56–62.

Kandel, E.R., Schwartz, J.H. and Jessell, T.M. (2000). *Principles of Neural Science*. 4th edition, New York: McGraw-Hill, pg.1414. ISBN 0-8385-7701-6.

Keisch, B. (1972). The *Atomic Fingerprint: Neutron Activation Analysis.* USERDA Technical Information Center, Washington, DC. First edition, Published by U.S. Energy Research and Development Administration, Washington, D.C. Pg. 56. Washington, Atomic Energy Commission, Office of Information Services,

Kiernan, J.A. (2008). Histological and Histochemical Methods: *Theory and Practice*. 4th ed. Bloxham, UK: Scion publishing Limited, Pg.606. ISBN-10: 1904842429.

Klapdor, K. and van der staay, F.J. (1996). The Morris water-escape task in mice: strain differences and effects of intra-maze contrast and brightness. *Physiology of Behavior,* 60, 1247-1254.

Klein, G.L. (1993). Aluminium and hepatobiliary complications of total parenteral nutrition. *Gastroenterology*, 10: 1583-1584.

Kloppel, H., Fliedner, A. and Kordel, W. (1997). Behaviour and endotoxicology of aluminium in soil and water. Review of the scientific literature. *Chemosphere,* 35: 353-363.

Kruger, P. (1971). *Principles of Activation Analysis*. Wiley Inter-science: New York, NY. Mumuni, Vol. 1, pg. 42.

Lankoff, A., Banasik, A. and Duma, A. (2006). "A comet assay study reveals that aluminium induces DNA damage and inhibits the repair of radiation-induced lesions in human peripheral blood lymphocytes". *Toxicology Letters,* 161 (1): 27–36.doi:10 .1016 /j.t oxlet.2005.07.012(http://dx.doi.org/10.1016%2Fj.toxlet.2005.07.012).PMID1613996 9.

Lam, Y.W. and Sherman, S.M. (2010). Functional Organization of the Somatosensory Cortical Layer 6 Feedback to the Thalamus. *Cerebral Cortex Journal*, 20:13-24. doi: PMID 19447861.

Laschkarew, W. H. (1930). The Chemical Elements and their Compounds. *Zeitschrift fuer Anorganische und Allgemeine Chemie Journal (Z. Anorg. Chem.),* 193:270.Oxford University Press.

Lashley, K.S. and Clark, G. (1946). The Cyto-architecture of the cerebral cortex of Ateles. A critical Examination of Architectonic Studies. *Journal of Comparative Neurology*, 85: 223-305.

Lettermann, R.D. and Driscoll, C.T. (1988), Survey of residual aluminium in filtered water. *Journal of America Water Works Association*, 80,154.

Lin, Y, Wong, K. and Calame, K. (1997). Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation. *Science,* 276(5312):596-9.

Li Runshen, (1981). *New chemical for water treatment, poly-aluminium chloride*, china architecture industry publisher.

Liyu, W. (2004). *WINSPAN 2004,* A Multi-Purpose Gamma-Ray Spectrum Analysis Software. CIAE, Beijing, China.

Llewellyn, B.D. (2009). Nuclear staining with alum-hematoxylin. *Biotechnology and Histochemistry,* 84: 159-177.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 53:275-287.

Luna L.G. (1968). Manual of histologic staining methods of the armed forces institute of pathology: *Theory and Practice of Histological Techniques*. 3rd edition, New York: McGraw-Hill, pp. 175–176.

Mari, S., Golub, and Stacey, L. (2001). Long-term consequences of developmental exposure to Aluminium in a suboptimal diet for growth and behavior of Swiss Webster mice. *Neurotoxicology and Teratology*, 23:365–372.

Mark, S.B., Harder, J. A., Kelly, M. E., Cheng, C. H. K. and Costall, B. (1996). Combined pCPA and muscarinic antagonist treatment produces a deficit in rat water maze acquisition. Pharmacology,Biochemistry and Behavior, 55, 61-65. *Behavioral Neuroscience,* Vol. 122, Iss 6 - http://content.apa.org/journals/bne.

Markesbery, W.R., Ehmann, W.D., Hossain, T.I., Alauddin, M. and Goodin, D.T. (1981). Instrumental neutron activation analysis of brain Aluminium in Alzheimer disease and aging. *Annals of Neurology*, 10: 511-516.

Matyja, E. (2000). Aluminium enhances glutamate-mediated neurotoxicity in organotypic cultures of rat hippocampus. *Folia Neuropathology*, 38(2):47-53.

McDonald, R.J. and White, N.M. (1994). Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. *Behavioral Neural Biology, 61,* 260-270.

Mettler, F.A. (1935). Coticifugal fiber connections of the cortex of Macaca mulata. *Journal of comparative neurology*, 63: 23-47.

Meyer, D.R. and Woolsey, C.N. (1952). Effects of localized cortical destruction on auditory discriminative conditioning in cat. *Journal of Neurophysiology*, 15: 149-162.

Miller, R.G., Kopfler, F. C., Kelty, K. C., Stober, J. A. and ULmar, N. S. (1984). The occurrence of aluminium in drinking water. *Journal of America water Association*, 76: 84-91.

Milton, H. (1974). Nervous System. In: *Analysis of Vertebrate Structure Text*. Pp.77. vol: 9, pg. 351.

Morris, R.G.M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, 1981;12:239–260.

Morris, R. (1984).Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods, 11,* 47-60.

Morris, R, G.M., Garrud, P., Rawlins. J.N.P. and O'Keefe. J. (1982). Place-navigation impaired in rats v~ith hippocampal lesions, *Nature* (Lond.), 297: 681-683.

Mountcastle, V. (1997). The columnar organization of the neocortex. *Journal of Neuroscience,* 120 (4): 701-722. doi: 10.1093/brain/120.4.701.

Muller, G., Bernuzzi, V., Desor, D., Hutin, M.F., Burnel, D. and Lher, P.R. (1990). Developmental alteration in offspring of female rats orally intoxicated by Aluminium lactate at different gestation periods. *Teratology,* 42:253–261.

Neri, L.C. and Hewitt, D. (1991). Aluminium, Alzheimer's disease, and drinking water. *Lancet,* 338:390.

Newairy, A.S., Salama, A.F., Hussien, H.M., and Yousef, M.I. (2009). Propolis alleviates aluminium-induced lipid peroxidaion and biochemical parameters in male rats. *Food Chemical Toxicology*, 47(6):1093-8.

Nigerian Industrial Standard (NIS) (2007). Drinking Water Quality Standard Used in Nigeria. ICS 13.060.20. Price group D SON 2007. Approved by SON governing Council; Nigeria Water Resources Act No 101, (1993). Corperate headquarter, plot 1687, Lome street, Wuse Zone 7 Abuja.OR Operational Headquarters 13/14 Victoria Arobieke street, Off Admiraltry way, Lekki Peninsula Scheme 1 Lekki Lagos.

Niu, Q., Yang, Y., Zhang, Q., Niu, P., He, S., Di Gioacchino, M. and Boscolo, P. (2007). "The relationship between Bcl-2 gene expression and learning & memory impairment in chronic Aluminium-exposed rats. *Journal of Biological Chemistry*, Volume 12, Number 3, 163-169, DOI: 10.1007/BF03033913.

Owen, E.H., Logue, S.F., Rasmussen, D.L. and Wehner, J. (1997). Assessment of learning by the Morris water task and fear conditioning in inbred mouse strains and F1 hybrids: implications of genetic backgrounds for single gene mutations and quantitative trait loci analyses. *Neuroscience, 80,* 1087-1099.

Pardee, W., Melikian, H.E., Rasmussen, D.L., Kenneson, A., Conn, P.J. and Warren, S.T. (1999).Fragile X mouse: strain effects of knockout phenotype and evidence suggesting deficient amygdala function. *Neuroscience, 94,* 185-192.

Parry, S.J. (1991). *Activation Spectrometry in Chemical Analysis*. John Wiley and Sons: New York, NY.Pg.264. ISBN: 0471638447.

Pavlov, I.P. (1927).Conditional Reflexes: *An Investigation of the Physiological Activity of the Cerebral Cortex*. Oxford University Press, London.Translated and Edited by G. V. Anrep. London.

Pawlek, R.P. (1998). The Primary Aluminium Industry at the Turn of the Year 1997/98. "Light Metals Age" p8, *Journal of Applied Electrochemistry*,28:781–789. Published/Hosted by Springer. ISSN (printed): 0021-891X. ISSN (electronic): 1572-8838.

Paylor, R., Baskall-Baldini, L., Yuva, L. and Wehner, J.M. (1996). Developmental differences in place-learning performance between C57BL/6 and DBA/2 mice parallel the ontogeny of hippocampal protein kinase C. *Behavioural Neuroscience, 110*, 1415-1425.

Pellow, S., Chopin, P., File, S.E. and Briley, M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, 14, 149–167 | Article | PubMed | ISI | ChemPort.

Penfield, W. (1950). Observations on the anatomy of memory. Fol. Psychiatry Neurology. *Neurochirurgica Nearl,* 53: 349-351.

Pennington, J.A.T. and Schoen, S.A.(1995). Estimates of dietary exposure to aluminium. *Food Additives and Contaminants*, 12(1):119-128.

Perlman, I. and Asaro, F. (1969). Pottery analysis by neutron activation. *Archaeometry* 11: 21–52

Proudfoot, A.T. (2009). Aluminium and zinc phosphide poisoning. *Clinical Toxicology, (Phila)*, 47 (2):89-100.

Provan, S.D. and Yokel, R.A. (1992). Aluminium inhibits glutamate release from transverse rat hippocampal slices: role of G proteins. Ca channels and protein kinase C. *Neurotoxicology*, 13:413–420.

Rakic, P. (1988). "Specification of cerebral cortical areas". *Science* 241, (4862): 170–176. doi:10.1126/science.3291116.PMID 3291116.http://www.sciencemag.org/cgi/ content / abstract/241/4862/170.

Rankin, J., Sedowofia, K., Clayton, R. and Manning, A. (1993). Behavioural effects of gestational exposure to aluminium. *Annual Ist Super Sanita*, 29: 147-152.

Richard, C.M. (1999). "Weight and gravity - the need for consistent definitions". *The Physics Teacher,*;37:51. Bibcode 1999.doi: 10.1119/1.880152.

Rifat, S.L., Eastwood, M.R., McLachlan, D.R.C. and Corey, P.N. (1991). Effect of exposure of miners to Aluminium powder. *Lancet.* 336:1162–1165.

Roberts, E. (1986). Alzhermer's disease may begin in the nose and may be caused by aluminosilieates. *Neurobiology of Aging*, 7:561–567.

Rubio-Garrido, P., Pérez-de-Manzo, F., Porrero, C., Galazo, M.J. and Clascá, F. (2009). Thalamic input to distal apical dendrites in neocortical layer 1 is massive and highly convergent. *Cerebral Cortex Journal*, 19(10):2380-95. PMID 19188274 doi: 10. 1093/ cercor/bhn259.

Sahin, G., Varol, I. and Temizer, A. (1994). Determination of aluminium levels in the kidney, liver, and brain of mice treated with aluminium hydroxide. *Biological Trace Element Research,* 41:129–135.

Sarin, S., Gupta, V. and Gill, K.D. (1997). "Alterations in lipid composition and neuronal injury in primates following chronic aluminium exposure". *Biological Trace Element Research,* **59** (1-3): 133–43. doi:10.1007/BF02783238 (http://dx.doi.org /10.1007 %2FBF02783238) . PMID 9522055.

Savory, J. and Garruto, R.M. (1998) Aluminum, tau protein, and Alzheimer's disease: an important link?The Evidence That Aluminum Causes AD Is Compelling. *Nutrition,* 14(3):313-4.

Sayre, E.V. and Dodson, R.W. (1957).Neutron Activation Study of Mediterranean Potsherds: Nuclear Activation Analysis. Pg 7, Published by Bobbs-Merrill reprint series in social sciences.

Schmidt, M.L., Zhukavera,V., Perl, D.P., Sheridan, S.K. and Schuck, T. (2001). Spinal cord neurofibrillary pathology in alzheimer disease and guam parkinsonism-dementia complex. *Journal of Neuropathology and Experimental Neurology*, 60: 1075-1086.

Sepe, A., Costantini, S., Ciaralli, L., Ciprotti, M., Giordano, R. (2001). Evaluation of aluminium concentration in samples of chocolate and beverages by electrothermal atomic absorption spectrometry. *Food Additives Contaminant*, 18 (9), 788.

Shehla, K.F., Prabhavathi, P.A., Padmavathi, P. and Reddy, P.P. (2001). Analysis of chromosomal aberrations in men occupationally exposed to cement dust. *Mutation Research*, 490: 179-186.

Shepherd, J.K., Grewal, S.S., Fletcher, A., Bill, D.J. and Dourish, C.T. (1994). Current Topics in Behavioral Neuroscience. *Psychopharmacology,* 116: 56-64.

Shi, B. and Haug, A. (1990). Aluminium uptake by neuroblastoma cells. *Journal of Neurochemistry,* 55:551-558.

Shipp, and Stewart,(2007). Structure and functions of the cerebral cortex. *Current Biology,* 17(12)**:**R443–9.doi:10.1016/j.cub.2007.03.044 PMID 17580069 http:/ /www.cellcom/ current-biology/retrieve/pii/S0960982207011487.

Sim, M., Dick, R., Russo, J., Bernard, B., Mueller, C. and McCammon, C. (1997). Are Aluminium potroom workers at increased risk of neurological disorders? *Occupation and Environmental Medicine*, 54:229–235.

Sisson, S. and Grossman, J.D. (1953). Nervous System. In: *The Anatomy of the Domestic Animals*. 4th Ed. W.B. Saunders Company. Philadelphia, PP.788-811.

Smith, C. M. and Swash, M. (1978). Possible biochemical basis of memory disorder in Alzheimer Disease. *Annals of Neurology*, 3, 471-473.

Soete, D., Gijbels, R. and Hoste, J. (1972). *Neutron Activation Analysis*, Wiley Interscience, New York. Science, pg. 836, Vol. 37

Solomon, B., Koppel, R. and Jossiphov, J. (2001). Immunostaining of calmodulin and Aluminium in Alzheimer's disease-affected brains. *Brain Research Bulletin*, 55:253–256.

Somova, L.I., Missankov, A. and Khan, M.S. (1997). Chronic Aluminium intoxication in rats: dose dependent morphological changes. *Methods Find Experimental and Clinical Pharmacology,* 19: 599-604.

Starska, K. (1993). Aluminium in food. *Roczniki Panstwowego Zakladu Higieny's Journal,Poland, (Rocz.Państw.Zakł.Hig.),* 44 (1), 55.

Stobart, P.D. (1986). Chemistry of Aluminium, Gallium, Indium, Thallium.P.D. *Centenary of the Hall and Heroult Processes*, 1886-1986,International Primary Aluminium Institute, London, 1986. 10. L.G. Boxall, A.V., P.D. Stobart, ed.,Cooke books.

Stephen, C. N., Alexander, C. F., Tanily, A. W., Ryan, S. D. and Arnold, R. K. (2001). "Neurons derived from radial glial cells establish radial units in neocortex". *Nature* 409(6821):714–720.doi:10.1038/35055553.PMID 11217860.http://www.nature.com /nature /journal/v409/n6821/abs/409714a0.html.

Sutherland, R.J., Whishaw, 1.Q. and Regehr. J.C. (1982) Cholinergic receptor blockade impairs spatial Iocalisation using distal cues in the rat, *Journal of Comparative and Physiological Psychology*. 96: 563-573.

Sutherland, R.J., Whishaw, I.Q. and Kolb, B. (1983). A behavioural analysis of spatial Iocalisation following electrolytic, kainate-, or colchicine-induced damage to the hippocampal formation in the rat. *Behavioural Brain Research*, 7: 133-153.

Tahara, H. (2004). Osteomalacia and vitamin D deficiency in hemodialyzed patients. *Clinical Calcium*. 14:42–45.

Theordore, W.T. and Alan, F. (1979). Nervous system development. In: *The morphogenesis of vertebrates.* 4th edition, John Wiley and Sons Ltd European Distribution Centre New Era Estate Oldlands Way Bognor Regis West Sussex. Pp.457-531.

Tomer, R., Denes, A.S., Tessmar-Raible, K. and Arendt, D. (2010)."Profiling by image registration reveals common origin of annelid mushroom bodies and vertebrate pallium". *Cell*, 142 (5): 800–809. doi:10.1016/j.cell.2010.07.043.

Upchurch, M. and Wehner, J.M. (1988). Differences between inbred strains of mice in Morris water maze performance. *Behavioral Genetics, 18,* 55-68.

Verstraeten, S.V., Aimo, L., and Oteiza, P.I. (2008). Aluminium and lead: molecular mechanisms of brain toxicity. *Archives of Toxicology*, Nov 2008; 82(11):789-802.

Vértes, A., Nagy, S. and Süvegh, K. (1998). *Nuclear Methods in Mineralogy and Geology*, Plenum Press, New York and London.Pg.558, ISBN-10: 0306458322.

Von Lubitz, D., Paul, I., Bartus, R. and Jacobson, K. (1993). Effects of chronic administration of adenosine A1 receptor agonist and antagonist on spatial learning and memory. *European Journal of Pharmacology, 249,* 271-280.

Wolfer, D., Stagljar-Bozicevic, M., Errington, M. and Lipp, H-P. (1998). Spatial memory and learning in transgenic mice: fact or artifact? *News in Physiological Science, 13*, 118-123.

Waller, M.B., Waller, P.F. and Brewster, LA. (1960). A water maze for use in studies of drive and learning. *Psychological Reports*. 7:99 102.

Walton, J., Tuniz, C. and Fink, D. (1995). Uptake of trace amounts of Aluminium into the brain from drinking water. *Neurotoxicology,* 16(1):187-190.

Wenk, G. and Stemmer, K. (1981). The influence of ingested Aluminium upon norepinephrine and dopamine levels in the rat brain". *Neurotoxicology*, 2, 347-353.

Wenk, G. and Stemmer, K. (1982). Activity of the enzymes dopamine -beta-hyrdoxylase and phenyl-ethanolamine-N-methyl-tranferase in discrete brain regions following Aluminium ingestion", *Neurotoxicity,* 3: 93-99.

Wever. E.G. (1932). Water temperature as an incentive to swimming activity in the rat. *Journal of Comparative Psychology*. 14: 219-224.

Whitehouse, P. J., Struble, R. G., Hedreen, J. C., Clark, A. W. and Price, D. L. (1985). Alzheimer's disease and related dementias: Selective involvement of specific neuronal system C.R.C. Critical Review. *Clinical Neurobiology*, 1, 319-339.

White, D.M., Longstreth, W.T., Rosentock, L., Keith, H.J., Clay-poole, H.J., Brodkin, C.A. and Townes, B.D. (1992).Neurological syndrome in 25 workers from an Aluminium smelting plant. *Archives of Internal Medicine,* 152:1443–1448.

WHO, (1997). Aluminium. Geneva, World Health Organization, International Programme on Chemical Safety. *Environmental Health Criteria 194*.

WHO, (1998). Guidelines for drinking water quality. second edition. *Addendum to Vol.2*., Published by World Health Organization, Geneva. Pg 283.

WHO, (2000). Aluminium in drinking Water; *Guidelines for Aluminium*; www.hc- sc.gc.ca/ ehp/ehd/catalogue/general/iyh/alwater.htm.

Williams, R.J.P. (1992). Aluminium and biological systems: an introduction. *Coordination Chemistry Reviews (Coord Chem Rev),* 149:1–9.

Wills, M.R., Hewitt, C.D., Sturgill, B.C., Savory, J. and Herman, M.M. (1993). Long-term oral or intravenous aluminium administration in rabbits.I. Renal and hepatic changes. *Annals of Clinical and Laboratory Science*, 23:1–16.

WWW.Net.Doctor (2004).

Yang, Y.X., Niu, Q., Niu, P.Y. and Lou, J. (2006). Effects of Aluminium on lipid peroxidation in rat's brain and its sex - related difference. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. (Chinese journal of industrial hygiene and Ocupational diseases),* 24(5):281 3.

Yen-Koo, H.C. (1992).The effect of Aluminium on conditioned avoidance response (CAR) inmice. *Toxicology and industrial health,* 8:1-7.

Yokel, R.A. (1985). Toxicity of gestational Aluminium exposure to the maternal rabbit and offspring. *Toxicology and Applied Pharmacology*, 79:121–133.

Yokel, R.A. (2000).The toxicology of Aluminium in brain: review, *Neurotoxicology* .21 (5): 813-828.

Yokel, R.A. (2002). Brain uptake, retention, and efflux of aluminium and manganese. *Environmental Health Perspectives,* 110:699–704.

Yokel, R.A. and McNamara, P.J. (2001). Aluminium toxicokinetics: an updated mini-review. *Annual Review of Pharmacology and Toxicology***,** 88: 159-167.

Zattal, P.F., Nicolini, M. and Corain, B. (1991). Aluminium (III) toxicity and blood-brain barrier permeability: *In aluminium in chemistry, biology and medicine* cortina international, Verona and Reven Press, New York Pg. 97-112.

Zecevic, N. and Rakic, P. (2001). "Development of layer I neurons in the primate cerebral cortex". *Journal of Neuroscience,* 21 (15): 5607–19. PMID 11466432. www. jneurosci. org/cgi/ content /full/21/15/5607.

Zhang, K. and Zhou, Q. (2005). Toxic effects of Al-based coagulants on Brassica chinensis and Raphanus sativus growing in acid and neutral conditions. *Environmental Toxicology,* 20:179–187.

Zubenko, G.S. and Hanin, I. (1989). "Cholinergic and noradrenergic toxicity of intraventricular Aluminium chloride in the rat hippocampus". *Brain Research,* 498 (2): 381–4. PMID 2790490 (http://www.ncbi.nlm.nih.gov/pubmed/2790490)20.

APPENDIX I

Tissue Preparation Method for Histological Analysis

Tissue preparation method for histological analysis has been differentiated into the following stages; fixation, tissue processing, sectioning, staining and photomicrography. The tissue preparation was done using standard techniques in accordance with Gurr, (1962), Culling (1963) and Bradbury (1977). The outlines are listed below:

a) Fixation

The cerebral hemisphere was carefully separated from the other component of the brain and a section of the two cerebral hemispheres was made. These sections were fixed in Bouin's fluid.

b) Tissue Processing

Both manual and automatic tissue processor were employed after the area of the brain section showing the cerebral cortex has been trimmed.

The manual processing techniques used where outlined by Culling 1963 and Bradbury (1977) while some samples were processed using the Automatic tissue processor called Histokinnette Bench Model automatic tissue processor of the Department of Human Anatomy, A.B.U. Zaria. Xylene was used in clearing the tissue. After impregnation of the tissue in molting paraffin wax in two changes for 3 hours each, the tissue was embedded in paraffin wax, blocked and trimmed.

c) Sectioning and mounting

The tissue sections were done using Rotary Microtome at 5 microns. The cut sections were floated in hot water bath and they were picked up on clean slides for staining.

d) Staining

The staining techniques used were general (routine),Heamatoxylin and Eosin (H & E) and Hirano-Zimmerman were the routine technique employed in order to demonstrate outline of the cerebral cortex.

e) Photomicrography

Photomicrographs at X100 and X250 magnifications were taken from all the groups with the help of light microscope and digital camera, fitted with a laptop.

APPENDIX II
Haematoxylin and Eosin Staining Methods

Preparation of Harris Haematoxylin: Haematoxylin-2 gm, Alcohol (95%)- 50 mls, Ammonium aluminum sulphate- 30gm, Distilled water- 500mls; Dissolved ammonium aluminum sulphate in water; and then bring to boil. Add the haematoxylin in alcohol, while hot; add 1 gm of mercuric oxide red to oxidize the Haematoxylin to haematin. Once dark purple color appears, plunge the beaker containing the stain immediately into cold water to stop the oxidation. One to four drops of glacial acetic acid could be added. 2. Staining procedure for the haematoxylin and eosin.

- Dewax section in xylene for 3 minutes
- Deep in xylene again for another 3 minutes
- Then in 100% alcohol for 1 minutes
- In 95% alcohol for 1 minute
- 70% alcohol for 1 minute
- Harris Haematoxylin for 7 minutes
- Rinse in distilled water for 3 minutes
- 35% alcohol for 3 minutes
- 70% alcohol containing 15 drops of H_2SO4 conc. per 600mls solution.
- 50% alcohol containing 0.5% NaHCO3 (that is 3grams sodium bicarbonate per 600mls of 5% alcohol
- 95% alcohol, Eosin alcohol
- 100% alcohol for 2 changes (i.e. twice)
- Xylene for 2 changes (i.e. twice)
- Mount in Canada basam and cover with cover slip (Kiernan, 2008; Llewellyn, 2009).

APPENDIX III

Hirano-Zimmerman Methods for Nerve Cells

Fixation: 10% Buffered Formalin

Sections: Paraffin @ 12-15 microns

Staining procedure for Hirano-Zimmerman method:

1. Deparaffinize and hydrate to distilled water.

2. **Silver Nitrate, 10%** for 2 hours or longer.

3. Place sections directly into **Ammonia Water** for 3 minutes.

4. Place sections directly in **Formalin Solution** for 3 minutes. Tissue becomes black-brown. Rinse in distilled water, two changes.

5. Place sections again into **Silver Nitrate, 10%** for 3-5 minutes.

6. Repeat steps 3 and 4. Tissue becomes darker in color.

7. Tone in **Gold Chloride Solution** for 20 to 30 minutes.

8. Place sections directly into **Sodium Thiosulfate, 5%** for 1 minute. Rinse in distilled water.

9. Dehydrate in 95% alcohol, absolute alcohol and clear in **Xylene** two changes each.

10. Mounting

Staining Results: Nucleolus and nuclear membrane- Black

Neurofibrils, dendrites and axis cylinders -Black

Cytoplasm of astrocytes and the cytoplasmic membranes of macrophages-Gray.

APPENDIX IV

The Statistics of Spatial Learning and Memory of Wistar Rats during the First Run of Training

Descriptive

First Run

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	4	32.0000	6.73300	3.36650	21.2863	42.7137	24.00	40.00
Group II	4	24.0000	11.16542	5.58271	6.2333	41.7667	10.00	33.00
Group III	2	47.0000	.00000	.00000	47.0000	47.0000	47.00	47.00
Group IV	4	21.2500	18.39157	9.19579	-8.0151	50.5151	4.00	47.00
Group V	5	22.4000	4.39318	1.96469	16.9452	27.8548	19.00	30.00
Total	19	27.1053	12.41815	2.84892	21.1199	33.0906	4.00	47.00

ANOVA

First Run

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1173.839	4	293.460	2.565	.084
Within Groups	1601.950	14	114.425		
Total	2775.789	18			

Post Hoc Tests

Multiple Comparisons

First Run

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	8.00000	7.56389	.824	-15.5687	31.5687
	Group III	-15.00000	9.26384	.510	-43.8656	13.8656
	Group IV	10.75000	7.56389	.625	-12.8187	34.3187
	Group V	9.60000	7.17574	.674	-12.7592	31.9592
Group II	Group I	-8.00000	7.56389	.824	-31.5687	15.5687
	Group III	-23.00000	9.26384	.151	-51.8656	5.8656
	Group IV	2.75000	7.56389	.996	-20.8187	26.3187
	Group V	1.60000	7.17574	.999	-20.7592	23.9592
Group III	Group I	15.00000	9.26384	.510	-13.8656	43.8656
	Group II	23.00000	9.26384	.151	-5.8656	51.8656
	Group IV	25.75000	9.26384	.091	-3.1156	54.6156
	Group V	24.60000	8.94972	.096	-3.2868	52.4868
Group IV	Group I	-10.75000	7.56389	.625	-34.3187	12.8187
	Group II	-2.75000	7.56389	.996	-26.3187	20.8187
	Group III	-25.75000	9.26384	.091	-54.6156	3.1156
	Group V	-1.15000	7.17574	1.000	-23.5092	21.2092
Group V	Group I	-9.60000	7.17574	.674	-31.9592	12.7592
	Group II	-1.60000	7.17574	.999	-23.9592	20.7592
	Group III	-24.60000	8.94972	.096	-52.4868	3.2868
	Group IV	1.15000	7.17574	1.000	-21.2092	23.5092

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX V

The Statistics of spatial learning and memory of Wistar rats for the second run

Descriptive

Second Run

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	6	4.1667**	10.11270	4.12849	5.0540	26.2793	6.00	35.00
Group II	6	4.4000**	4.89898	2.00000	5.8588	16.1412	4.00	19.00
Group III	5	6.6100**	1.51658	.67823	2.5169	6.2831	3.00	7.00
Group IV	5	11.000**	3.50714	1.56844	2.2453	10.9547	3.00	10.00
Group V	6	17.5000**	6.15630	2.51330	11.0394	23.9606	11.00	24.00
Total	28	11.4286.	7.63243	1.44239	8.4690	14.3881	3.00	35.00

ANOVA

Second Run

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	693.624	4	173.406	4.536	.008
Within Groups	879.233	23	38.228		
Total	1572.857	27			

Post Hoc Tests

Multiple Comparisons

Second Run

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	4.66667	3.56967	.690	-5.8854	15.2187
	Group III	11.26667*	3.74390	.045	.1996	22.3337
	Group IV	9.06667	3.74390	.145	-2.0004	20.1337
	Group V	-1.83333	3.56967	.985	-12.3854	8.7187
Group II	Group I	-4.66667	3.56967	.690	-15.2187	5.8854
	Group III	6.60000	3.74390	.418	-4.4671	17.6671
	Group IV	4.40000	3.74390	.765	-6.6671	15.4671
	Group V	-6.50000	3.56967	.386	-17.0520	4.0520
Group III	Group I	-11.26667*	3.74390	.045	-22.3337	-.1996
	Group II	-6.60000	3.74390	.418	-17.6671	4.4671
	Group IV	-2.20000	3.91037	.979	-13.7592	9.3592
	Group V	-13.10000*	3.74390	.015	-24.1671	-2.0329
Group IV	Group I	-9.06667	3.74390	.145	-20.1337	2.0004
	Group II	-4.40000	3.74390	.765	-15.4671	6.6671
	Group III	2.20000	3.91037	.979	-9.3592	13.7592
	Group V	-10.90000	3.74390	.055	-21.9671	.1671
Group V	Group I	1.83333	3.56967	.985	-8.7187	12.3854
	Group II	6.50000	3.56967	.386	-4.0520	17.0520
	Group III	13.10000*	3.74390	.015	2.0329	24.1671
	Group IV	10.90000	3.74390	.055	-.1671	21.9671

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX VI

The Statistics of spartial learning and memory wistar rats for the third run of testing

Descriptive

Third run

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	6	3.1667	1.16905	.47726	1.9398	4.3935	2.00	5.00
Group II	6	5.6667	3.88158	1.58465	1.5932	9.7401	3.00	13.00
Group III	6	7.0000	4.06202	1.81659	1.9563	12.0437	4.00	14.00
Group IV	6	7.2000	2.48998	1.11355	2.1083	8.2917	3.00	9.00
Group V	6	9.6867	4.13118	1.68655	5.3313	14.0021	6.00	16.00
Total	30	6.1429	3.82694	.72322	4.6589	7.6268	2.00	16.00

ANOVA

Third run

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	137.129	4	34.282	3.053	.037
Within Groups	258.300	23	11.230		
Total	395.429	27			

Post Hoc Tests

Multiple Comparisons

Third run

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	-2.50000	1.93481	.698	-8.2193	3.2193
	Group III	-3.83333	2.02924	.351	-9.8318	2.1652
	Group IV	-2.03333	2.02924	.852	-8.0318	3.9652
	Group V	-6.50000*	1.93481	.021	-12.2193	-.7807
Group II	Group I	2.50000	1.93481	.698	-3.2193	8.2193
	Group III	-1.33333	2.02924	.964	-7.3318	4.6652
	Group IV	.46667	2.02924	.999	-5.5318	6.4652
	Group V	-4.00000	1.93481	.268	-9.7193	1.7193
Group III	Group I	3.83333	2.02924	.351	-2.1652	9.8318
	Group II	1.33333	2.02924	.964	-4.6652	7.3318
	Group IV	1.80000	2.11947	.912	-4.4652	8.0652
	Group V	-2.66667	2.02924	.685	-8.6652	3.3318
Group IV	Group I	2.03333	2.02924	.852	-3.9652	8.0318
	Group II	-.46667	2.02924	.999	-6.4652	5.5318
	Group III	-1.80000	2.11947	.912	-8.0652	4.4652
	Group V	-4.46667	2.02924	.215	-10.4652	1.5318
Group V	Group I	6.50000*	1.93481	.021	.7807	12.2193
	Group II	4.00000	1.93481	.268	-1.7193	9.7193
	Group III	2.66667	2.02924	.685	-3.3318	8.6652
	Group IV	4.46667	2.02924	.215	-1.5318	10.4652

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX VII

Effects of aluminium chloride on anxiety-related behaviour of wistar rats (Time spent on open arms of the elevated plus maze)

Descriptive

Open Arms

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	2	285.00***	7.07107	5.00000	221.4690	348.5310	280.00	290.00
Group II	2	265.00***	7.07107	5.00000	201.4690	328.5310	260.00	270.00
Group III	2	195.00***	7.07107	5.00000	131.4690	258.5310	190.00	200.00
Group IV	2	170.00***	14.14214	10.00000	42.9380	297.0620	160.00	180.00
Group V	2	130.00***	14.14214	10.00000	2.9380	257.0620	120.00	140.00
Total	10	209.00***	61.72520	19.51922	164.8445	253.1555	120.00	290.00

ANOVA

Open Arms

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	33740.000	4	8435.000	76.682	.000
Within Groups	550.000	5	110.000		
Total	34290.000	9			

Post Hoc Tests

Multiple Comparisons

Open Arms
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	20.00000	10.48809	.416	-22.0730	62.0730
	Group III	90.00000**	10.48809	.002	47.9270	132.0730
	Group IV	115.00000**	10.48809	.001	72.9270	157.0730
	Group V	155.00000***	10.48809	.000	112.9270	197.0730
Group II	Group I	-20.00000	10.48809	.416	-62.0730	22.0730
	Group III	70.00000**	10.48809	.006	27.9270	112.0730
	Group IV	95.00000**	10.48809	.001	52.9270	137.0730
	Group V	135.00000***	10.48809	.000	92.9270	177.0730
Group III	Group I	-90.00000**	10.48809	.002	-132.0730	-47.9270
	Group II	-70.00000**	10.48809	.006	-112.0730	-27.9270
	Group IV	25.00000	10.48809	.257	-17.0730	67.0730
	Group V	65.00000**	10.48809	.008	22.9270	107.0730
Group IV	Group I	-115.00000**	10.48809	.001	-157.0730	-72.9270
	Group II	-95.00000**	10.48809	.001	-137.0730	-52.9270
	Group III	-25.00000	10.48809	.257	-67.0730	17.0730
	Group V	40.00000	10.48809	.060	-2.0730	82.0730
Group V	Group I	-155.00000***	10.48809	.000	-197.0730	-112.9270
	Group II	-135.00000***	10.48809	.000	-177.0730	-92.9270
	Group III	-65.00000**	10.48809	.008	-107.0730	-22.9270
	Group IV	-40.00000	10.48809	.060	-82.0730	2.0730

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX VIII

Effects of aluminium chloride on anxiety-related behaviour of wistar rats (Time spent on closed arms of the elevated plus maze)

Descriptive

Close Arms

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	2	15.0000	7.07107	5.00000	-48.5310	78.5310	10.00	20.00
Group II	2	35.0000	7.07107	5.00000	-28.5310	98.5310	30.00	40.00
Group III	2	105.0000	7.07107	5.00000	41.4690	168.5310	100.00	110.00
Group IV	2	130.0000	14.14214	10.00000	2.9380	257.0620	120.00	140.00
Group V	2	170.0000	14.14214	10.00000	42.9380	297.0620	160.00	180.00
Total	10	91.0000	61.72520	19.51922	46.8445	135.1555	10.00	180.00

ANOVA

Close Arm

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	33740.000	4	8435.000	76.682	.000
Within Groups	550.000	5	110.000		
Total	34290.000	9			

Post Hoc Tests

Multiple Comparisons

Close arms

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	-20.00000	10.48809	.416	-62.0730	22.0730
	Group III	-90.00000**	10.48809	.002	-132.0730	-47.9270
	Group IV	-115.00000**	10.48809	.001	-157.0730	-72.9270
	Group V	-155.00000***	10.48809	.000	-197.0730	-112.9270
Group II	Group I	20.00000	10.48809	.416	-22.0730	62.0730
	Group III	-70.00000*	10.48809	.006	-112.0730	-27.9270
	Group IV	-95.00000**	10.48809	.001	-137.0730	-52.9270
	Group V	-135.0000***	10.48809	.000	-177.0730	-92.9270
Group III	Group I	90.00000**	10.48809	.002	47.9270	132.0730
	Group II	70.00000*	10.48809	.006	27.9270	112.0730
	Group IV	-25.00000	10.48809	.257	-67.0730	17.0730
	Group V	-65.00000*	10.48809	.008	-107.0730	-22.9270
Group IV	Group I	115.00000**	10.48809	.001	72.9270	157.0730
	Group II	95.00000*	10.48809	.001	52.9270	137.0730
	Group III	25.00000	10.48809	.257	-17.0730	67.0730
	Group V	-40.00000	10.48809	.060	-82.0730	2.0730
Group V	Group I	155.00000*	10.48809	.000	112.9270	197.0730
	Group II	135.00000*	10.48809	.000	92.9270	177.0730
	Group III	65.00000*	10.48809	.008	22.9270	107.0730
	Group IV	40.00000	10.48809	.060	-2.0730	82.0730

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX IX

Effects of Aluminium Chloride on Anxiety-Related Behaviour of Wistar Rats (Number of Faecal boli)

Descriptive

Faecal Boli

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	2	.0000**	.00000	.00000	.0000	.0000	.00	.00
Group II	2	1.5000**	.70711	.50000	-4.8531	7.8531	1.00	2.00
Group III	2	2.0000**	.00000	.00000	2.0000	2.0000	2.00	2.00
Group IV	2	3.0000**	.00000	.00000	3.0000	3.0000	3.00	3.00
Group V	2	3.5000**	.70711	.50000	-2.8531	9.8531	3.00	4.00
Total	10	2.0000**	1.33333	.42164	1.0462	2.9538	.00	4.00

ANOVA

Faecal Boli

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	15.000	4	3.750	18.750	.003
Within Groups	1.000	5	.200		
Total	16.000	9			

Post Hoc Tests

Multiple Comparisons

Faecal Boli
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	-1.50000	.44721	.095	-3.2940	.2940
	Group III	-2.00000*	.44721	.033	-3.7940	-.2060
	Group IV	-3.00000**	.44721	.006	-4.7940	-1.2060
	Group V	-3.50000**	.44721	.003	-5.2940	-1.7060
Group II	Group I	1.50000	.44721	.095	-.2940	3.2940
	Group III	-.50000	.44721	.793	-2.2940	1.2940
	Group IV	-1.50000	.44721	.095	-3.2940	.2940
	Group V	-2.00000*	.44721	.033	-3.7940	-.2060
Group III	Group I	2.00000*	.44721	.033	.2060	3.7940
	Group II	.50000	.44721	.793	-1.2940	2.2940
	Group IV	-1.00000	.44721	.299	-2.7940	.7940
	Group V	-1.50000	.44721	.095	-3.2940	.2940
Group IV	Group I	3.00000**	.44721	.006	1.2060	4.7940
	Group II	1.50000	.44721	.095	-.2940	3.2940
	Group III	1.00000	.44721	.299	-.7940	2.7940
	Group V	-.50000	.44721	.793	-2.2940	1.2940
Group V	Group I	3.50000**	.44721	.003	1.7060	5.2940
	Group II	2.00000*	.44721	.033	.2060	3.7940
	Group III	1.50000	.44721	.095	-.2940	3.2940
	Group IV	.50000	.44721	.793	-1.2940	2.2940

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX X

Effects of aluminium chloride on the the number of time on close arm entries of elevated plus maze.

Descriptive

Number of time on closed arm entries

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	2	4.000***	.00000	.00000	4.0000	4.0000	4.00	4.00
Group II	2	6.500***	.70711	.50000	.1469	12.8531	6.00	7.00
Group III	2	8.500***	.70711	.50000	2.1469	14.8531	8.00	9.00
Group IV	2	9.500***	.70711	.50000	3.1469	15.8531	9.00	10.00
Group V	2	11.000***	.00000	.00000	11.0000	11.0000	11.00	11.00
Total	10	7.900***	2.60128	.82260	6.0392	9.7608	4.00	11.00

ANOVA

Closed arms entries

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	59.400	4	14.850	49.500	.000
Within Groups	1.500	5	.300		
Total	60.900	9			

Post Hoc Tests

Multiple Comparisons

Number of time on closed arm entries

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	-2.50000*	.54772	.030	-4.6972	-.3028
	Group III	-4.50000**	.54772	.002	-6.6972	-2.3028
	Group IV	-5.50000***	.54772	.001	-7.6972	-3.3028
	Group V	-7.00000***	.54772	.000	-9.1972	-4.8028
Group II	Group I	2.50000*	.54772	.030	.3028	4.6972
	Group III	-2.00000	.54772	.070	-4.1972	.1972
	Group IV	-3.00000*	.54772	.014	-5.1972	-.8028
	Group V	-4.50000**	.54772	.002	-6.6972	-2.3028
Group III	Group I	4.50000**	.54772	.002	2.3028	6.6972
	Group II	2.00000	.54772	.070	-.1972	4.1972
	Group IV	-1.00000	.54772	.450	-3.1972	1.1972
	Group V	-2.50000*	.54772	.030	-4.6972	-.3028
Group IV	Group I	5.50000***	.54772	.001	3.3028	7.6972
	Group II	3.00000**	.54772	.014	.8028	5.1972
	Group III	1.00000	.54772	.450	-1.1972	3.1972
	Group V	-1.50000	.54772	.178	-3.6972	.6972
Group V	Group I	7.00000***	.54772	.000	4.8028	9.1972
	Group II	4.50000**	.54772	.002	2.3028	6.6972
	Group III	2.50000*	.54772	.030	.3028	4.6972
	Group IV	1.50000	.54772	.178	-.6972	3.6972

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX XI

Effects of aluminium chloride on the the number of time on open arm entries of elevated plus maze.

Descriptive

Number of time on Open Arms Entries

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	2	2.00***	.00000	.00000	2.0000	2.0000	2.00	2.00
Group II	2	4.00***	.00000	.00000	4.0000	4.0000	4.00	4.00
Group III	2	5.50***	.70711	.50000	-.8531	11.8531	5.00	6.00
Group IV	2	8.00***	.00000	.00000	8.0000	8.0000	8.00	8.00
Group V	2	9.00***	.00000	.00000	9.0000	9.0000	9.00	9.00
Total	10	5.70***	2.71006	.85700	3.7613	7.6387	2.00	9.00

ANOVA

Time on open arms entries

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	65.600	4	16.400	164.000	.000
Within Groups	.500	5	.100		
Total	66.100	9			

Post Hoc Tests

Multiple Comparisons

Time on open arms entries
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	-2.00000**	.31623	.008	-3.2685	-.7315
	Group III	-3.50000***	.31623	.001	-4.7685	-2.2315
	Group IV	-6.00000***	.31623	.000	-7.2685	-4.7315
	Group V	-7.00000***	.31623	.000	-8.2685	-5.7315
Group II	Group I	2.00000**	.31623	.008	.7315	3.2685
	Group III	-1.50000*	.31623	.026	-2.7685	-.2315
	Group IV	-4.00000***	.31623	.000	-5.2685	-2.7315
	Group V	-5.00000***	.31623	.000	-6.2685	-3.7315
Group III	Group I	3.50000***	.31623	.001	2.2315	4.7685
	Group II	1.50000*	.31623	.026	.2315	2.7685
	Group IV	-2.50000**	.31623	.003	-3.7685	-1.2315
	Group V	-3.50000***	.31623	.001	-4.7685	-2.2315
Group IV	Group I	6.00000***	.31623	.000	4.7315	7.2685
	Group II	4.00000***	.31623	.000	2.7315	5.2685
	Group III	2.50000**	.31623	.003	1.2315	3.7685
	Group V	-1.00000	.31623	.115	-2.2685	.2685
Group V	Group I	7.00000***	.31623	.000	5.7315	8.2685
	Group II	5.00000***	.31623	.000	3.7315	6.2685
	Group III	3.50000***	.31623	.001	2.2315	4.7685
	Group IV	1.00000	.31623	.115	-.2685	2.2685

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX XII

Effects of oral administration of aluminium chloride on brain aluminium uptake

Descriptive

Aluminium Uptake

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	2	26.00***	1.41421	1.00000	13.2938	38.7062	25.00	27.00
Group II	2	328.50***	12.02082	8.50000	220.4973	436.5027	320.00	337.00
Group III	2	782.50***	3.53553	2.50000	750.7345	814.2655	780.00	785.00
Group IV	2	903.50***	4.94975	3.50000	859.0283	947.9717	900.00	907.00
Group V	2	4249.50***	13.43503	9.50000	4128.7911	4370.2089	4240.00	4259.00
Total	10	1258.00***	1611.26720	509.52743	105.3689	2410.6311	25.00	4259.00

ANOVA

Aluminium Uptake

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.337E7	4	5841318.500	80237.891	.000
Within Groups	364.000	5	72.800		
Total	2.337E7	9			

Post Hoc Tests

Multiple Comparisons

Brain Aluminium Uptake
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	-302.500***	8.53229	.000	-336.7273	-268.2727
	Group III	-756.500***	8.53229	.000	-790.7273	-722.2727
	Group IV	-877.500***	8.53229	.000	-911.7273	-843.2727
	Group V	-4223.500***	8.53229	.000	-4257.7273	-4189.2727
Group II	Group I	302.500***	8.53229	.000	268.2727	336.7273
	Group III	-454.000***	8.53229	.000	-488.2273	-419.7727
	Group IV	-575.000***	8.53229	.000	-609.2273	-540.7727
	Group V	-3921.000***	8.53229	.000	-3955.2273	-3886.7727
Group III	Group I	756.500***	8.53229	.000	722.2727	790.7273
	Group II	454.000***	8.53229	.000	419.7727	488.2273
	Group IV	-121.000***	8.53229	.000	-155.2273	-86.7727
	Group V	-3467.000***	8.53229	.000	-3501.2273	-3432.7727
Group IV	Group I	877.500***	8.53229	.000	843.2727	911.7273
	Group II	575.000***	8.53229	.000	540.7727	609.2273
	Group III	121.000***	8.53229	.000	86.7727	155.2273
	Group V	-3346.000***	8.53229	.000	-3380.2273	-3311.7727
Group V	Group I	4223.500***	8.53229	.000	4189.2727	4257.7273
	Group II	3921.000***	8.53229	.000	3886.7727	3955.2273
	Group III	3467.000***	8.53229	.000	3432.7727	3501.2273
	Group IV	3346.000***	8.53229	.000	3311.7727	3380.2273

Multiple Comparisons

Brain Aluminium Uptake

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	-302.500***	8.53229	.000	-336.7273	-268.2727
	Group III	-756.500***	8.53229	.000	-790.7273	-722.2727
	Group IV	-877.500***	8.53229	.000	-911.7273	-843.2727
	Group V	-4223.500***	8.53229	.000	-4257.7273	-4189.2727
Group II	Group I	302.500***	8.53229	.000	268.2727	336.7273
	Group III	-454.000***	8.53229	.000	-488.2273	-419.7727
	Group IV	-575.000***	8.53229	.000	-609.2273	-540.7727
	Group V	-3921.000***	8.53229	.000	-3955.2273	-3886.7727
Group III	Group I	756.500***	8.53229	.000	722.2727	790.7273
	Group II	454.000***	8.53229	.000	419.7727	488.2273
	Group IV	-121.000***	8.53229	.000	-155.2273	-86.7727
	Group V	-3467.000***	8.53229	.000	-3501.2273	-3432.7727
Group IV	Group I	877.500***	8.53229	.000	843.2727	911.7273
	Group II	575.000***	8.53229	.000	540.7727	609.2273
	Group III	121.000***	8.53229	.000	86.7727	155.2273
	Group V	-3346.000***	8.53229	.000	-3380.2273	-3311.7727
Group V	Group I	4223.500***	8.53229	.000	4189.2727	4257.7273
	Group II	3921.000***	8.53229	.000	3886.7727	3955.2273
	Group III	3467.000***	8.53229	.000	3432.7727	3501.2273
	Group IV	3346.000***	8.53229	.000	3311.7727	3380.2273

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX XIII

Effects of Aluminium Chloride on Body weights of Both Sexes (Male and Female) of Wistar Rats

Groups	Mean Values(g)	N	Std Deviation	Std error mean
I Before	164.3000	10	24.46789	7.73743
I After	190.8000***	10	19.26886	6.09335
II Before	179.3000	10	40.92826	12.94265
II After	172.2000**	10	39.40897	12.46221
III Before	157.0000	10	38.95582	12.31891
III After	151.0000**	10	34.77228	10.99596
IV Before	174.1111	10	34.18130	11.39377
IV After	160.7778***	9	31.96004	10.65335
V Before	176.5556	10	40.16563	13.38854
V After	162.5556***	9	35.81240	11.93747

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX XIV

Effects of Aluminium Chloride on the Body Weights of Male Wistar Rats

Groups	Mean Values(g)	N	Std Deviation	Std error mean
I Before	177.0000	5	24.25902	10.84896
I After	197.8000**	5	19.35717	8.65679
II Before	200.8000	5	46.79423	20.92702
II After	191.0000*	5	46.96275	21.00238
III Before	167.6000	5	44.79174	20.03148
III After	160.2000*	5	41.88317	18.73072
IV Before	199.4000	5	16.41036	7.33894
IV After	185.2000*	5	11.07700	4.95379
V Before	208.0000	5	20.26080	9.06091
V After	190.0000**	5	20.29778	9.07744

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX XV

Effects of Aluminium Chloride on Body weights of Female Wistar Rats

Groups	Mean Values(g)	N	Std Deviation	Std error mean
I Before	151.6000	5	18.84940	8.42971
I After	183.8000***	5	18.39021	8.22435
II Before	157.8000	5	20.58397	9.20543
II After	153.4000	5	20.13206	9.00333
III Before	146.4000	5	33.57529	15.01533
III After	141.8000	5	27.47180	12.28576
IV Before	142.5000	5	18.92969	9.46485
IV After	130.2500**	4	17.98842	8.99421
V Before	137.2500	5	6.84957	3.42479
V After	128.2500	4	6.80074	3.40037

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX XVI

Effects of Aluminium Chloride on The Brain Weights of Wistar Rats

Descriptive

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group I	10	1.6560	.14901	.04712	1.5494	1.7626	1.45	1.92
Group II	10	1.5880	.07584	.02398	1.5338	1.6422	1.50	1.65
Group III	10	1.6270	.08166	.02582	1.5686	1.6854	1.50	1.72
Group IV	9	1.6460	.08553	.02705	1.5848	1.7072	1.52	1.76
Group V	9	1.6460	.03950	.01249	1.6177	1.6743	1.59	1.70
Total	50	1.6326	.09267	.01310	1.6063	1.6589	1.45	1.92

* P<0.05, ** P<0.01, *** P<0.001

ANOVA

Brain Weights

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.029	4	.007	.841	.506
Within Groups	.391	45	.009		
Total	.421	49			

Post Hoc Tests

Multiple Comparisons

Brain Weights

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	.06800	.04171	.487	-.0505	.1865
	Group III	.02900	.04171	.956	-.0895	.1475
	Group IV	.01000	.04171	.999	-.1085	.1285
	Group V	.01000	.04171	.999	-.1085	.1285
Group II	Group I	-.06800	.04171	.487	-.1865	.0505
	Group III	-.03900	.04171	.882	-.1575	.0795
	Group IV	-.05800	.04171	.637	-.1765	.0605
	Group V	-.05800	.04171	.637	-.1765	.0605
Group III	Group I	-.02900	.04171	.956	-.1475	.0895
	Group II	.03900	.04171	.882	-.0795	.1575
	Group IV	-.01900	.04171	.991	-.1375	.0995
	Group V	-.01900	.04171	.991	-.1375	.0995
Group IV	Group I	-.01000	.04171	.999	-.1285	.1085
	Group II	.05800	.04171	.637	-.0605	.1765
	Group III	.01900	.04171	.991	-.0995	.1375
	Group V	.00000	.04171	1.000	-.1185	.1185
Group V	Group I	-.01000	.04171	.999	-.1285	.1085
	Group II	.05800	.04171	.637	-.0605	.1765
	Group III	.01900	.04171	.991	-.0995	.1375
	Group IV	.00000	.04171	1.000	-.1185	.1185

* P<0.05, ** P<0.01, *** P<0.001

APPENDIX XVII

Nigerian Industrial Standard (NIS), 2007):

Nigerian Drinking Water Quality Standards for Aluminium and other elements in the consumables, as well, as their Health Impact)

Table 2 - Inorganic Constituents Parameter	Unit	Maximum Permitted	Health Impact	Notes
Aluminum (Al)	mg/L	0.2	Potential Neuro-degenerative disorders	Note 1
Arsenic (As)	mg/L	0.01	Cancer,	
Barium	mg/L	0.7	Hypertension	
Cadmium (Cd)	mg/L	0.003	Toxic to the kidney	
Chloride (Cl)	mg/L	250	None	
Chromium (Cr6+)	mg/L	0.05	Cancer	
Conductivity	µS/cm	1000	None	
Copper (Cu+2)	mg/L	1	Gastrointestinal disorder,	
Cyanide (CN-)	mg/L	0.01	Very toxic to the thyroid and the nervous system	
Fluoride (F-)	mg/L	1.5	Fluorosis, Skeletal tissue (bones and teeth) morbidity	
Hardness (as CaCO3)	mg/L	150	None	
Hydrogen Sulphide (H2S)	mg/L	0.05	None	
Iron (Fe+2)	mg/L	0.3	None	
Lead (Pb)	mg/L	0.01	Cancer, interference with Vitamin D metabolism, affect mental development in	

				infants, toxic to the central and peripheral nervous systems
Magnesium (Mg+2)	mg/L	0.20		Consumer acceptability
Manganese (Mn+2)	mg/L	0.2		Neurological disorder

Parameter	**Unit**	**Maximum Permitted**	**Health Impact**	**Notes**
Mercury (Hg)	mg/L	0.001		Affects the kidney and central nervous system
Nickel (Ni)	mg/L	0.02		Possible carcinogenic
Nitrate (NO3)	mg/L	50		Cyanosis, and asphyxia („blue-baby syndrome") in infants under 3 months syndrome") in infants under 3 months
Nitrite (NO2)	mg/L	0.2		Cyanosis, and asphyxia („blue-baby syndrome") in infants under 3 months
pH	-	6.5-8.5		None
Sodium (Na)	mg/L	200		None
Sulphate (SO4)	mg/L	100		None
Total Dissolved Solids	mg/L	500		None
Zinc (Zn)	mg/L	3		None

Note 1: Parameter to be monitored only if aluminium chemicals are used for water. References from: (i) Water Resources Act No 101 (1993), (ii) Standards Organisation of Nigeria (SON) Act- Retained as Cap412, (iii) Public Health Act (1958), (iv) Federal Environmental Protection Agency- Retained as Cap 131, (v) Federal Environmental Protection Agency (Amendment) Act (1992-No. 59) and (vi) Federal Environmental Protection Agency (Amendment) Act (1992-No. 14).