EFFECT OF ORAL ADMINISTRATION OF ALUMINIUM CHLORIDE ON THE HIPPOCAMPUS (BRAIN) OF WISTAR RATS

BY

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APPRECIATION

My sincere appreciation goes to the University Board of Research, Ahmadu Bello University, Zaria for the Sponsorship of this Research work.

DECLARATION

I BURAIMOH ADEBAYO ADEKUNLE hereby declare that this research work was carried out by me under the guidance and supervision of Professor J. O. Hambolu and Dr. S. S. Adebisi. References made of other investigators and other sources of information have been duly acknowledged. No part of this thesis has been submitted or accepted elsewhere for a higher degree or diploma.

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CERTIFICATION

This is to certify that this is an original work carried out by BURAIMOH ADEBAYO ADEKUNLE of registration number M.Sc /MED /31686 /2001 – 2002 under our care and supervision. This thesis meets the requirements and regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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This thesis is dedicated to God through my Saviour JESUS CHRIST and my beloved father, Late Pa ABOLARINWA BURAIMOH, I will ever remember you for the rest of my days.

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ABSTRACT

The study of the effect of oral administration of aluminium chloride on the Hippocampus of wistar rats was carried out in order to ascertain whether the small daily amount of aluminium that gain access to the body produce any accumulation damage to the hippocampus and if there is any association between drinking water containing aluminium and brain damage. This investigation was carried out using 50 female adult wistar rats. The animals were divided into five groups; 10 rats per group. Stock solution of aluminium chloride was prepared (2g/l or 2mg/ml).Different concentrations of aluminium was administered to different groups orally. Group I was control, while Groups II– V were given 2.3mg/kg(0.4mg),5.7mg/kg(1mg),11.4mg/kg (2mg),and17.1mg/kg (3mg) respectively with an average weight of between 150-200g for duration of twelve (12) weeks. Blood was collected from the tails of the rats for haematological indices. The animals were sacrificed using chloroform and then the brain tissues were fixed immediately in Bouin's fluid. The hippocampus sections were processed through the routine tissue processor. The stained samples were examined by means of light microscope for histological changes. Histological examination showed clumping of cell neurons, or reduced pyramidal cells and scanty neurofibrillary tangle which was an indication of neurodegeneration in the treated groups and which may suggest or tend to link accumulation of aluminium on brain cells to incidence or aetiology of Alzheimer's disease .The haematological results showed that the differences in parked cell volume, white blood cell and neutrophils of the control and experimental groups were statistically significant at $P<0.05$. The increase in PCV, neutrophil and WBC observed in this study could be as a result of the destruction of the hippocampal cells due to oral administration of aluminium chloride. It was however, concluded that the oral administration of aluminium

chloride could induce brain damage which may possibly impair memory and learning as seen in Alzheimer disease.

Chapter 1

INTRODUCTION

Aluminium is present in small amounts in mammalian tissues; research studies to establish its specific positive functional effect to the mammalian body is yet to be ascertained (Beavon, 2004). 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 (Parkinson *et al*., 1981). 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 Chloride according to Wells (1963), is used as a catalyst in the process of Friedel crafts(dye making process).According to him,it has an electron deficient molecule forming only 3 bonds and has no lone pairs. The catalyst acts as an electron acceptor for a lone pair on the halide atom. It is widely used in other manufacturing of petrochemicals such as alkylbenzene, ethylbenzene, alkylarylkertone, ethylchloride (Greenwood, 1990). It is also used in the manufacturing pharmaceuticals, dyes intermediates and other organic chemicals such as anthraquinone, phthalocyanines, acetophenone, buytl rubber, phenylethyl alcohols (Lide, 1996). It is used as a nucleus inhibitor in the production of titanium dioxide (West, 1999).

Aluminium Chloride is also used in the production of aluminium, in the metallurgical industry and as a flux in aluminium smelting, in production of rubber, lubricants and wood preservatives and in cosmetics as an astringent; active ingredient in antiperspirants (Cotton *et al.*, 1999).

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 (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 aircraft production, 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 (Net Doctor, 2004).

Frank (2006),describing Aluminum toxicity in U.S.A.stated that “from the earliest days of food regulation, the use of alum (aluminum sulphate) in foods has been condemned. It is universally acknowledged as a poison in all countries. If the Bureau of chemistry, U.S.A. had been permitted to enforce the law, no food product in the country would have any trace of any aluminium. No soft drink would contain caffeine: no bleached floor would be in interstate commerce. Our food and drugs would be wholly without adulteration and the health of our people would be vastly improved and their life greatly extended”.

Dr. Wiley in U.S.A vehemently resisted crime against food laws, was the prime mover behind the original pure food law and Director of Food and Drug Administration (FDA) in 1929, and resigned in disgust in 1912 over exceptions granted to the law and lack of enforcement particularly on aluminium where aluminium has been exempted from testing for safety by the FDA under a convoluted logic wherein it is classified as GRAS (Generally Regarded as Safe). It has never been tested by the FDA on its safety and there are no restrictions whatever on the amount or use of Aluminium (Wiley, 1975).Frank also noted that 4 ppm of aluminium in human blood can cause it to inhibit learning (Frank, 2006.)

The functions of certain learning and memories have been associated with different areas of the brain like the hippocampus prefrontal cortex and cerebellum. Hippocampus is associated with memory of new words, faces, places and event. Cerebellum has been associated with the memory of learning new skills like playing an instrument etc while the prefrontal cortex is linked with retention of mental image and delayed response (Brodal, 1992).

Williams (1992), opines that aluminium compounds 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 (Williams, 1992). 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” (Tam and Williams, 1986). 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 (Mclachlan *et al.*, 1991b). Aluminium has also been implicated as a toxic agent in aetiology of Alzheimer's disease and parkinsonism-dementia (Hewitt *et al.*, 1990). It is argued that glutamate and aspartate complexes of Aluminium (Al^{3+}) play roles in Alzheimer's diseases and other Aluminium related conditions (Deloncle and Guillard, 1990).

1.1 Significance of the Study

This study becomes important in order to ascertain whether the small daily amount of aluminum chloride that gains access to the body produce any accumulated damage to brain tissues (hippocampus) of wistar rats.

1.2 Objectives of the Study Include the Following

a. To determine the relationship between drinking water containing Aluminium chloride and hippocampus damage of wistar rats by looking for histological changes in the hippocampus.

b. To compare the haematological indices of the treated and control of wistar rats.

Chapter 2

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, Trichloroaluminum. 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 Al 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 in China and found to have a coagulating effect as good as 2-3 times of traditional aluminium salt (Zhang, 1998). 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;

- 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 effect 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 (Zhang, 1998). Considering the practice of liquid Aluminium chloride used in Shenzhen (China), 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. (Wenbin *et al*., 2002).

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. 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). More than 100 toxic of aluminium have been identified and many are damaging to the human brain (Mclachlan *et al*., 1991b).

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) and 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 plaques and ultimately the mental deterioration known as AD (Versieck and Cornelis, 1980). However, there are at least two recognized synergistic ways that these factors contribute to brain damage (Gonatas, 1967).

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 plaques 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 plaques neurofibillary tangles (Flaten and Odegard, 1989).

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 (Sorenson et al., 1974; Fulton and Jeffery, 1990.).

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 (Pennington, 1987).

2.1 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.

Table 2.1: 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)

2.2 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 less than five, the amount of soluble aluminium in water tends to increase (Jones and Benneta, 1986).

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 less than < 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 of naturally present in water. However, the median level of aluminium in the finished water samples studied was very low (less than < 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 variables.One possible confounder is silicon. Waters that are high in aluminium tend to be low in silicon, and vice versa (Birchall, 1992).

2.3 Food Additive as a Source of Aluminium

Food additives are major sources of dietary aluminium in the United States, although aluminium containing additives are present in only a limited number of foods (Greger 1985, Pennington 1987). Variability in the usage of these foods makes it very difficult to estimate aluminium intake from these sources.

In 1982 approximately four million pounds weight of aluminium was used in food additives in the United States (Committee on food Additives survey data 1984). This means that the average US citizen theoretically consumed 21.5 mg aluminium daily in food additives (Gregger, 1985). The use of industrial production figures in this manner tends to over estimate intakes. However, calculation based on standardized menu such as those used in the total diet study of the food and drug administration (FDA) may underestimate the use of certain processed foods, such as processed cheese which can contain very high levels of aluminium.

The data compiled by the committee on the GRAS list survey Phase II (1979) indicated that individual usage of aluminium containing food additives varied greatly. About 5% of adults in the United States consumed more than 95 mg aluminium in food additive daily; 50% consumed 24 mg or less aluminium daily in food additives.

According to Pennington and Jones (1989), the three foods that contributed the most aluminium in the total diet study menu were processed American cheese, home made cornbread, and yellow cake with white icing. Food additives were the major source of aluminium in these three foods. Only 9-15g of each of these foods was included in the standardized menu. Individual consuming standard servings of processed cheese (23g), cornbread (45g) and cake (60g) would consume proportionally more aluminium from additives.

2.4 Unprocessed Foods as Source of Aluminium

Most foods, except for herb and tea leaves, contain less than $5\,\mu g$ aluminium per gram. Furthermore only small quantities of herbs are consumed by most individuals

and most of the Aluminium in tea leaves is insoluble. Thus most individuals consume only 1 to 10 mg of aluminium from natural sources daily (Versiech and Cornelis, 1980).

2.5 Packaging and Utensils as Sources of Aluminium

Many foods accumulate statistically significant amounts of aluminium when cooked or stored in aluminium pans, trays or foil as compared to similar batches of processed in stainless steal container. However, most foods accumulate less than 20 μg /Al /g food during preparation and storage (Greger, *et al.*, 1985).

Several factors appear to influence the accumulation of aluminium by foods cooked in aluminium pans, such as pH, length of cooking period and the use of new pans or pressure cookers (Greger *et al.*, 1985, Inove *et al.*, 1988). Thus, investigators have found that tomato sauces cooked for several hours in aluminium pans accumulated 3-6 mg aluminium per 100 g serving and Chinese noodle accounted 2.6 mg aluminium per serving.

Organic acids, including citric acid and copper in foods may increase the solubilization of aluminium from pans and foil (Baxter *et al.*, 1988, Flatten and Odegard 1989, Ellen *et al.*, 1990). The addition of fluoride increased slightly the amount of aluminium leached from pans when citric acid solution was heated in the pans. However, this effect of fluoride was not observed when fluoride was added to acidic foods (Savory *et al.*, 1987, Baxter *et al.*, 1988).

2.6 Pharmaceutical Products as Sources of Aluminium

The typical quantities of aluminium consumed in food and beverages amount to less than 1% of the quantities consumed in pharmaceutical agents such as antacids, buffered analgesic, anti-diarrhoeal agents and certain anti-ulcer drugs (Lione, 1983; 1985). Lione (1985) estimated that 126-728 mg and 840-5000 mg were possible daily doses of aluminium in buffered analgesics and antacids, respectively.

According to the committee on Nutrition, American Academy of paediatrics (1986), aluminium containing phosphate binding gels have been used for years to treat hyperphosphataemia in patients with chronic renal failure. Unfortunately, very high doses (100 mg /kg /day) were needed to control hyperphosphetaemia in some paediatric cases, and symptoms of aluminium-associated bone diseases were sometimes observed. Thus, the use of these aluminium containing pharmaceutical agents is now discouraged among children with chronic renal failure.

Of special concern are parenterally delivered pharmaceutical because the protective barrier of the gut has been bypassed (Greger, 1987). Several substances administered intravenously including albumin and calcium and phosphate salts, can contain significant quantities of aluminium (Sedman *et al*., 1985). Parenterally administered products intended for repeated use should be tested for aluminium contamination and patients, especially those with impaired renal function should be monitored for aluminium intoxication (Hoieberg, 1989).

2.7 Infant Formulae

According to (Koo *et al*., 1988, McGraw *et al*., 1986, Simmer *et al*., 1990) of recent a number of investigators have become concerned about the aluminium content of infant formulae. The Aluminium content of human or cow's milk is negligible (< 0.05 µg/ ml) Koo *et al.*, 1988). Dabeka and McKenzie (1990) reported that ready to use milk-based and soy-based formulae contain 0.01-0.36 and 0.4-6.4 µg Al/ g, respectively. Thus 1-3 month old infants consuming certain soy-based formulae could take in as much as 2.1 mg Al daily, whereas infants fed human or cow's milk would consume only 3 µg Al daily. The infants prone to the risk would be preterm infants with impaired renal function because they would be less able to excrete absolved aluminium (Greger, 1987; Koo *et al*., 1988).

2.8 Bioavailability of Aluminium

The potential negative effects of exposure to aluminium reflect not only the dose, but also the percentage of the dose that is absorbed and retained. The effectiveness of the gut as a protective barrier is illustrated by the observation that only 0.01-0.05% of oral doses of aluminium appears to be retained in rats (Ecelbarger and Greger, 1991).

The bioavailability of aluminium is dependent on the total dietary milieu, not just on the chemical form in which aluminium is ingested (Dayde *et al*., 1990; Greger, 1988). For example, the elevation of dietary calcium levels has been found to decrease the retention of aluminium in bone in rats (Ecelbarger and Greger, 1991). Citrate is another dietary factor that can influence aluminium retention in tissues.

Investigators have reported that citrate increased the tissue retention of aluminium in animals that were gavage fed aluminium daily (Slanina and Falkerborn, 1984; Slanina *et al*., 1985) or given Aluminium in their drinking water (Fulton and Jeffery, 1990).

Excelbarger and Greger (1991) demonstrated that the addition of citrate (10-31 μmol/ g diet) to the diets of rats increases their zinc absorption and the retention of aluminium in their bones. Their data suggest that citrate decreased the pH in the gut and increased the solubility of trace elements such as zinc and aluminium, in the gut. This increased solubility resulted in greater absorption. Moreover, in a second study, rats with one kidney removed and reduced kidney function were more sensitive to the effects of dietary citrate on aluminium retention than intact rats. These modest but consistent effects of dietary citrate on aluminium retention are probably important in practical situation. Americans are estimated to consumed 4g of preformed citrate in foods daily.

The assessment of aluminium exposure is complex. Scientists need to define the dietary, physiological and pathological factors that affect the absorption and retention of dietary aluminium, because any assessment of dietary aluminium exposure is incomplete if it does not include an assessment of the bioavailability of aluminium (Flemming, 1987).

Little is known about the relative bioavailability of aluminium from different dietary sources and it is possible that the aluminium in drinking water makes a disproportionate contribution to the amount absorbed from the gastrointestinal tract. Despite these uncertainties about bioavailability, studies of the risk of Alzheimer's

disease associated with the use of aluminium containing products are of great interest (Frank, 2006).

2.9 Epidemiology of Alzheimer's Diseases in Relation to Aluminium

Many aluminium salts and compounds are used in a wide variety of industries. In 1982, 4 x 10^6 lb of aluminium were used in food additives in the USA (Greger, 1988); the amount of aluminium salts used in water treatment in the UK is of the order of 10^5 tonnes per year.

When aluminium becomes available to organisms through the acidification of surface waters, it is toxic to plants, affecting roots development (Taylor, 1988) and to fish, affecting gill function (Driscoll *et al*., 1980). Its toxicity to human was first clearly recognised in renal medicine, when the element was identified as the causal agent in the neurological and bone disorders observed in patients dialysed with aluminium-containing water (Kerr *et al*., 1992). The use of reverse osmosis and deionization has removed this problem, although the continued use of oral aluminium hydroxide as a phosphate binding still produces aluminium overload in some patients (Flemming *et al*., 1982).

According to Roth *et al*. (1984), in dialysis induced aluminium overload (when plasma aluminium level can rise from less than 1 μmol /litre to above 5 μmol /litre) Aluminium accumulates in many tissue, including kidney, liver, skeletal muscle, heart, brain and bone. The cellular toxicity of aluminium is undoubted, but the mechanisms of its toxicity are not fully understood. The question of current interest is whether exposure to environmental aluminium in food, water, medication and in other

ways is a causal agent in various diseases, in particular Alzheimer's disease. The daily human intake of aluminium is estimated to be about 20 mg (Jones and Bennett, 1985) and absorption is influenced inter alia by dietary constituents such as citrate and maltol.

However, Epidemiological studies relating aluminium in water to the incidence of Alzheimer's disease (Martyn *et al*., 1989; Martyn, 1992) raise several problems, notably a lack of correlation between dose and effect and the fact that the intake of aluminium from water is but a fraction of that from foods water that contains significant amounts of aluminium (Soft waters and that from upland areas) contain little silicic acid whereas water high in silicic acid (hard) contain little aluminium,. The epidemiological studies therefore suggest an inverse relationship between silicon in water and Alzheimer's disease, with a high silicic acid intake restricting the absorption of aluminium from food (Birchall and Chappell, 1989)

The possible relationship between aluminium and Alzheimer's disease was first suggested by the demonstration of neurofibriliary degeneration in rabbits after direct exposure of central nervous system to aluminium salt (Klatzo *et al*., 1965). Subsequently, increased aluminium level were found in the brains of Alzheimer's disease patients; later came the identification of Aluminium in tangle-bearing hippocampal neurons (Perl and Brody, 1980; Perl and good, 1992) and at the core of senile plaques (Candy *et al*., 1986; Edwardson *et al*., 1992).

2.10 Relationship between Alzheimer's Disease and Aluminium in Drinking Water

Two surveys undertaken in Norway in the early 1980s compared geographical variation in mortality with number of measured variables in drinking water (Vogt, 1986; Flaten, 1990). Many of the data used were common to both surveys and the conclusion reached by the investigators did not differ very much. One of the few statistically significant correlations revealed by this study was between concentrations of aluminium in water and mortality from dementia. There are, however, as the investigator frankly acknowledged, some problems in interpreting this result. The association observed was between aluminium concentration and dementia in general rather than Alzheimer's disease as a specific cause of dementia (Kidman, 1979).

In England and Wales, unlike in Norway, most of the aluminium in water is derived not from natural sources but from treatment processes. The highly coloured water from upland catchment is often treated with aluminium sulphate in order to remove colour and particulate matter. Such waters are soft and contain little natural buffer. Their pH varies considerably over short periods of time. Because the solubility of aluminium in water is highly dependent on pH, high concentrations of aluminium may pass into supply. A survey of England and Wales exploited the geographical variation in the type of water (and hence its aluminium content) supplied to different parts of these countries to investigate a possible association between rates of Alzheimer's disease and the concentration of aluminium in drinking water (Martyn *et al.*, 1989).

Incident cases of Alzheimer's disease, other dementing illnesses and late onset epilepsy in people between ages of 40 and 70 years were identified from the records

of neuroradiology centres. Seven areas of the country were included; South Wales, East Anglia, Hampshire, Merseyside, Northumbria, Nottinghamshire and Devon and Cornwall. In all of these areas the incidence of Alzheimer's disease, other forms of dementis and epilepsy was estimated for each of the country districts within the locality served by the CT scanner. These estimates of incidence were age-standardized and adjusted for the distance that patients had to travel to reach the neurological centre and for differing availability of CT scans in different parts of the country. The mean aluminium concentration of the water supplied to the country district for ten 10 years prior of the survey was obtained from records made available by the local water companies and water authorities. The 88 country districts within the study where divided into five groups according to their water aluminium concentrations. Relative risks for probable Alzheimer's disease, possible Alzheimer's disease, other causes of dementia and late onset epilepsy where shown with their associated 95% confidence intervals. The risk of probable Alzheimer's disease was raised in districts in which water aluminium concentration exceed 0.01 mg /l when compared with districts where the water aluminium concentration was lower. When the analysis is restricted to the subgroup of cases under the age of 65 years, a stronger relationship between water aluminium concentration and risk of probable Alzheimer's disease is seen (see table below).

Table 2.2: Relative Risks (95% Confidence interval) of Alzheimer's disease, dementia from other causes and epilepsy in UK patients lagged 40-64 (risk adjusted from distance form CT scanning unit and CT scanning rate)

Aluminium concentration mg /l	Probable Alzheimer's disease (n=307)	Possible Alzheimer's disease (n=153)	Other causes of dementia (n=372)	Epilepsy (n=2461)
0-0.01	1	1	1	1
0.02-0.04	1.4 (1.0-2.2)	0.9 (0.5-1.5)	1.2 (0.8-1.7)	1.0 (0.8-1.1)
0.05-0.07	1.4 (1.0-2.2)	1.1 (0.7-1.8)	1.1 (0.8-1.6)	0.9 (0.8-1.1)
0.08-0.11	1.6 (1.0-2.5)	0.6 (0.3-1.2)	1.2 (0.8-1.8)	1.0 (0.9-1.2)
>0.11	1.7 (1.1-2.7)	0.9 (0.5-1.6)	1.2 (0.8-1.8)	1.0 (0.8-1.1)

Source: Martyn *et al.*, (1989)

A French group subsequently attempted to replicate these findings in Aquitaine (Michael *et al.*, 1991). They used data collected in a population – based study of ageing and showed that prevalence rates of Alzheimer's disease correlated positively with aluminium concentrations in water (Table 2.3 below). The number of cases from which these ratios were calculated was small but the results were statistically significant.

Table 2.3: Prevalence Percentage of dementia among those 65 years and over in areas of Aquitaine according to aluminium concentration in drinking water.

	Aluminium Concentration (mg /l)			
	0.01	0.02-0.04	0.05-0.07	≥ 0.08
Probable Alzheimer	0	1.2	1.6	5.8
Possible Alzheimer	0	0.3	0.6	1.3
Other dementia	0	0.4	1.4	0.6
Not dementia	100	98.1	96.4	92.3
Total	147	1984	506	155

(Modified from Michel *et al*., 1991)

According to Neri and Hewit (1991), an association between Alzheimer's disease and aluminium in drinking water had been found in a Canadian case-control study. The methods of this study have only been given in outline: cases of Alzheimer's disease and presenile dementia were identified from discharged records of general hospitals in the province of Ontario and matched by age and by sex to controls with a non-psychiatric diagnosis. Aluminium exposure was estimated from water quality surveillance data corresponding to the locality of place of residence of the subject.

2.11 Aluminium and Pathogenesis of Alzheimer's disease (AD)

There is a now a general agreement that aluminium is both highly neurotoxic in experimental models and present in toxic concentrations in affected brain regions in Alzheimer's disease (AD) (Price, 1986). In brain biopsies containing neuro-fibrillary tangles from early AD and AD-affected tissue from advanced terminal disease at post-mortem aluminium where found in bulk neocortical grey matter (Crapper *et al*., 1973,

1976; Trapp *et al*., 1978; Yoshimasu *et al.*, 1981; Ward and Mason, 1986). Aluminium had been reported to be elevated in five AD-affected cell or tissue compartments: In the nucleus (Crapper *et al*., 1980), on dinucleosomes enriched in repressed (untranscribed) genes (Lukiw *et al.*, 1991); in neurofibrillary tangle (Perl and Brody, 1980, Perl and Pendlebury, 1984); in the core of senile plaques (Duckett and Galle, 1976; Masters *et al.*, 1985, Candy *et al*., 1986, Edwardson and Candy, 1990). And in pia blood vessels (Crapper *et al*., 1976). Two laboratories failed to find elevated concentrations of aluminium in AD-affected tissue (McDermott *et al.*, 1979; Markesbery *et al.*, 1981). In the former study, failure to defect increased amounts of Aluminium may have been related to the sample size and the criteria for case selection for both controls and AD subjects (McLachlan *et al*., 1980). In the latter report (Markesbery *et al*., 1981), the failure to defect Al may be related to the difficulty in using certain nuclear reactors for neutron activation analysis applied to the measurement of aluminium (Krishnan *et al*., 1987). Instrumental neuron activation is based on the thermal neutron reaction $^{27}\mathrm{Al}(\eta\gamma)^{28}\mathrm{Al}$. Without pro irriadation separation, the assay of aluminium in tissue is difficult and often inaccurate because of the interfering isotopes: ($^{31}\mathrm{P}(\eta,\alpha)^{28}\mathrm{Al}$) reaction from fast neutrons generated in most of the conventional reactors generally used for biological assays. In plain language, phosphorus in most reactors decay to ^{28}Al. since the concentration of phosphorus is about 12,000 times higher than that of aluminium in biological specimens; it is often difficult to measure low concentrations of naturally occurring Aluminium accurately by this method.

While most workers agree that elevated aluminium concentrations are present in AD-affected brain, the precise role of aluminium in the degenerative process of AD is controversial (Boutton, 1992). Aluminium by itself does not induce AD-type neuro-

fibrillary tangles compose of paired helical filaments (PHFS), or neuritic plaques with amyloid cores (Kidd, 1963).However, aluminium is a 'dementing ion', capable of inducing changes in learning and memory and possibly contributing to the dementia of AD (McLachlan *et al*., 1991a). Aluminium does induce some biochemical changes which may contribute to the production of AD type tangles and amyloid accumulation. Reports strengthen this possibility. In evaluating the role of this environmental agent in AD, Flatten and Garruto (1991) have argued that taking all the evidence into account, Al neurotoxicity does fulfil the criteria put forward by Sir Austin Bradford Hill (1965) to evaluate the cause and effect relationships between a disease (AD) and a potential causative factor (Al). Hill suggested eight (8) criteria which include:

(a) Strength of Association. A statistically significant association between the concentrations of aluminium in drinking water and the number of cases of AD evaluated by epidemiological techniques, has been shown in five countries and in eight studies (Martyn, 1992). The relative risk of AD in geographical regions characterized by high aluminium concentrations ranges between 1.5 and 4, when compared to regions with low aluminium concentration in drinking water. A survey conducted in Ontario by Neri and Hewitt (1991) is perhaps the most convincing, for several reasons:

   i. A significant association was found for processed water but not raw intake water.

ii. The diagnosis of probable AD was taken as the hospital discharge diagnosis (2344 AD patients who were matched for age, sex and place of residence to 2233 randomly selected non-psychiatric controls). Ontario has universal health care and all resident have equal access to the appropriate diagnostic facilities and laboratory tests required for the accurate diagnosis of AD. Second opinions by specialists are frequent and the diagnostic criteria for AD proposed McKhann *et al*., (1984) are widely used. Thus the accuracy of the clinical diagnosis of AD was likely to be high.

iii. The study was case controlled.

iv. The aluminium, concentration in water was taken as the average for a 12-month period as measured and published by the Ontario Ministry of the Environment. This procedure greatly reduced the uncertainty concerning annual variation in Al concentration in the finished drinking water (Yuping, 1998).

v. The postal code used to give the place of residence in this study also gives precise information about the water source of drinking water, thereby reducing the uncertainty about the source.

vi. There was a clear dose-response effect for aluminium in this study. Importantly, an epidermiological study from Zurich failed to find a relation between cognitive impairment and the aluminium content of drinking water (Wettstein *et al*., 1991). The reason for this apparent

absence of a relation had not been investigated, but it could be related to the presence of naturally occurring protective factors. For instance, according to a study on population of 77 year old males in Ontario by Forbes *et al*. (1991), those persons who show no indication of impaired mental functioning were more likely to have resided in areas where the aluminium concentrations in drinking water were relatively low and where fluoride concentrations in drinking water are relatively high. This study suggested that fluoride may protect against a toxic effect of aluminium. Silicon may also protect against Al neurotoxicity and may be a confounding factor in epidemiological studies that would reduce the estimated risk (Birchall, 1992).

(b) Consistency. Although analytical difficulties remain elevated levels of Al in brain tissue from AD patients have been reported by at least 10 laboratories employing six different analytical techniques on tissues obtained from various geographical localities in four continents (McLachlan, 1986) and this appeared to be a consistent finding.

(c) Biological gradient. A clear dose-response gradient had been observed in epidemiological studies from Norway (Vogt, 1985; Flaten, 1990) and Canada (Neri and Hewitt, 1991).

(d) Specificity. Elevated brain aluminium content occurred after prolonged renal failure requiring dialysis treatment. However, aluminium did not accumulate in brain diseases other than AD as a result of non-specific tissue damage (Traub *et al*., 1981). Epidemiological studies indicate that several other neurological

disorders, such as epilepsy, Parkinsonism, amytrophic lateral sclerosis and medical diseases predisposing to multi-infarct dementia, were not associated with increased concentrations of Al in drinking water.

(e) Temporality. Humans were exposed to aluminium over a lifetime and there was no known method for detecting when brain aluminium levels become elevated in living people for aluminium to become a risk. For aluminium to become a risk factor for AD, one would suppose that either the blood brain barrier to aluminium becomes defective very early in the natural history of this disease or aluminium was a contributing factor to the release of the aetiological 'causal' factors responsible for the disease in a cell nuclei from temporal lobe, the mean aluminium content of dinucleosomes extracted from control brains free of neuro-fibrillary degeneration was 518 μg Al/ g DNA (mean age 79 years, n = 7), compared with 2850 μg Al/ g in cell nuclei from temporal lobes of AD patients (16 AD brains, mean age 79.8 years) (P = 0.0001 on analysis of variance) (Lukwi *et al*., 1991). The observation that aluminium treated human neurons in tissue culture develop epitomes suggests that aluminium acts early in the development of AD neurofibrillary tangles (Guy *et al*., 1991)

(f) Coherence. The progression of neurological signs after a single intracranial injection of an Al dose lethal to 50% of animals did not seriously conflict with the generally known facts about the naturally history of AD, although the time course is much slower. Indeed, the clinical course of the experimental model more closely reproduces AD than the encephalopathy associated with dialysis in renal failure. After Al injection, cats and rabbits remained asymptomatic by

behavioural and electro-physical criteria for seven (7) to 15 days. Cats first exhibit selective deficits in performance of tasks measuring short term memory and impairment in motor control after jumping. Several days later there was evidence of defects in motor control required for retrieving food and the progressive development of an increased 'lead pipe' type of tone in limb and truncal muscles. These animals might survive for over three years with chronic neurological damage associated with the impaired acquisition of tasks measuring new learning. An aluminium induced chronic neurodegenerative condition in rabbits which was not accompanied by seizures had been developed (Struble *et al.*, 1985). This aluminium-induced neurobehavioural and motor control deficits were similar in character and sequence of progression to those found in AD, although the time course was much shorter (Terry and Davis, 1980).

(g) Plausibility. Alzheimer's disease occurred in all population throughout the world who survived to the age of risk. It was therefore plausible that a common environmental toxic agent might contribute to the AD degenerative process. At the chemical level, Al affected several enzymes and biomolecules relevant to AD; it was toxic to many species by several routes of exposure and it was present in several subcellular structures. Al also altered protein kinase activity and results in altered phosphorylation of cytoskeletal proteins which may be important in the formation of AD-type neurofbrillary degeneration (Jope and Johnson, 1992).

(h) Experimental intervention. A treatment trial employing a trivalent metal ion chelating agent, desferrioxamine, indicated that lowering the body and brain Al content retarded the progression of Alzheimer's disease (McLachlan *et al*., 1991b). This was the strongest observation in support of causative relationships.

A randomized two-year prospective, single-blind clinical trial was conducted to determine whether the sustained use of low dose dose desferrioxamine would slow the progression of the dementia (Mclachlan *et al*., 1991b). A total of 48 people living at home in the early stages of the illness were randomly assigned to three treatment groups: desferrioxamine (125 mg injected intramuscularly twice daily, five days per week, for two years), Lecithin (oral) dose 1.0 g /day and no treatment. A structured performance test measuring daily living skills was video-taped in the home and was taken as a reliable measure of outcome over the two-year initial observation period. The tapes were analysed at random by trained behaviour raters who were unaware of the purpose protocol of the study. There was no statistical difference in the average rate of decline in performance between the group receiving lecithin and the untreated group. However, the average two year decline in the desferrioxamine infected group was both practically and statistically significantly slower than that observed for the combined untreated and lecithin-treated groups, designated as 'no-treatment' group (McLachlan *et al*., 1991a).

The effect appears to be due to aluminium, because an analysis of the trace metal content of brains of patients from a previous study who died with advanced AD while receiving desferrioxamine has demonstrated that aluminium, become the only trivalent metal that had been removed from the brain after prolonged treatment. This

clinical trial strongly supported the conclusion that aluminium was indeed an important factor in the complex series of event associated with Alzheimer Disease (AD) (McLachlan et al 1991a).

2.12 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive disease that destroys the mind with forgetfulness in early stages, followed by inability to communicate and provide self-care. AD is the most common cause of dementia in Western civilization. It affected more women than men, and the clinical course generally lasts approximately five years. The younger the individual is at the onset of the disease, the more severe the deficits for patient. One famous contemporary who suffered from the disease was former US President Ronald Reagan. (http://www.sci.uidaho.edu/med532/alzheimer:htm 2006

The cerebral cortex and some other forebrain regions atrophy so severely that the brain may weight less than 1000g at death. Shrinkage was most pronounced in the frontal and temporal lobes. The insula and the medial part of the temporal lobe tended to demonstrate the highest number of plaques. The disease often caused vacuolization of the subpial layers of the temporal and parietal lobes. The spongy state was associated with neuronal loss (med. science 532, 2006).

Researches continued to search for causes and cures for AD. The gene that codes for the B-amyloid protein located on chromosome 21 was implicated in the 20% of patients for whom there was a family history of AD. Head injury had been implicated in 3-5% of AD cases. There was a 70-90% decrease in the production of the enzyme that made acetylcholine. Other neurotransmitter abnormalities had also been

implicated (Med. Sci 532, 2006). The practical steps that were already known to stop this disease (AD) included a healthful diet and avoidance of the consumption of the toxic metal, Aluminium. However, as seen, even with diseases that are well established to be caused by diet, like heart disease, obesity, type-2 diabetes, and many common cancers, controversy abounds not because data from scientific research fail to provide safe and effective behaviours for us to follow but because money and politics rule and important people defend their own dinner plates (Dougall, 2004)

The five drugs currently approved by the Food and Drug Administration (FDA) for the treatment of AD patients (tacrine, donepezil, rivastigmine, galantamind and memantine) at most improve symptom and none has been seen to slow the progression of AD. Therefore, it is imperative that our attention should be, focused on practical matters, such as our diet and avoidance of toxic substances,this translates immediately into cost-free, highly effective non-toxic approaches for prevention and treatment of any disease (Dougall, 2004).

2.13 Plaques and Tangles-the Pathology of AD

AD is generally characterized by the death of brain cells. The diagnosis is firmly established by seeing on microscopic examination two characteristic changes that follow years of repeated injury and this resulting chronic inflammation. The main feature of AD is clumps of protein, called beta-amyloid deposits, which are commonly referred to as senile plaques. These deposits were found in space between the brain cells. The senile plaques were so important for the diagnosis of AD that they were often referred to as pathognomonic lesions-this word means, if you see the pathology, then you name the disease-in other words, finding a significant number of

these senile plaques on microscopic examination establishes that the patient had AD (Perry, 1985).

Injury resulted in damage within the brain cells to tiny (micro) tubules made up the structure of the inside of the brain cells, and serve to transport substances into and out of the cells. When damaged they clump together forming filamentous snarls, as neurofibrillary tangles (Dougall, 2004). Thus our focus of attention was upon the scientific research that identify the sources of injury that result in these two characteristic lesions of AD (senile plaques and neurofibrillary tangles) (Beal et al., 1989).

Chapter 3

MATERIAL AND METHODS

The subjects used in this research work were 50 female adult wister rats (Rattus Novegicus). The rats were purchased from the Faculty of Vet. Medicine A.B.U.Zaria with an average weight of between 150-200g at the beginning of the research work. They were kept in the animal house of Dept of Human Anatomy, Ahmadu Bello University, Zaria for three weeks under (normal) standard environmental conditions (22 ± 1^0C, relative humidity of 60%, 12h-12h light-dark cycle with light on at 08:00h) with sufficient food, water and under a good ventilation in order for the animals (wistar rats) to acclamatized. The animals were all fed with grower's mash obtained from Nassara Feeds, Kaduna. The nutritional composition of the feed is shown on Appendix I.

3.1 Experimental Groups

Stock solution of Aluminium Chloride used was 2g/L (2mg/mL). The animals (wistar rats) were divided into five groups of 10 rats per cage (group). Different concentrations of Aluminium chloride were administered to different groups as stated below:

Group I is the control

Group II is 2.3mg/kg (0.2ml)

Group III is 5.7mg/kg (0.5ml)

Group IV 11.4mg/kg (1ml)

Group V 17.1mg/kg (1.5ml)

The duration of administration was twelve (12) weeks. The method of administration was through oral intubation.

3.2 Experimental Procedure

The animals were taken to Human Physiology Department for Hematological indices after oral administration of various concentration of Aluminum chloride to each group of the treated animals except group I (the control) that was administered with distilled water. This is done by collecting the blood sample through the tails of the rats.

Packed cell volume of each sacrificed animal was done by filling heparinized capillary tube with some blood collected into the EDTA bottles. The lower end was sealed with plasticin and the tube centrifuge using the Hawskley microhaematocrit centrifuge. The PCV value was read using the Hawsksley Haematocrit reader (GELMAN HAWSKLEY LTD. ENGLAND).

From the blood in the EDTA bottles, total white blood cell count was determined with the aid of the WBC pippette and Neubar Haemocytometer (Dancie and Lewis 1991). The (RBC) Red Blood Cell, differential blood counts were also determined.

The animals were sacrificed with the use of chlororform in a closed tight box. Section of the brain was dissected and then fixed in Bouin's solution immediately in order to prevent enzymatic and other postmortem changes that degrade tissue and also to harden the brain so that it can be sectioned without tearing.

3.3 Tissues Preparation for Microscopic Examination

3.3.1 Materials

Formaline (10% formal saline), Absolute Alcohol, 95% alcohol, 70% alcohol, Toluene paraffin, wax, egg albumin, rotary microtome, slides, cover slips, oven water bath, automatic tissue processor (Histokinette Bench model) light microscope, Digital camera, air tight sampling bottles, Bouin's fluid, aqueous potassium permanganate, aqueous potassium metabisulphite, aqueous ferric ammonium sulphate (iron alom).

3.3.2 Method

Tissue preparation method for histological analysis has been 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).

3.3.3 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. The fixed cerebral hemispheres were then carefully dissected to expose the area of the brain of the wistar rats showing the hippocampus.(by the temporal lobe).

3.3.4 Tissue Processing

Both manual and automatic tissue processor, were employed after the area of the brain section showing the hippocampus has been trimmed. The manual processing techniques used were 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.

Toluene was used in clearing the tissue instead of the usual Xylene. After impregnation of the tissue in molting paraffin wax in two changes for 3 hours each, the tissue was embedded in paraffin, blocked and trimmed.

3.3.5 Section and mounting

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

3.3.6 Staining

The staining techniques used were general (routine). Heamatoxylin and Eosin (H & E) was the routine technique employed in order to demonstrate outline of the hippocampal region and the layers.

3.3.7 Photomicrography

Photomicrographs at x 250 magnification were taken from all the groups with the help of light microscope and digital camera, fitted with a laptop.

3.4 Statistical Analysis

ANOVA was used to determine whether the differences that existed among the treated and the control groups were merely due to chance or whether the differences were statistically significant. The level of significance was taken to be less 5% ($P<0.05$).

Chapter 4

RESULTS

4.1 Haemotological Indices

The result of the haematological indices are represented in Table 4.1.

4.1.1 Packed Cell Volume (PCV)

The result showed that the PCV mean value for group I was 40.67%, Group II was 42.67%, group III was 46.00%, group IV 46.33% and Group V 46.33%. There was statistical significant difference in the PCV value between the control and the treated groups at $P<0.05$ (Tables 4.1 and Figure 1).

Table 4.1: Results of the Haematological Indices Noting those Results that Showed Significance between Treatments

Groups	PVC	RBC	WBC	Neutrophil
Control	40.667*	0.84133E+13	0.58000E+10*	17.333*
Group I	42.667*	0.73567E+13	0.69500E+10*	24.000*
Group II	46.000*	0.69967E+13	0.73000E+10*	28.000*
Group III	46.333*	0.66600E+13	0.82167E+10*	32.000*
Group IV	46.333*	0.61733E+13	0.11700E+11*	35.000*

*P<0.05

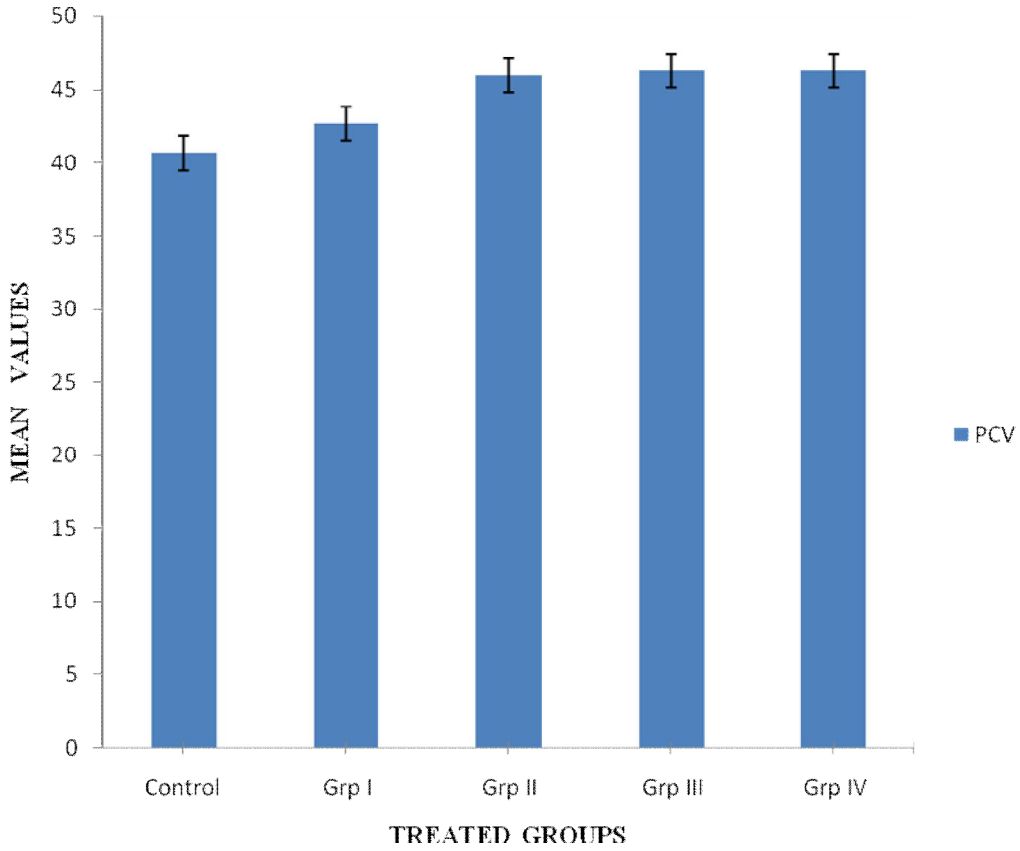


Fig 1: A Graph of Packed Cell Volume (PCV) Comparing the Treated with the Control

4.1.2 Red Blood Cells (RBC)

The results showed that the mean value of RBC for Group I was 8.413 x 10^9 /L, Group II 7.356 x 10^9 /L, Group III 6.997 x 10^9 /L, Group IV 6.670 x 10^9 /L and Group V 6.173 x 10^9 /L. There was no statistical significant difference in the RBC value between the control and the treated groups at $P<0.05$ (Table 4.1 and Figure 2).

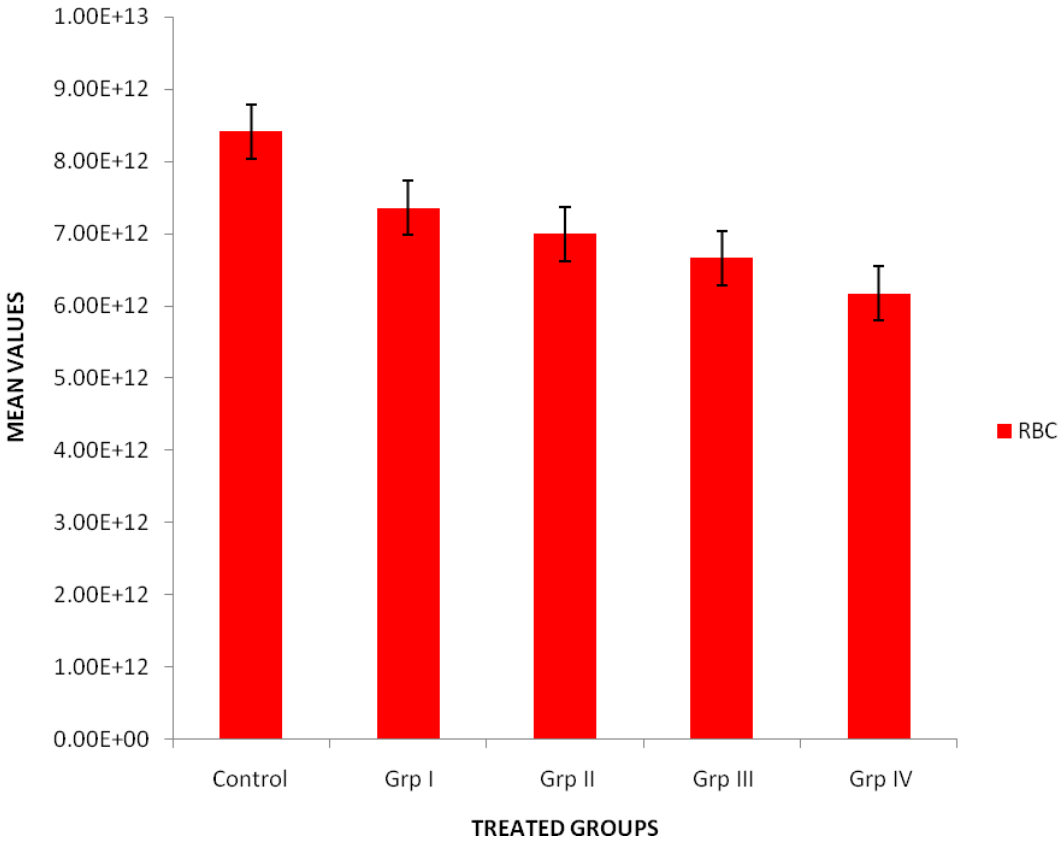


Fig 2: A Graph of Red Blood Cell (RBC) Comparing the Treated with the Control

4.1.3 White Blood Cell (WBC) Count

The result showed that the mean value for group I control was 5.8 x 10^9 /L, Group II 6.95 x 10^9 /L, Group III 7.3 x 10^9 /L, Group IV 8.22 x 10^9 /L and Group V with mean value of 11.0 x 10^9 /L. There was statistical significant difference in the WBC value between the control and the treated groups at $P<0.05$ (Tables 4.1 and Figure 3).

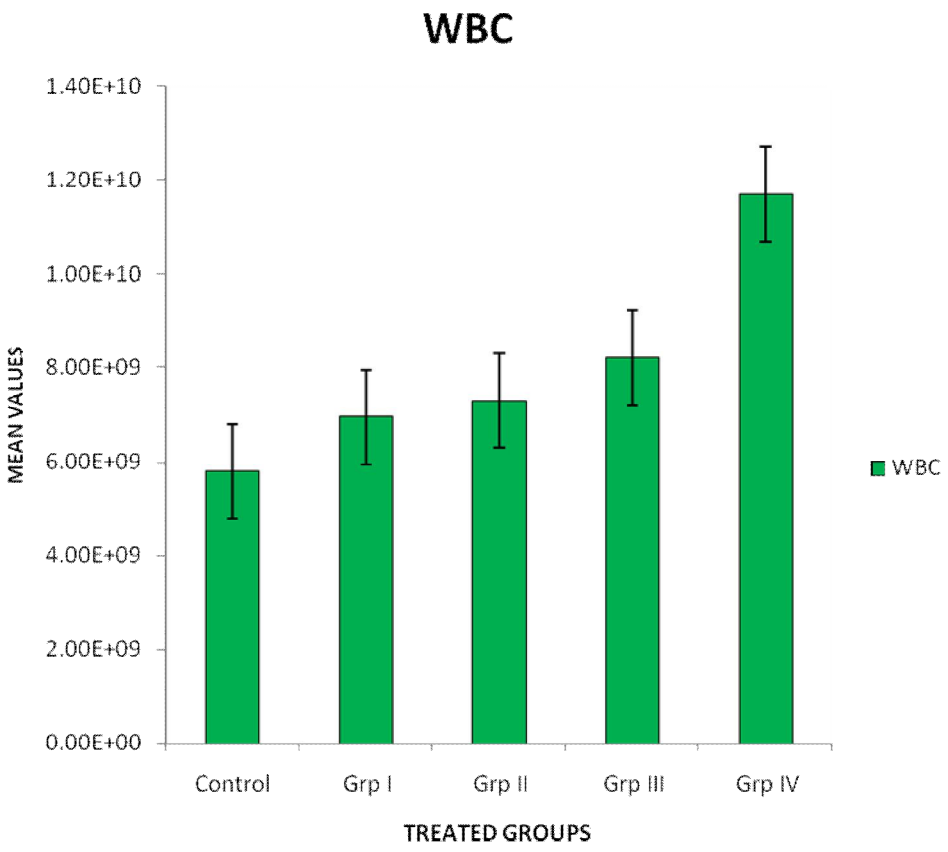


Fig 3: A Graph of White Blood Cells (WBC) Comparing the Treated with the Control

4.1.4 Neutrophil

The result showed that the mean value of neutrophil for group I was 17.33%, Group II was 24%, group III was 28%, Group IV 32% and Group V was 35%. There was statistical significant difference in the neutrophil value between the control and the treated groups at $P<0.001$ (Tables 4.1 and Figure 4).

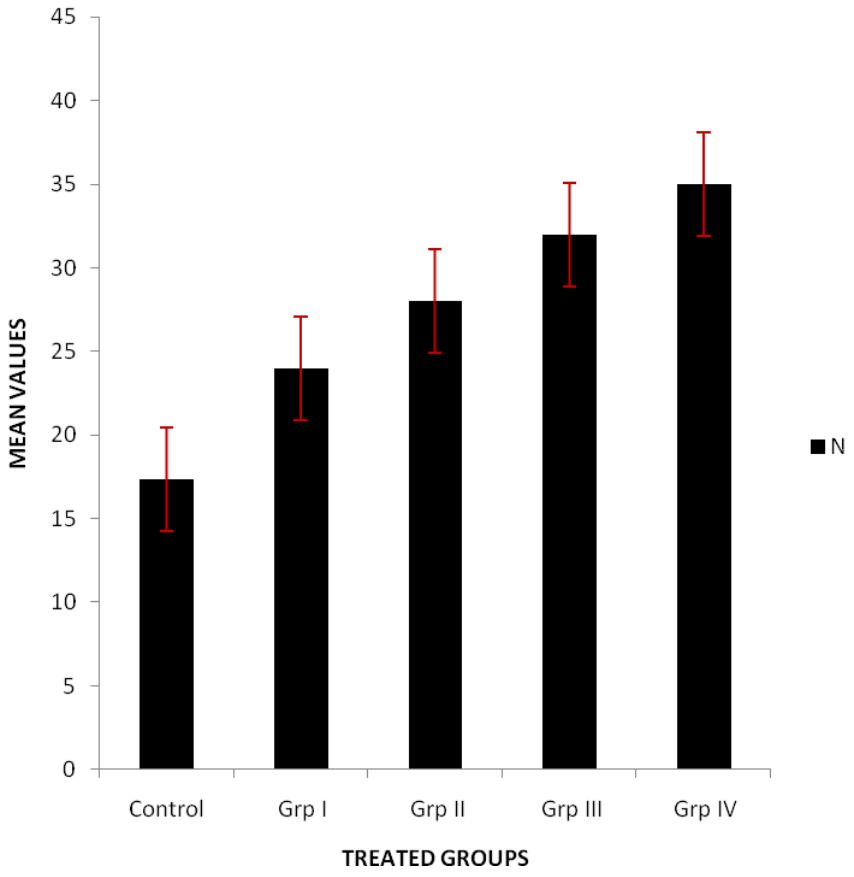


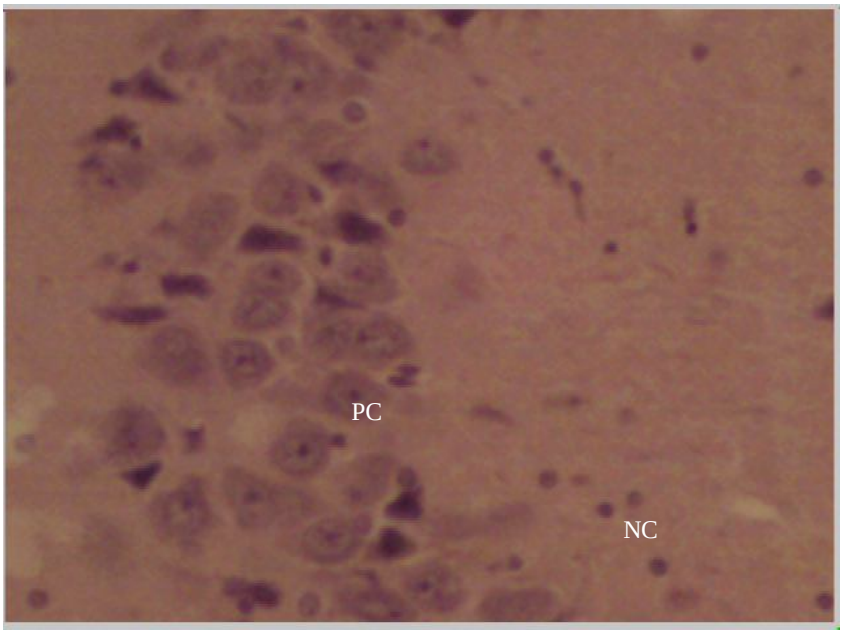
Fig 4: A Graph of Neutrophil Comparing the Treated with the Control

4.2 Microscopic Examination of Tissues

4.3

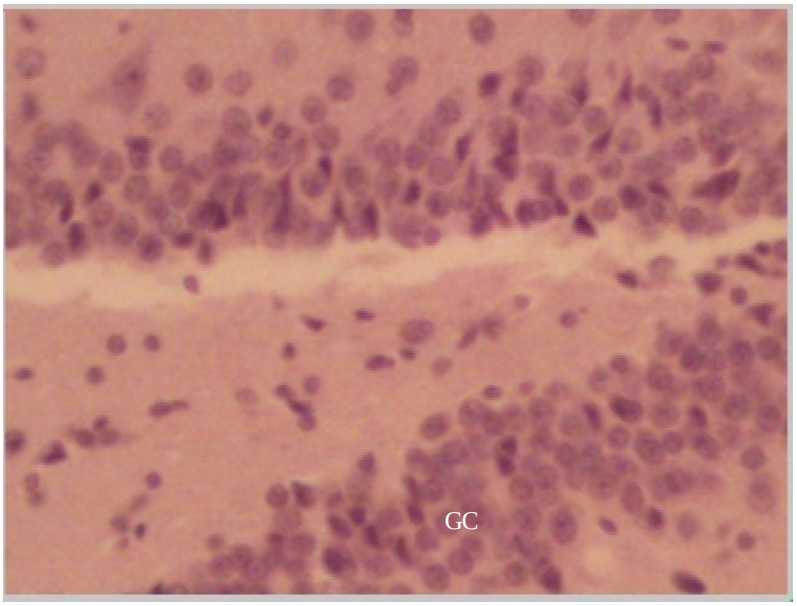
The result of the microscopic examination of the pyramidal cell layer and the granular cells of the Dentate gyrus of hippocampus in Group I (control) showed the general outline of the normal histology of the well arranged without distortion of the pyramidal cell (PC) and neuroglial cell (NC) (Plates I and II).

Plate III of Group II showed no distortion in the pyramidal cells of the hippocampus. Plate IV to X (group III to V) showed reduced pyramidal cells and high level of neurodegeneration.



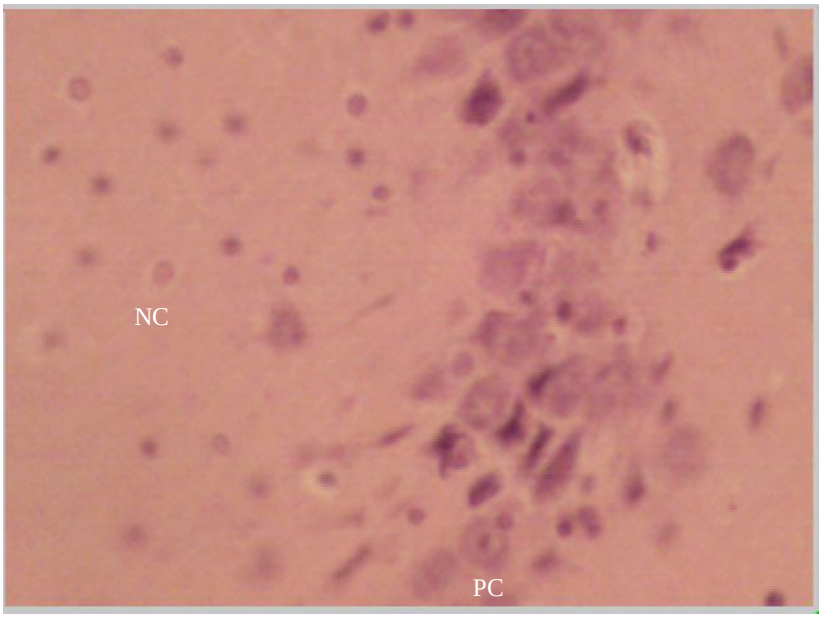
PC = Pyramidal cells
NC = Neuroglial cells

Plate I: Showing the Normal Histology of the Pyramidal cell layers of the Hippocampus of wister rat. Control section stained with H and E at higher magnification; mag. X 250.



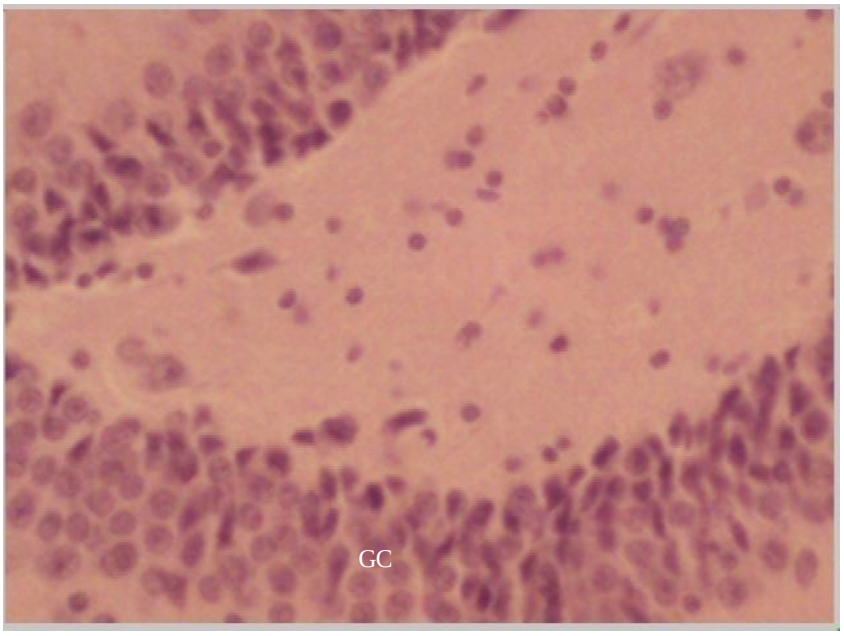
GC=Granular Cells

Plate II: Section of the normal Histology of granular cells of Dentate gyrus of the hippocampus of the control group stained with H and E at higher magnification; mag. X 250.



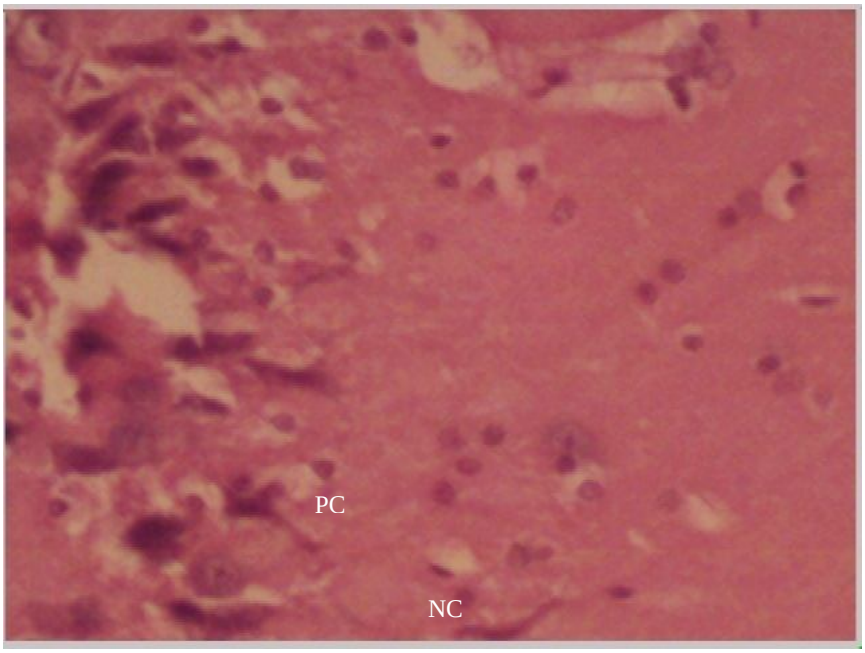
PC = Pyramidal cells
NC = Neuroglial cells

Plate III: Section showing the histology of the pyramidal cell layers of the Hippocampus of Group II stained with H and E at higher magnification; mag. X 250



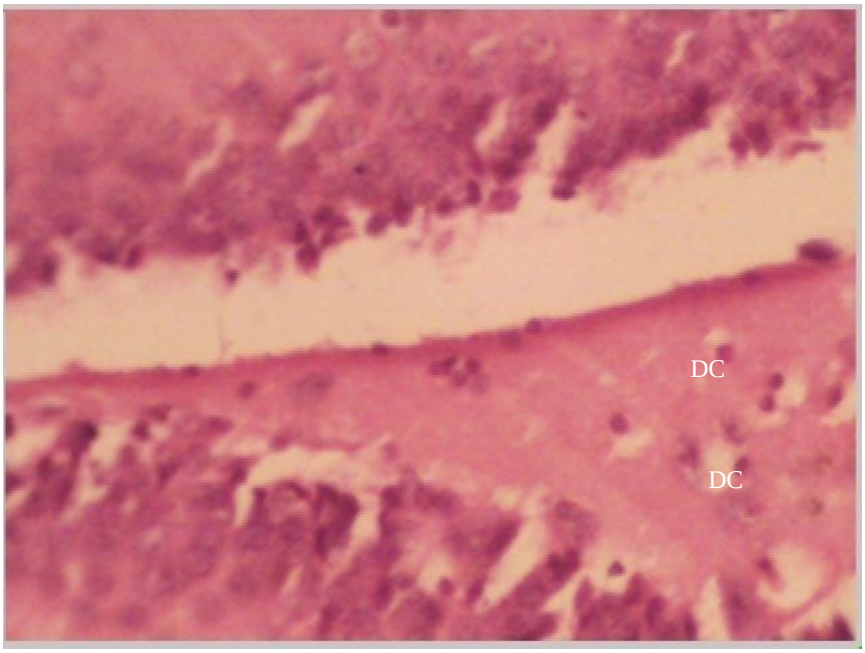
GC=Granular Cells

Plate IV: Showing the Histology of the granular cells of Dentate gyrus (DG) of the Hippocampus of Group II stained with H and E at higher magnification; mag. X 250



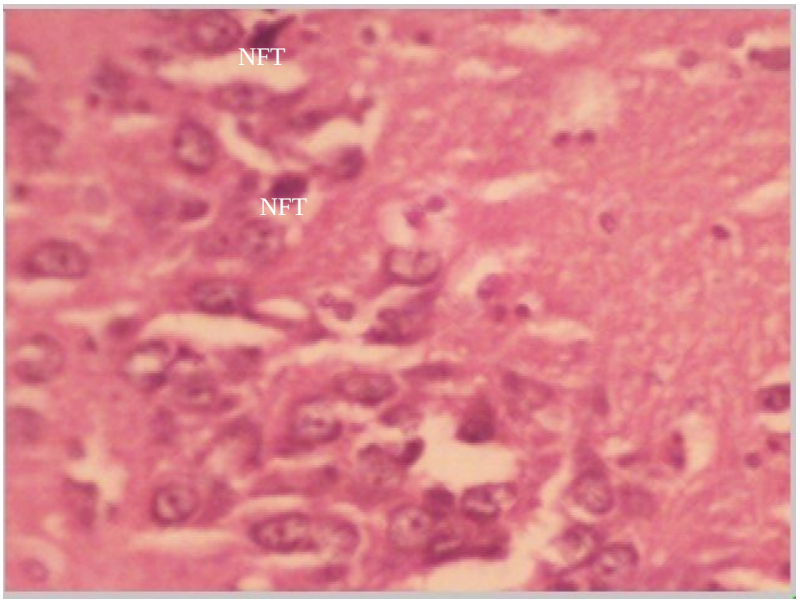
PC = Pyramidal cells
NC = Neuroglial cells

Plate V: Showing the Histology of pyramidal cell layers of the Hippocampus of group III stained with H and E; mag X 250 and neurodegeneration of the pyramidal cells.



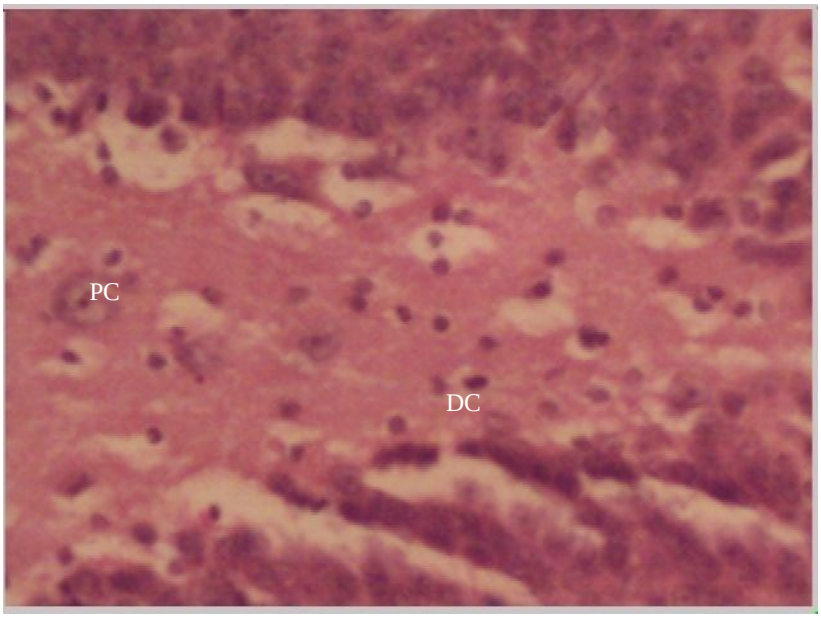
Distorted cells

Plate VI: Showing the Histology of the granular cell layers of the Hippocampus of group III with some level of Neurodegeneration. mag. X 250



NFT= Neurofibrillary Tangle

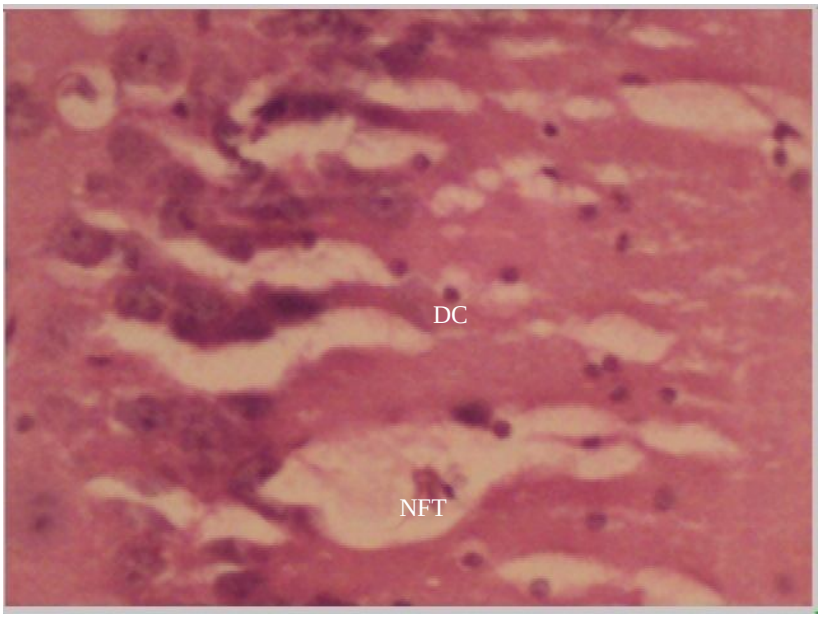
Plate VII: Showing Histology of the pyramidal cell layers of the Hippocampus of group IV with Degenerating cells; Neurofibrillary tangle (NFT). Mag X250



DC= Distorted cells

PC= Pryamidal cells

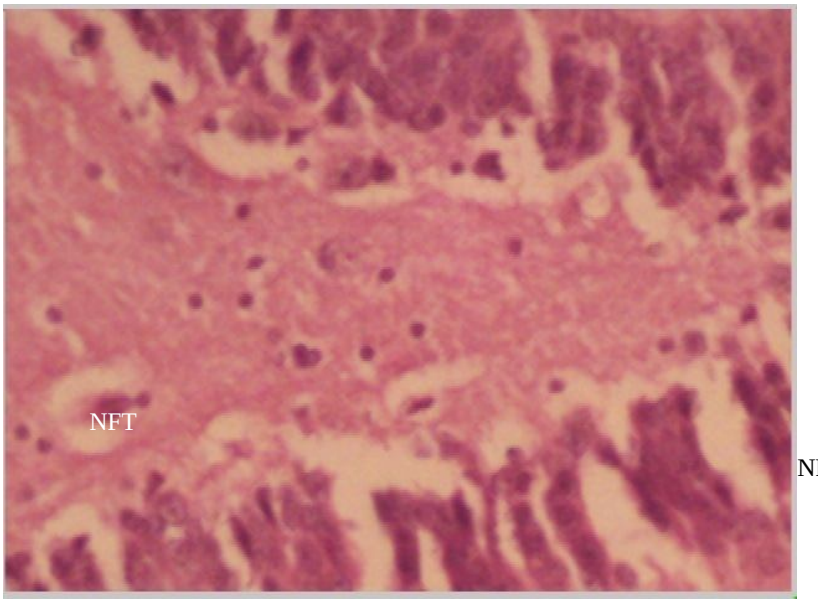
Plate VIII: Showing Histology of granular cell of Dentate gyrus of Hippocampus of group IV with cells distortion (Neurodegeneration). mag. X 250



DC = Distorted cells

NFT = Neurofibrillary tangle

Plate IX: showing Histology of pyramidal cell layers of Hippocampus of group V with cell degenerations; reduced pyramidal cells and few neurofibrillary tangle. mag. X 250



NFT = Neurofibrillary tangle

Plate X: Showing Histology of granular cells of the Dentate gyrus of Hippocampus with cell distortion and few Neurofibrillary tangle. mag. X 250

Chapter 5

DISCUSSION

This study showed degeneration of the pyramidal cells of the hippocampus due to exposure of the brain to aluminium. This may support a hypothetical statement by Yokel (1983) that Aluminium exposure has neuro-degenerating effect resulting in learning deficits in rats and also the documentation compiled by Frank (2006) which stated that in human aluminium inhibits learning.This result of this study may cause learning disability. There were also few neurofibrillary tangles in groups IV and V which suggested the possible effect of oral administration of aluminium chloride on the brain of the rats. This is in line with Muller-Hill and Beyreuther (1989) who suggested that aluminium might have a role in the aetio pathogenesis of Alzeheimer's disease although based on circumstantial evidence.

According to Brodal (1992), the functions of certain learning and memory have been associated with different areas of the brain like the hippocampus and cerebellum. While the hippocampus is associated with memory of new words, faces, place and event, cerebellum has been associated with memory of learning new skills like playing an instrument etc.

Crapper *et al*. (1980), observed that aluminium concentration was elevated in neurons containing neurofibrillary tangles and perhaps within senile plaques, however, aluminium might accumulate in neurons secondarily to intracellular degenerating changes and the neuropathological and behavioural changes following the aluminium exposure were similar to those observed in Alzheimer's disease and the

neurofibrillary changes observed in Alzheimer's disease were found mostly within the cortical and hippocampal neurons: this suggests why there were clumping of neurons in the pyramidal layer of the groups III, IV and V respectively. This is an indication of neurodegeneration as was found in Alzheimer.

The neurofibrillary tangles observed on the treated group in this study was very scanty which do not totally agree with what Crapper *et al.,* (1980) observed. The scanty neurofibrillary tangle observed in this experiment could be as a result of the fact that the aluminium was administered orally unlike Crapper et al. (1980), who injected directly into the hippocampus. However, our results showed that there was a correlation between drinking water containing aluminium and brain (hippocampus) damage as observed from the photomicrographs of the treated groups when compared with the control. This supports the works of Cowling *et al.* (1991).

The packed cell volume (PCV) is the percentage ratio of Red Blood Cell (RBC) volume to the total blood volume. It is about 46% in adult males and 42% in females and about 64% in newly born infants (that is 20% more) due to increase number and size of red cells caused by oxygen lack during intrauterine life (Abdel-Rehim *et al.,* 2004). There was an increase in the packed cell volume from control group to the treated groups in this study. The difference among the groups was statistically significant at $P<0.05$. Increase in PCV can either be as a result of dehydration due to decrease plasma volume as in severe vomiting and diarrhoea or may also be due to polycythaemia. This substantiated the epidemiological studies carried out by Parkinson *et al.*, 1981 who indicated that acute aluminium intoxicated patients were non specifically ill but nausea and vomiting were the common symptom during

haemodialysis and this further substantiated the general phenomena that increase PCV could due to decreased plasma volume as in severe vomiting and diarrhoea (dehydration) (Abdel Rehim *et al.*, 2004). There was a decrease in the RBC count and the decrease was not statistically significant at $P<0.05$ when the control was compared with the treated group. The decrease in RBC count is an indication of anaemia which was in support of the works carried by Short et al., (1980) in Edinburgh who observed a progressive development of anaemia as aluminium load increase and the reversal of the process over a few months when effective water treatment was introduced. Generally, RBC is kept constant by a balance between the rate of RBC destruction and rate of RBC formation.

In haemorrhage the RBC are corrected by contraction of spleen which will lead to injection of the stored RBC into circulation and also by the stimulation of the bone marrow to form new RBC (Abdel Rehim, *et al.*, 2004) The oral administration of aluminium chloride at different dose has effect on the White Blood Cell (WBC) count. There was general increase of the WBC from the control to the treated groups which was statistically significant at $P<0.05$. Increase WBC count usually occur in leucocytosis which could either be physiological caused by emotions, pregnancy, muscular exercise, exposure to cold or pathological caused by increase neutrophillic count that usually occur with tissue destruction (Ganong, 2005). The mean value of neutrophil was statistically significant at P less than 5% ($P<0.05$). The increase in the neutrophil in the treated groups is referred to as neutrophilia which normally occurs with tissue destruction for example acute inflammation of tissues with pus formation. The increase of PCV, WBC, and neutrophil observed in this study could be as a result of destruction of hippocampal cells due to oral administration of aluminium chloride.

Chapter 6

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary and Conclusion

Alzheimer's disease is a neurodegenerative disorder characterized by a complex array of neuropathological biochemical and behavioural sequelae (Foltein and Whitehouse, 1983). It is a socioeconomic problem that has significant effects on a large percentage of an increasingly more aged populations.

Three microscopic abnormality characterized Alzheimer disease: they are neurofibrillary tangle, neurotic or senile plaque and granulovacuolar degeneration. All three may be present in small number in hippocampus of normal elderly patients and however, neurofibrllary tangle occupy the perikaryon of medium and large pyramidal cell (Robert and Terry, 1979).

The oral administration of Aluminium chloride in the experimental groups have shown some level of neurodegeneration (or granulovacuolar) on the hippocampus of the treated rats when compared with the control group I.

However, Aluminium has been linked with Alzheimer's disease and this has led to concern about levels of aluminium in drinking water. Despite the fact that drinking water constitutes only a minor source of total dietary water aluminium intake; recent epidemiological studies have indicated a positive correlation between drinking water aluminium level and Alzheimer's disease incidence (Cowling *et al.*, 1991).

From careful observation of the photomicrograph of the slides, it can be concluded that oral administration of aluminium chloride has a neurodegenerating effect on the hippocampus and hence could suggest a link between exposure to aluminium and Alzheimer's disease, which is in line with the observation of Cowling et al (1991).

Comparing the haematological indices of the control and experimental groups, it could also be concluded that oral administration of aluminium chloride result in increased white blood cells and neutrophil were statistically significant at P less than 5% ($P<0.05$); PVC was not statistically significant at P less than 5% ($P<0.05$) but at P less than 10%($P<0.10$),the RBC was however was not significant statistically.

6.2 Recommendation

- From this experimental work, I therefore recommend that injection of Aluminium Chloride above 300mg in Human may not be safe.

- Public processed drinking water should be regulated so that the concentration of aluminium is very minimal.

- The regulatory body like National Agencies For Food, Drug, Administration and Control (NAFDAC) in Nigeria should encourage manufacturers to indicate the aluminium content of packages of all substances marketed for human consumption and contact, including processed foods, drinking fluid, cosmetics, toothpaste and pharmaceuticals.

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APPENDIX I

PREPARATION OF 10% FORMALIN

1. Preparation of Normal Saline
    - Sodium chloride - 0.9 gm
    - Distilled water to make - 100mls
    - Total - 100mls
2. Preparation of 10% formalin
    - Formal dehyde - 10mls
    - Normal saline - 90mls
    - Total - 100mls

APPENDIX II

HAEMATOXYLIN AND EOSIN STAINING METHOD

1. Preparation of Harris Haematoxylin
   - Haematoxylin - 2 gm
   - Alcohol (95%) - 50 mls
   - Ammonium aluminuim sulphate - 30gm
   - Distilled water - 500mls
   - ➢ Dissolved ammonium aluminuim 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 colour 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

APPENDIX III

Composition of Rat feeds (Nasara feeds Kaduna) Grower's mash
Contents

- Protein (CP) % 15
- Calcium % 0.7
- Methionine % 0.4
- Phosphorus % 0.5
- Energy Kcal/kg 2,600

APPENDIX IV

Table 1: Analysis of variance of Packed Cells Volume

	SS	DF	MS	F	P-Value
BETWEEN	80.933	4	20.233	3.2287	0.0604
WITHIN	62.667	10	6.2667		
TOTAL	143.60	14	10.257		

Table 2: Analysis of Variance of Red Blood Cells Counts

	SS	DF	MS	F	P-Value
BETWEEN	0.85551E+25	4	0.21388E+25	1.5370	0.2644
WITHIN	0.13915E+26	10	0.13915E+25		
TOTAL	0.22470E+26	14	0.16050E+25		

Table 3: Analysis of Variance of White Blood Cells

	SS	DF	MS	F	P-Value
BETWEEN	0.60508E+20	4	0.15127E+20	3.7783	0.0401
WITHIN	0.40037E+20	10	0.40037E+19		
TOTAL	0.10054E+21	14	0.71817E+19		

Table 4: Analysis of variance of Neutrophil

	SS	DF	MS	F	P-Value
BETWEEN	576.27	4	144.07	18.791	0.001
WITHIN	76.667	10	7.6667		
TOTAL	652.93	14	46.638		