EVALUATION OF THE EFFECTS OF AQUEOUS EXTRACT OF *Psidium guajava* LEAVES ON LEAD ACETATE-INDUCED NEUROTOXICITY IN ADULT WISTAR RATS

BY

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BY

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DEPARTMENT OF HUMAN ANATOMY,
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OCTOBER, 2015

DECLARATION

I declare that the research work in this dissertation entitled "**Evaluation of the effects of aqueous extract of *Psidium guajava* leaves on lead acetate-induced neurotoxicity in adult Wistar rats**" has been carried out by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree at this or any other Institution.

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Iliyasu Musa Omoyine	**Date**

CERTIFICATION

This dissertation entitled "**EVALUATION OF THE EFFECTS OF AQUEOUS EXTRACT OF *Psidium guajava* LEAVES ON LEAD ACETATE-INDUCED NEUROTOXICITY IN ADULT WISTAR RATS"** by **Musa Omoyine ILIYASU** meets the regulations governing the award of the degree of Master of Science (M.Sc.) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Almighty ALLAH for His favour, guidance and protection.

To my late father (Mr. Musa Omoyine). May ALLAH have mercy on him and grant him eternal rest.

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ABSTRACT

Lead poisoning is known to cause a number of adverse effects including brain damage, mental impairment and severe behavioural anomalies, impaired cognitive functions in human and experimental animals. The toxic effects of lead are treated by chelation therapy which also depletes the body store of essential cations and as such there is need to look for alternative therapy to lead poisoning. The present study was aimed at evaluating the effects of aqueous extract of *Psidium guajava* leaves (EAPG) on lead acetate induced changes in behaviour, cerebellum, cerebrum and hippocampus of adult Wistar rats. Thirty five (35) adult male Wistar rats were divided into 7 Groups of 5 rats per group. The rats were trained for spatial learning and memory, and motor activity using Morris water maze and beam Balance methods, respectively. Group 1 was administered with distilled water from 1st to 35th day, Group 2 was administered with 1000 mg/kg body weight (bwt) of EAPG only from 1st to 35th day, Groups 3 to 7 were administered with 120 mg/kg bwt of lead acetate from 1st to 21st. While the rats in Group 3 were sacrificed at the 22nd day of the administration, Group 4 was administered with distilled water from the 22nd to 35th day, Group 5 was treated with 500 mg/kg bwt of EAPG from the 22nd to 35th day, Group 6 was treated with 1000 mg/kg bwt of EAPG from the 22nd to 35th day and Group 7 was treated with 10 mg/kg bwt of Succimer from 22nd to 35th day. The administrations were carried out orally once per day for 35 days. The rats were sacrificed and blood samples were collected via cardiac puncture for haematological and biochemical analysis. Brain tissues were removed and fixed in Bouin’s fluid and processed for histopathological studies using Haematoxyline and Eosin (H and E), and Cresyl Echt Violet stains. The results showed that there were significant deficits in spatial learning, memory and motor activities in rats exposed to lead acetate when compared with the control rats and those exposed to lead acetate and treated with EAPG ($P < 0.05$). Oxidative stress was induced in rats exposed to lead acetate by increasing lipid peroxidation and decreasing catalase, superoxide dismutase and reduced glutathione, and the effects were reversed in lead exposed rats treated with EAPG. There were changes in haematological indices of rats exposed to lead acetate and these changes were reversed in rats treated with EAPG. The study also revealed histopathological changes in hippocampus, cerebellar and cerebral cortices of rats exposed to lead acetate and the changes were ameliorated when treated with EAPG. Thus, the present study has established that aqueous extract of *Psidium guajava* leaves at doses of 500 mg/kg bwt and 1000 mg/kg bwt was able to ameliorate the effects of lead acetate induced toxicity and may likely be beneficial to the population in endemic areas that are exposed to lead poisoning.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Lead poisoning has been a recurrent problem in the society for many centuries, and its deleterious effects on central nervous system (CNS) are known as lead encephalopathy or lead neuropathy (Flora *et al.*, 2006). Neurobehavioural impairments, hyperactivity, alterations in brain structure, and learning and cognitive deficits in children have been observed even with low blood lead levels of 10–20µg/dl (Needleman, 1993).

The metal (lead) is primarily found in leaded gasoline, (Tong *et al.*, 2000). Automobile emissions have been an important source of lead exposure to urban residents, particularly in areas with congested traffic. The main source of adult human exposure is through contaminated food, which is believed to account for over 60% of blood levels; while air inhalation accounts for approximately 30% and water of 10% (John *et al.*, 1991).

Lead poisoning is also known as plumbism, colica Pictonum, saturnism, Devon colic, or painter's colic, which is a medical condition caused by increased levels of the heavy metal lead in the body (Rossi, 2008). Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestine, kidneys, reproductive and nervous systems. Interference with the development of the nervous system could lead to permanent learning and behaviour disorders. Symptoms of lead toxicity include abdominal pain, confusion, headache, anaemia, irritability, and in severe cases seizures, coma, and death (Barbosa *et al.*, 2005).

It has been reported that lead exposure produces free radicals, such as, active oxygen species ($O_2^{\bullet -}$, $^{\bullet}OH$ or lipid peroxyl radical, $LOO^{\bullet}$), cause oxidative damage to lipids, proteins, and nucleic acids and may lead to oxidative stress, biological carcinogenesis, mutagenesis, aging, atherosclerosis, neuro-degenerative diseases and stress-induced depression (Sapolsky, 2000; Bilici *et al.*, 2001).

Oxidative stress may adversely affect neuronal cells in which they may die when oxygen supply is reduced or eliminated (Ibegbu *et al.*, 2012). Oxidative stress has been implicated in nerve cell death that occurs in a variety of neurodegenerative disorders like dementias, multiple sclerosis, Alzheimer's disease and Parkinson's disease (Maher, 2001; Prentice *et al.*, 2011). Oxidative stress leads to metabolic cellular processes in which oxidative species such as super oxide radical anions, hydrogen peroxide and lipid peroxides are generated intracellularly (Chen and Buck, 2000; Lee *et al.*, 2012). These reactive species, if not eliminated, may damage DNA, proteins or membrane lipids and cause oxidative cell death. Endogenous antioxidative enzymes as well as antioxidants are required for cells to survive (Semenza, 2005), while exogenous antioxidants have been shown to effectively prevent oxidative cell death in cultured cells (Du *et al.*, 2012).

It is generally known that the consumption of a variety of local herbs and vegetables by man contributes significantly to the improvement of human health, in terms of prevention, and or treatment of diseases because, plants have long served as a useful and natural source of therapeutic agents (Chevellier, 1996). About 80% of the World population depends on herbal based alternative systems of medicine. The activities of these curative plants are evaluated by their chemical components. Indian Ayurveda utilizes about 2000 plants to cure different ailments (Daniel, 2006). Some of these curative plants have natural antioxidants, which neutralize free radical**s**, are receiving more attention from nutritionists and medical researchers for their potential effects in the prevention of chronic and degenerative changes, such as cancer, cardiovascular disease and aging (Young and Woodside, 2001).

Psidium guajava leaf is an important part of guava tree which is useful in treating many health problems. Guava leaves have anti-oxidant, anti-cancer, antibacterial, and anti ulcer properties needed in treatment of many diseases (Vibha *et al.,* 2012).

1.2 STATEMENT OF RESEARCH PROBLEM

Lead remains a considerable occupational and public health problem, which is known to cause a number of adverse effects like brain damage, mental impairment and severe behavioural anomalies, neuromuscular weakness, decreased hearing, impaired cognitive functions in experimental animals and coma (Liuji *et al.,* 2002; Verina *et al.,* 2007; Flora *et al.,* 2007). The toxic effects of lead are treated by chelation therapy which also depletes the body store of essential cations (Ruff *et al.,* 1996) and as such there is need to look for alternative solution to lead poisoning. There are reports on lead induced toxicity in experimental studies but there are scanty works on medicinal plants as treatment options for lead induced brain damage in our environment.

1.3 JUSTIFICATION OF THE STUDY

The recent increase in lead exposure in our environment, especially the cases of Zamfara (Elaine, 2010) and Niger States (Lucia, 2015) has placed a heavy burden on health risk in both adults and children which may result to oxidative stress and severe behavioural anomalies. This incessant exposure to lead and related products require investigation on locally available modes of treatment. *Psidium guajava* has been reported to elicit strong antioxidative activity of which the present study intends to evaluate, following lead exposure.

1.4 SIGNIFICANCE OF THE STUDY

The present study would be of importance in the identification and evaluation of available natural remedy that may possibly be used as alternatives to the presently used chelation therapy to treat lead poisoning, which are not always free from adverse effects. Therefore, from the result of the present study, *Psidium guajava* leaves could be of help in the management of lead poisoning.

1.5 SCOPE OF THE STUDY

This study was be limited to the study of the general histomorphological and degenerative changes in the cerebellum, cerebrum and hippocampus and the study of spatial learning and memory; motor coordination; oxidative stress markers; and haematological analysis.

1.6 AIM AND OBJECTIVES OF THE STUDY

1.6.1 Aim of the study

The aim of the study was to evaluate the effects of aqueous extract of *Psidium guajava* leaves on lead induced changes in brain tissues mainly cerebellum, cerebrum and hippocampus, behaviour, biochemical and haematological parameters of adult Wistar rats.

1.6.2 Objectives of the study

The objectives of the study were to:

i. Evaluate the effects of *Psidium guajava* aqueous leaves extract on lead induced changes in spatial learning, memory and motor coordination in adult Wistar rats.

ii. Study the effects of the extract on lead induced alterations in haematological and biochemical parameters of adult Wistar rats.

iii. Determine the effects of the extract on lead induced changes in brain tissues mainly cerebellum, cerebrum and hippocampus of adult Wistar rats.

1.7 STUDY HYPOTHESIS

Aqueous extract of *Psidium guajava* leaves has ameliorative effects on lead acetate induced changes on the brain tissues, behaviour, haematological and biochemical parameters of adult Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 FORMS IN WHICH LEAD EXIST

Lead is a poisonous metal which occurs in both inorganic and organic forms (Shalan *et al.*, 2005). Inorganic lead is the form of lead found in old paint, soil, dust and various consumer products. The colour varies, depending on the chemical form, and the most common forms are white lead (a lead carbonate compound), yellow lead (lead chromate, lead monoxide) or red lead (lead tetraoxide) and lead acetate has a sweetish taste (WHO, 2010).

Organic leads are tetra-ethyl lead and are used in leaded gasoline. Organic forms of lead are extremely dangerous, as they are absorbed through the skin and are highly toxic to the brain and central nervous system. Organic lead can be more toxic than inorganic lead (WHO, 2010) because the body more readily absorbs it and as such potential exposures to organic lead should be taken very seriously (Shalan *et al.,* 2005).

2.2 ENVIRONMENTAL LEAD EXPOSURE

2.2.1 Sources of lead in the environment

There are different Sources of lead in the environment and these include natural sources, Industrial sources, consumable products, and diffusion and combustion sources.

2.2.1.1 Natural sources

Lead (Pb) occurs in the earth's crust (Sohair *et al.*, 2010) as the end-product of the radiometric decay of three naturally-occurring radioactive elements: Uranium, Thorium, and Actinium. Lead, primarily in the form of lead-sulfide in galena ore, constitutes approximately 10 to 17 milligrams per kilogram (mg/kg) or 0.001 to 0.002 percent of the earth's crust. A common natural means of releasing lead to the atmosphere is via windborne dusts created by the weathering of deposits. Other natural sources of lead emissions are sea and salt lake aerosols, forest fires, and volcanic eruptions. Since lead does not dissipate, biodegrade, and decay, lead deposited into dust and from Pb paint, leaded gasoline and industrial or occupational source of lead become a long term source of lead exposure (Habal, 2002).

2.2.1.2 Industrial sources

Leaded gasoline is still widely used in many countries and contributes to elevated blood lead level especially in urban children. Lead contamination may arise from cottage industries that recycle lead. Paint industrial source may dramatically increase air and soil level in part of the world where environmental controls have not been effectively implemented (Gurer and Ercal, 2000; Meyer *et al.*, 2003).

2.2.1.3 Consumable products

Lead in food comes from several sources in addition to lead solder, the soil in which the plant is grown, air, rain and food processing. Poorly glazed pottery could result in high food lead level. Cosmetics as lipstick and lead-based paints are the most common high dose sources of lead exposure (Habal, 2002).

2.2.1.4 Diffusion and combustion sources

Burning of fossil fuels (petrol and gas), batteries disposal and burning lead painted wood contribute to lead exposures to man and animals (D'souza *et al.*, 2011).

2.2.2 Exposure to lead compounds

2.2.2.1 Oral exposures

Consumption of contaminated food, water or alcohol with lead is an important mode of exposure. Ingestion of dust and soil during meals and playtime activity appears to be a more significant pathway than inhalation for young children (D'souza *et al.,* 2011). Lead in drinking water is probably absorbed more completely than lead in food. Adults absorb 35%-50% of the lead and the absorption rate for children may be greater than 60% (Rogan, 2001; Wu, 2008; Pilsner *et al.,* 2009). Introduction of many non-food items through hand-to-mouth activities of children also contributed to their high rate of absorption (EPA, 2002; WHO, 2010). Lead can be ingested through fruits and vegetables contaminated by high levels of lead in the soils. Soil is contaminated due to the lead in pipes, lead dust from old paints and residual lead from gasoline (D'souza *et al.,* 2011).

2.2.2.2 Inhalation exposure

Inhalation is the second major pathway of exposure, especially for workers in lead-related occupations. Almost all inhaled lead is absorbed into the body, at the rate of 20–70% and usually more in children than adults. The inhalation of Pb could be from industrial action of fossil fuel powder odour (EPA, 2002; Hu *et al.,* 2007), inhalation of tobacco smoke and dust and in many occupational settings such as pigments and batteries production, galvanization and recycling of electrical tools (Frisbie *et al.,* 2002; Valverde *et al.,* 2002; Lambert and Lane, 2004; Saper *et al.,* 2004).

2.2.2.3 Dermal exposure

Exposure to Pb through the skin may be significant for a narrow category of people working with organic lead compounds, but is of little concern for general population. The rate of skin absorption is also low for inorganic lead via the use of lead ointments or cosmetic which may not result in disease condition (EPA, 2002; WHO, 2010).

2.2.2.4 Occupational exposure

Lead is widely used in the production of batteries, metal products (solder and pipes), ammunition and devices to shield X-rays leading to its exposure to the people working in these industries. The use of lead in gasoline, paints and ceramic products, caulking, and pipe solder has been dramatically reduced in recent years because of health concerns (Meyer *et al.*, 2003).

2.3 TOXICOKINETICS OF LEAD ACETATE

2.3.1 Absorption of Lead

Occupational lead intoxication takes place by the respiratory route, where absorption is more rapid and complete compared to any other route (Taib *et al.*, 2004; Flora *et al.*, 2006). According to WHO, (2010) lead and its inorganic compounds were absorbed by humans via the lungs and gastrointestinal tract. At the workplace, inhalation is the most important route of absorption (WHO, 2010).

After absorption of Pb from the gastrointestinal tract or the lungs, it enters the blood stream. At first, lead attaches to proteins in the blood and carried to different tissues or organ systems in the body. It has been reported that most of the lead present in the blood is bound to the red blood cells (Brian and Fred, 1995).

2.3.2 Distribution of Lead

Absorbed lead is distributed to many soft tissues and organ systems of the body. It is important to remember that lead cannot be destroyed or changed to something else in the body. Therefore, lead is conjugated in the liver and passed to the kidney, where small quantity is excreted in urine and the rest accumulated in various body organs (Sidhu and Nehru, 2004; Flora *et al*., 2006). The amount of lead stored in the body has been described as the “body burden” of lead. About 95% of the total body stores of lead are found in teeth of adult and 70% in children. This lead is not simply stored away in bone forever, but moves in and out as the body functions normally (Brian and Fred, 2005).

Research has established that about 95% of the blood lead concentration is bound to the erythrocyte membrane and is distributed in this form in the organism. In the body, the lead is present as a displaceable and an irreversibly bound fraction (Brian and Fred, 2005). The rapid exchange pool represents the biologically effective body burden which correlates with the blood lead concentration, i.e. the lead concentration in the blood is in equilibrium with the lead concentration in soft tissue (Elias *et al.,* 2014). Lead readily passes the placental barrier, and foetal blood therefore has more or less the same lead concentration as the maternal blood (Gulson *et al.*, 2003; Ettinger *et al.*, 2009). Lead also passes the blood–brain barrier, but is not thought to accumulate in the brain (Brian and Fred, 2005).

Human hair is one of the important areas where lead is stored and many authors have reported that human hair mineral analysis is a good marker of environmental pollution. Therefore, using scalp hair as an indicator of the environmental exposure to several trace elements has become a common practice (Shah *et al.*, 2005; Ozden *et al.*, 2007). The advantage of hair is that it is a storage tissue and retains trace elements over an extended period of time (Foo *et al.,* 1993; Izabela *et al.,* 2014). Metal body burden of lead is better reflected in hair than in blood because hair gives a record of relatively long periods, while blood shows momentary levels that fluctuate with time. Furthermore, hair is inert and easier to sample than blood and can be stored without technical problems (Mehra and Juneja, 2004). The investigation of Nowak and Chmielnicka, (2000) revealed that among the investigated keratinous matrices (hair, nail, tooth), lead content in hair was the mostly meaningful environmental marker of exposure to this metal in the human organism and depended on sex and age.

2.3.3 Excretion of Lead

The absorbed lead is eliminated mainly (75–80 %) with the urine and in smaller amounts via gastrointestinal excretion (WHO, 2010). However, the rate of elimination is relatively slow. It is very difficult to give a biological half-life because of the constantly decreasing availability of the depot in the bones. The "Task Group on Metal Accumulation" gives a half-life for lead in human bones of about 10 years (Kaste *et al.,* 2003). Therefore, many people (and most occupationally exposed workers) are unable to get rid of as much lead as they take in. That is why the "body burden" of lead increases over the decades. Until late in life, most persons are steadily getting more and more lead in their tissues. Only among the elderly, for example those 70 to 80 years old, does the body lead burden begins to get less (Brian and Fred, 2005).

2.4 EFFECTS OF LEAD

The effects of lead toxicity are systemic and multiple organs are involved. These have documented to impact a variety of health outcomes including neurodevelopment impairments (Bellinger, 2008), cardiovascular disease (Navas-Acien *et al.,* 2007), neurodegenerative diseases and cognitive decline (Schwartz *et al.,* 2000; Weisskopf *et al.,* 2004) and immune system impairment (Dietert and Piepenbrink 2006). It affects the haematopoietic system where it causes anaemia (Anetor, 2002), in gastrointestinal system it causes gastric ulcer (Olaleye *et al.,* 2002), in renal system it causes renal damage and in the hepatic system it causes hepatitis and hepatic failure (White *et al.,* 2007).

2.4.1 Effects of lead on central nervous system

In the central nervous system, lead poisoning is a potential factor in brain damage, mental impairment and severe behavioural anomalies, neuromuscular weakness, decreased hearing and impaired cognitive functions in experimental animals (Liuji *et al.,* 2002; Verina *et al.,* 2007; Flora *et al.,* 2007). Oxidative stress, oxidative damage to cellular components and activation of the oxidant-sensitive transcription factor could, in part, underlie some of the toxic effects of Pb. The deleterious effects of Pb can involve both reactive oxygen and nitrogen species (Gurer and Ercal, 2000).

It has been understood, during the past decade how much the nervous system is affected by lead. That means, earlier recommendations on “safe” amounts of lead in blood were dangerously close to levels now considered very likely to cause mental retardation in children, (Brian and Fred, 2005). Elevated levels of lead (Pb^{2+}) have been shown to induce cognitive and behavioural deficits in adults and children with different brain targets by inhibiting the N-methyl-D-aspartate receptor (NMDAR) or VGCCs. Inhibition of NMDAR or VGCCs result in a significant reduction of Ca^{2+} entry into the cell which alter pathways involved in synaptic development and neurotransmitter release in brain (Toscano and Guilarte, 2005; Bouchard *et al.,* 2011).

Studies have shown that the toxicity of lead plays a major role in the communication between the astrocytes and the endothelial cells of the blood-brain barrier (Goldstein, 1990). When the blood-brain barrier is exposed to high levels of lead concentration, plasma moves into the interstitial spaces of the brain, resulting in edema. High blood lead toxicity of the central nervous system results in encephalopathy and edema that mainly affects the cerebellum of the brain (Teo *et al.,* 1997). Edema causes extreme pressure increases in the brain, which can lead to irreversible brain damage (Goldstein, 1984). This type of brain damage includes decreased attention, affects visual-motor reasoning skills and social behaviour and can damage mathematic skills and reading abilities (Toscano and Guilarte, 2005). Studies have also shown that lead intoxication drastically decreases cognitive ability, resulting in a loss of Intelligence Quotient (IQ) points anywhere from 0 to 5 per increase of 10 μg/dl in brain lead levels (Bellinger and Needleman 2003; Lanphear *et al.,* 2005; Bellinger, 2008).

2.4.2 Effects of lead on genetic make up

Environmental exposure to Pb especially through inhalation has been linked to the induction of chromosomal aberrations, cancers and birth defects (Vaglenov *et al.,* 2001; Valverde *et al.,* 2002).

2.4.3 Effects of lead on kidney

Impaired kidney functions have been reported as one of the most silent feature of lead toxicity (Chang *et al.,* 1980). High exposures to lead that produce acute poisoning can damage the kidney in both adults and children, by interfering with the reabsorption functions of the kidney. It is believed that when lead levels are reduced, the kidney is able to regain its functions. However, if the lead exposures in childhood continue for a long time and at high amounts, children may show kidney disease later in life as adults. Chronic nephropathy (kidney disease) in lead workers is now recognised as a separate disease. Chronically lead poisoned workers can show elevated blood urea nitrogen (Brian and Fred, 2005). Lead nephrotoxicity is characterized by proximal tubular nephropathy, glomerular sclerosis and interstitial fibrosis (Diamond, 2005).

In rats, proximal tubular injury involves the convoluted and straight portions of the tubule (Dieter *et al.,* 1993) with greater severity, at least initially, in the straight (S3) segment (Murakami *et al.,* 1983). Typical histological features include, in the acute phase, the formation of intranuclear inclusion bodies in proximal tubule cells, abnormal morphology of proximal tubular mitochondria (Fowler *et al.,* 1980), karyomegaly, cytomegaly and cellular necrosis, at sufficiently high dosage. These changes appear to progress, in the chronic phase of toxicity and with sufficient dosage, to tubular atrophy and interstitial fibrosis (Khalil-Manesh *et al.,* 1992 a and b). Glomerular sclerosis has also been reported (Khalil-Manesh *et al.,* 1992a). Adenocacinomas of the kidney have been observed in long-term studies in rodents in which animals also developed proximal tubular nephropathy (Koller, 1985; Goyer, 1993).

2.4.4 Effects of lead on reproductive system

Female workers with high lead exposures and the wives of male lead workers have a higher rate of miscarriages. Male workers with elevated blood concentration of lead up to 50 µg/dL have more abnormal sperm cells and lower sperm counts (Brian and Fred, 2005). These observations indicate the tendency of this heavy metal to induce infertility in affected persons.

2.4.5 Toxicological effects of lead on blood

Studies have shown that Pb intoxication has destructive effects on blood (Elias *et al.,* 2014). Mugahi *et al.* (2003) evaluated the effects of chronic lead acetate intoxication on blood indices of animals by exposing male adult rats to 1% lead acetate in 0.4% acetic acid. A decrease in red blood cell count and increase in leukocyte count were observed. In addition, the rats also had monocytosis, eosinopenia, neutrophilia and thrombocytosis (Mugahi *et al.,* 2003). Similarly, Okediran *et al.* (2010) evaluated the toxic effect of lead on blood cells in animals by administering lead at 0.5, 1.0, 1.5, 2.0 mg/kg bwt to rats for 14 days and observed higher blood lead concentration, decreased packed cell volume, decreased haemoglobin concentration and decreased red blood cell count. There were also leukocytosis, monocytosis and neutrophilia in rats with higher doses of lead.

Anuradha, (2007) found that lead induced anaemia, resulted from shortening of erythrocyte life span and inhibition of haemoglobin synthesis. Lead acts on haeme synthesis via its inhibitory effect on Aminolevulinic Acid Dehydratase (ALAD), a key enzyme involved in the final step of the pathway (Anuradha, 2007; Barber *et al.,* 2011). One of the factors that could be responsible for decreased in haemoglobin content is lead inhibition of the conversion of coproporphyrinogen III to protoporphyrin IV leading to reduction in haemoglobin production and shortened life span of erythrocytes (Klassen, 2001).

Progressive destruction of RBCs due to binding of lead compounds to RBCs, could lead to increase fragility and destruction, consequently produces decrease in haematological values (Rous, 2000). Anaemia, leucopaenia, neutropaenia, lymphopaenia and monocytopaenia were recorded with oral administration of lead acetate at various doses

(Hashem asnd El-Sharkawy, 2009). About 99% of the lead present in the blood is bound to erythrocytes, probably because of a high affinity and makes them more vulnerable to oxidative damage than many other cells. Moreover, erythrocytes can spread lead to different organs of the body (Sivaprasad *et al.,* 2003).

Study has established that the significant reduction in haematological parameters of RBC, Hb, HCT and an increase in platelet count and WBC was induced by lead poisoning. This is possibly as a result of marrow infiltration by lead with a reactionary increase in some parameters (Nwokocha *et al.,* 2011).

It has also been shown that lead toxicity facilitates conversion of Hb into met-Hb. During Hb oxidation in the presence of lead, H_2O_2 is generated, which may induce lipid peroxidation in the erythrocyte cell membrane (Vargas *et al.,* 2003). As a result, lead might induce generation of reactive oxygen species (ROS) by interacting with oxy-Hb, leading to peroxidative damage of erythrocyte membranes (Lyn-Patrick, 2006; Sharma *et al.*, 2011).

Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (Pande and Flora, 2002; Auman *et al.,* 2007; Waters *et al.,* 2008). Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation (Upasani *et al.,* 2001) and brain thiobarbituric acid-reactive substances and altered the antioxidant defense system (Adanaylo *et al.*, 1999).

Similar effects were also reported in the hepatic tissues (Sandhir and Gill, 1995). A number of studies confirmed the possible involvement of reactive oxygen species (ROS) in lead-induced toxicity (Gurer and Ercal, 2000). Several antioxidant enzymes and molecules have been used to evaluate lead-induced oxidative damage in animal and human studies. Reduced glutathione (GSH) and glutathione disulfide (GSSG) concentrations, as well as modifications in superoxide dismutase (SOD) activity are the most frequently used markers in tissues or in blood (Khaki *et al.,* 2010). Based on the observation that free radical was generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants, some of which exist naturally in plants, could be an alternative method to chelation therapy (Flora and Tandon, 1986; Khaki, 2010).

2.5 *Psidium guajava*

2.5.1 General description of *Psidium guajava* plants

Psidium guajava is a member of myrtaceae family which is an important food crop and medicinal plant (Gutierrez *et al.,* 2008). It grows in all tropical and subtropical parts of the world, and readily adapts to different climatic conditions (Sandra *et al.,* 2012). *Psidium guajava*, popularly known as Guava in English, gwaabaa in Hausa, ugwoba in Igbo and guafa in Yoruba (Begum *et al.,* 2002). It is a shrub or small tree of about 2–5 m in height. The bark peels off in flakes, revealing the characteristically smooth trunk (Van-Wyk *et al*., 2002) as shown in figure 2.1. The bark is thin, smooth, patchy, copper-coloured, and flakes off showing the greenish layer beneath (Kamath *et al*., 2008). The short petiolate, large leaves are formed opposite each other in pairs, with prominent veins, particularly on the lower side. The blade is oval with prominent pinnate veins, 5–12 cm long (Gutierrez *et al.*, 2008). The flowers are somewhat showy; petals whitish, up to 2 cm long and contain numerous stamens. The rounded or pear-shaped yellow fruits are fleshy, globose to ovoid berry, about 5 cm in diameter, with an edible pink mesocarp containing numerous small, hard seeds (Gutierrez *et al.*, 2008). The many-seeded fruits have delicious taste and are high in vitamin C content (Van-Wyk *et al.*, 2002).

Figure 2.1: Photography of *Psidium guajava* leaves and fruits (Joseph and Priya, 2011)

2.5.2 Scientific classification

Kingdom	:	Plantae
Order	:	Myrtales
Family	:	Myrtaceae
Subfamily	:	Myrtoideae
Genus	:	*Psidium*
Species	:	*Guajava*
Binomial name	:	***Psidium guajava*** L

(Joseph and Priya, 2011)

2.5.3 Phytochemical constituents of *Psidium guajava*

Extracts of *Psidium guajaya* leaves have been shown to be made up of essential oil, tannins, flavonoids, phenol compounds, carotenoids and vitamin C, quercetin, saponins, alkaloids, cardiac glycosides, phlobatannis and anthraquinones (Vibha *et al.,* 2012). Quercetin has hepatoprotective and anti-oxidant activity, tannins have anti-oxidant and antiviral, anti hyperglycaemic and anti hyperlipidemic activities (Vibha *et al.,* 2012).

The main active constituent in guava plant is reported to be quercetin (Lozoya *et al.,* 2002) which consists of three rings and five hydroxyl groups as shown in figure 2.2. It is found in many common foods including apple, tea, onion, nuts, berries, cauliflower and cabbage. Quercetin has several pharmacological actions which possesses dose dependent antioxidative, anti-inflammatory, antiviral and antitumour activities (Roger and Clive, 1994).

The extract contains flavonoids which are powerful antioxidant polyphenolic compounds. Torell *et al.* (1986) and Faure *et al.* (1991) have shown that flavonoids inhibit peroxidation of polyunsaturated fatty acids in cell membranes. Moreover, reports have shown that flavonoids from Helichrysum genus inhibit the formation of superoxide ions and hydroxy radicals, which are two strong peroxidation agents (Facino *et al.,* 1990).

Witness *et al.* (2008), reported in biomedical literature, that *Psidium guajava* crude leaf aqueous extract, which contains many flavonoids, especially quercetin, induces vascular smooth muscle relaxation, with potency lower than that of pure quercetin.

Figure 2.2: Chemical Structure of Quercetin the main active constituent of *Psidium guajava* leaves (Joseph and Priya, 2011).

2.5.4 Medicinal uses of *Psidium guajava*

Psidium guajava leaf is an important part of guava tree which is useful in treating many health problems (Vibha *et al.,* 2012). Guava leaf extracts introduces many biological activities including antioxidant, antibacterial, analgesic, anti-inflammatory, antimicrobial, phytotoxic, hepatoprotection, anti hyperglycaemic and anticancer activities (Sandra *et al.,* 2012). All these properties of the guava leaf extract make it useful in treating many diseases (Sandra *et al.,* 2012) like oxidative stress, infectious diseases, gastroenteritis, liver damage, tumour, jaundice, cirrhosis and diabetes mellitus (Vibha *et al.,* 2012).

Ayoola *et al.* (2008), evaluated some plants including *Psidium guajava* to investigate their free radical scavenging activity. It was observed that *Psidium guajava* is the most potent among the plants and the free radical scavenging (antioxidant) activities of the plant, probably might contribute to the effectiveness in malaria therapy.

In a study conducted by Friday *et al.* (2010), phytochemical screening of aqueous extract of *Psidium guajava* leaves was carried out using male and female rats administered with 200 mg/kg body weight of oral daily doses of aqueous extract of *Psidium guajava* leaves for a period of 30 days. Blood samples collected at the end of administration to investigate haematopoietic and liver functions, exhibited the same effect on lipid profile as ascorbic acid in rats and may also serve as an acceptable blood booster in an anaemic condition or prophylactic purpose. Although the specific mechanism(s) through which the extract facilitated the increase in these haematological indices was not ascertained in the study, the action is assumed to be a direct effect of the extract on the haematopoietic systems. It is possible that the extract contains such constituent(s) that can interact and stimulate the formation and secretion of erythropoietin, haematopoietic growth factors/committed stem cells. Specifically, stimulations of haematopoietic growth factors and erythropoietin systems have been reported to enhance rapid synthesis of blood cells (Murray, 2000).

The possible role of endothelium mediators such as cyclic nucleotides, in PGE-induced vasodilation was investigated by using endothelium-denuded aortic ring preparations. The vasorelaxant effect of *Psidium guajaya* leaf aqueous extract was found to be smaller in endothelium-denuded aortic ring preparations, suggesting that PGE-induced vasodilation might be mediated, at least in part, through EDRF mechanisms. Acute activation of endothelial nitric oxide synthase (eNOS) by ACh results in the synthesis and or release of endothelial nitric oxide (NO), which in turn leads to guanylate cyclase activation, cyclic guanosine monophosphate (cGMP) elevation, leading ultimately to vascular smooth muscle relaxation (Ajay *et al.,* 2006). Vascular beneficial effects of flavonoids are closely related to their free radical scavenging and antioxidant properties, which might protect NO from superoxide-induced inactivation (Ajay *et al.*, 2006). Quercetin is a potent antioxidant, and has been shown to protect nitric oxide from scavenging actions of superoxide anion (Ajay *et al.,* 2006).

In a study conducted by Neeraj *et al.* (2012) to evaluate the protective effect of aqueous extract of *Psidium guajava* leaves against sodium arsenite-induced toxicity in experimental rats by exposing the rats to arsenic ($NaAsO_2$) at 20 mg/kg bwt through drinking water. Treatment with aqueous extract of *Psidium guajava* at 100 mg/kg bwt ($AEPG_{100}$) significantly restored the changes in activities of oxidative stress markers like LPO levels, GSH levels, SOD, and CAT induced by sodium arsenite but having the limited protective activity of the herbal extract was observed on tissues architecture. It was therefore concluded that prophylactic co-administration of AEPG could provide specific protection from oxidative injury and to some extent on tissue damage. The findings of their study suggest a therapeutic role of *Psidium guajava* leaves in protecting from oxidative damage by ROS (reactive oxygen species) and prevent the arsenic-induced alterations in the oxidative stress markers value. The AEPG might reduce the peroxidation rate and restore the body's antioxidant capacity. Co-administration of AEPG also reduced the arsenic, increased concentration levels in blood, tissues and showed metal chelation property.

2.6 STANDARD DRUG: SUCCIMER (Meso-2,3-dimercaptosuccinic acid)

Meso-2,3-dimercaptosuccinic acid (DMSA), is a water-soluble, sulfhydryl containing compound which is an effective oral chelator of heavy metals (Aposhian, 1983). It is a dithiol and an analogue of dimercaprol a lipid-soluble compound also used for metal chelation. Oral dosing with DMSA creates a distinct advantage over dimercaprol, which has a small therapeutic index and must be administered in an oil solution via painful, deep intramuscular injection (Muckter *et al.,* 1997). On the other hand, DMSA has a large therapeutic window and is the least toxic of the dithiol compounds (Graziano, 1986), and has been used since the 1950s as an antidote for lead poisoning in Russia, Japan, and the Peoples Republic of China (Muckter, *et al.,* 1997).

Studies in the past have shown DMSA to be a safe and effective chelator of lead, reducing blood levels significantly (Aposhian, 1983; Graziano, 1986). At a dose of 10 mg/kg for five days in adult males, DMSA lowered blood lead levels 35.5% and at a more aggressive approach utilizing 30mg/kg lowered blood lead 72.5% with corresponding improvement in the clinical symptoms and biochemical indices of lead toxicity (Graziano, 1986).

An animal study indicated DMSA is an effective chelator of lead in soft tissue, but it may not chelate lead from bone (Cory-Slechta *et al.,* 2010). In a study of lead's pro-oxidant activity and the effect of thiol substances as antioxidants, five weeks of lead exposure in mice depleted hepatic and brain glutathione (GS) levels, and increased malondialdehyde (MDA), a marker of lipid peroxidation. A subsequent DMSA administration for seven days resulted in a reduction in blood, liver, and brain lead levels (Alan and Miller, 1998).

It has been observed that N-acetylcysteine (NAC) supplementation decreased MDA levels, indicating that it ameliorates oxidative stress but has no effect on lead levels (Alan and Miller, 1998). A suggested protocol for lead toxicity is to identify and remove the environmental exposure, and use DMSA 10 mg/kg three times a day for the first five days, followed by 14 days at 10 mg/kg twice a day (Alan and Miller, 1998), to alleviate symptoms including those arising from nerve cell damage.

2.7 THE CENTRAL NERVOUS SYSTEM

The Central Nervous System (CNS) is composed of the brain and spinal cord and is surrounded by bone mainly the skull and vertebrae. Fluid and tissue also insulate the brain and spinal cord. The brain is composed of three parts: the cerebrum, cerebellum, and medulla oblongata (Gross, 1993).

2.7.1 Hippocampus

2.7.1.1 Morphology and Formation of the Hippocampus

Anatomically, the hippocampus is an elaboration of the edge of the cerebral cortex (Gray, 2000; Chris, 2008). As shown in figure 2.3, the hippocampus, a horseshoe shape paired structure, with one hippocampus located in the left brain hemisphere and the other in the right hemisphere (Gross, 1993). The hippocampus, the dentate gyrus and die sibiculum collectively make up the hippocampal formation, which is said to be part of the limbic system. The hippocampus and the dentate gyrus belong to the allocortex and has simplified laminar pattern compared with the neocortex.

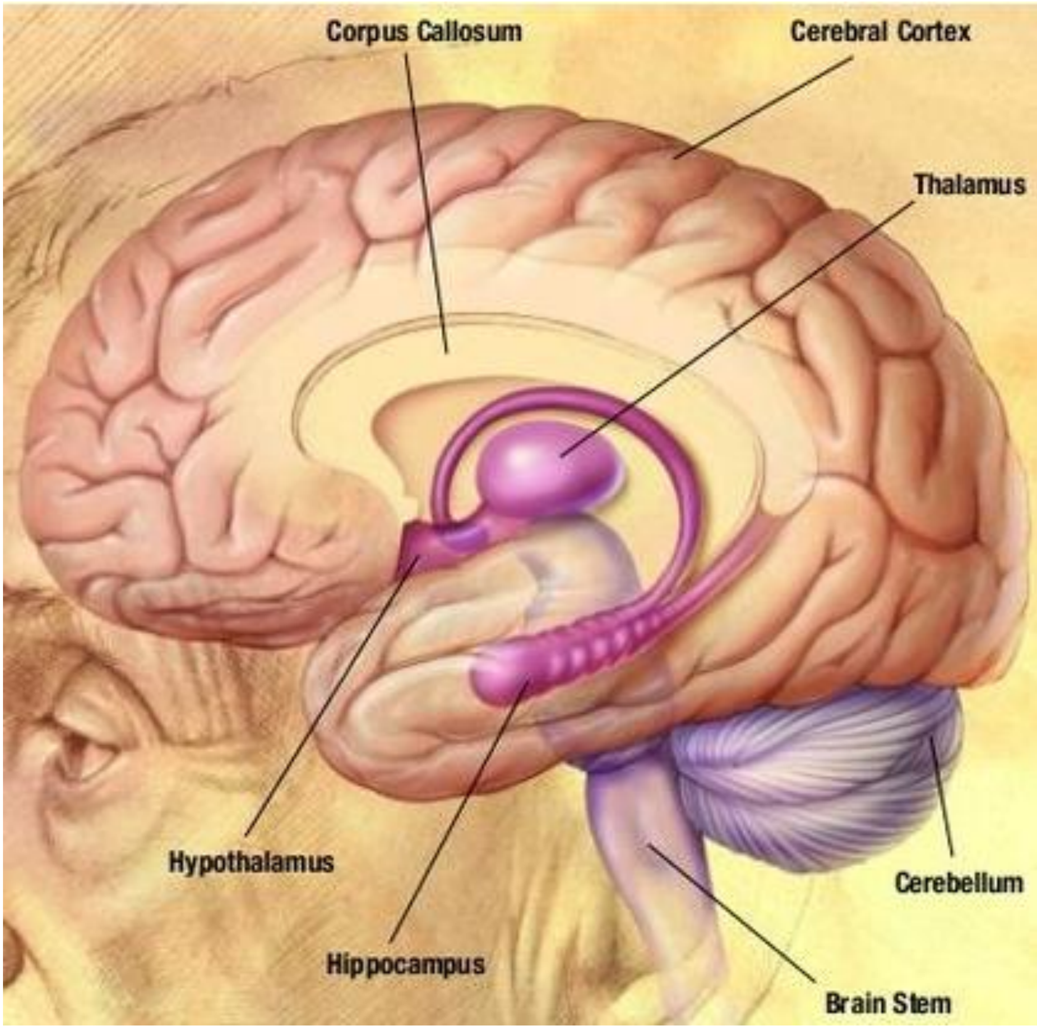

Figure 2.3: Human hippocampus and its associated structures (Chris, 2008).

2.7.1.2 The Cells and Layers of Hippocampus

The hippocampus is composed of multiple subfields as shown in figure 2.4. Though terminology varies among authors, the terms most frequently used are dentate gyrus and the cornu ammonis literally “Amun’s horns”, abbreviated CA. The dentate gyrus contains the fascia dentata and the hilus, while CA is differentiated into fields CA1, CA2, and CA3 (Chris, 2008). The horse shoe appearance of the hippocampus is caused by cell density differentials and the existence of varying degrees of neuronal fibers. The CA regions are also structured depth-wise in clearly defined strata (Philippe, 2007; Hiroaki, 2007; Annabelle, 2009).

The alveus is the deepest layer and contains the axons from pyramidal neurons, passing on towards the fimbria/fornix, one of the major outputs of the hippocampus. Stratum oriens is the next layer superficial to the alveus. The cell bodies of inhibitory basket cells and horizontal trilaminar cells, named for their axons innervating the three layers, the oriens, pyramidal, and radiatum are located in this stratum (Annabelle, 2009). The basal dendrites of pyramidal neurons are also found here, where they receive input from other pyramidal cells, septal fibers and commissural fibers from the contra lateral hippocampus usually recurrent connections, especially in CA3 and CA2 regions (Annabelle, 2009).

Stratum pyramidale contains the cell bodies of the pyramidal neurons, which are the principal excitatory neurons of the hippocampus. This stratum tends to be one of the more visible strata to the naked eye. In region CA3, this stratum contains synapses from the mossy fibers course through stratum lucidum. This stratum also contains the cell bodies of many interneurons, including axo-axonic cells, bistratified cells, and radial trilaminar cells (Chris, 2008). Stratum lucidum is one of the thinnest strata in the hippocampus and only found in the CA3 region. Mossy fibers from the dentate gyrus granule cells course through this stratum in CA3, though synapses from these fibers can be found in stratum pyramidale.

Stratum radiatum like stratum oriens, contains septal and commissural fibers. It also contains Schaffer collateral fibers, which are the projection forward from CA3 to CA1. Some interneurons that can be found in more superficial layers can also be found here, including basket cells, bistratified cells, and radial trilaminar cells. Stratum lacunosum is a thin stratum that too contains Schaffer collateral fibers, but it also contains perforant path fibers from the superficial layers of entorhinal cortex. Due to its small size, it is often grouped together with stratum moleculare into a single stratum called stratum lacunosum-moleculare (Chris, 2008). Stratum moleculare is the most superficial stratum in the hippocampus. Here the perforant path fibers form synapses onto the distal, apical dendrites of pyramidal cells.

The hippocampal sulcus or fissure is a cell-free region that separates the CA1 field from the dentate gyrus. Because the phase of recorded theta rhythm varies systematically through the strata, the fissure is often used as a fixed reference point for recording EEG as it is easily identifiable.

The Dentate Gyrus: The dentate gyrus is composed of a similar series of strata namely; Polymorphic layer is the deepest layer of the dentate gyrus and is often considered a separate subfield. This layer contains many intemeurons, and the axons of the dentate granule cells pass through this stratum on their way to CA3 (Annabelle, 2009). Stratum granulosum contains the cell bodies of the dentate granule cells.

Stratum moleculare, outer third is where both commissural fibers from the contralateral dentate gyrus run and form synapses as well as where inputs from the medial septum terminate on the proximal dendrites of the granule cells. Stratum moleculare, external two thirds is the deepest of the strata, sitting just superficial to the hippocampal fissure across from stratum moleculare in the CA fields. The perforant path fibers run through this stratum, making excitatory synapses with the distal apical dendrites of granule cells (Gross, 1993; Chris, 2008).

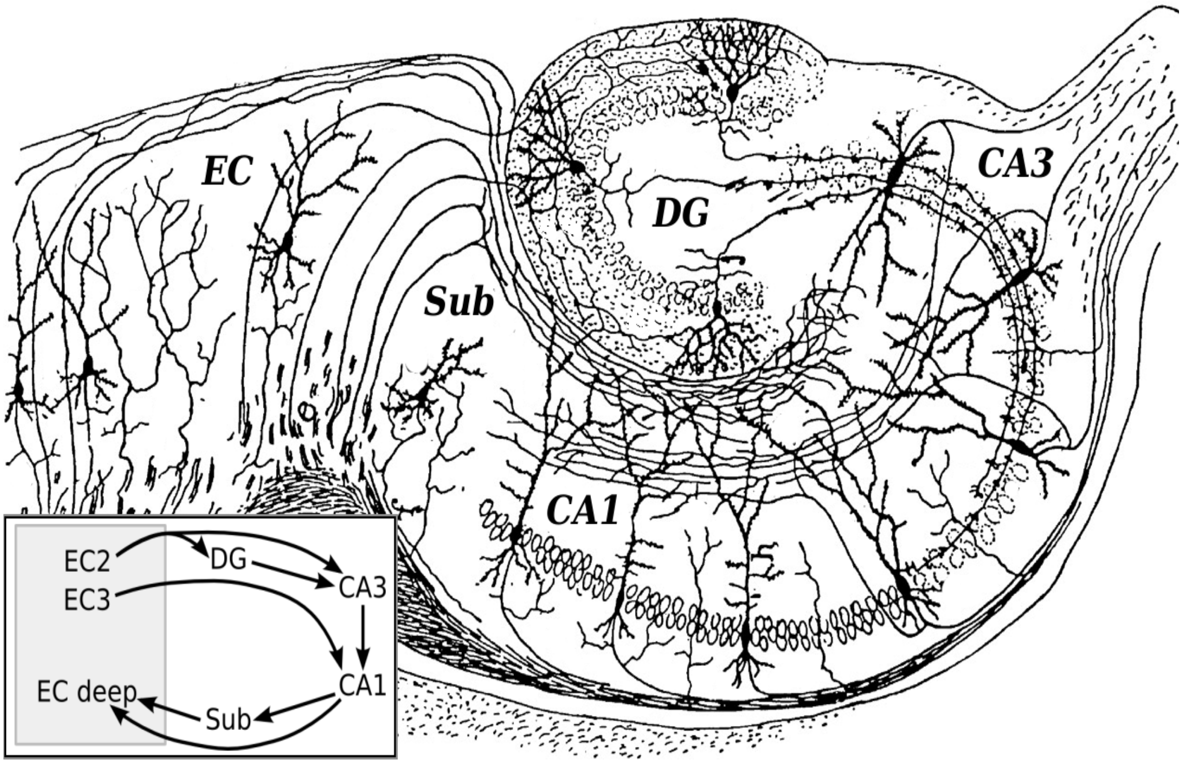

Figure 2.4: Modified drawing of the neural circuitry of the rodent hippocampus (Annabelle, 2009).

2.7.1.3 Functions of Hippocampus

The hippocampus is believed to play important role in several functions of the body. The hippocampus is the part of the brain that is involved in memory formation, organization and storage. Hippocampus is a limbic system structure that is particularly important in forming new memories and connecting emotions and senses, such as smell and sound, to memories. The hippocampus acts as a memory indexer by sending memories out to the appropriate part of the cerebral hemisphere for long-term storage and retrieving them when necessary (Annabelle, 2009).

2.7.2 Cerebellum

2.7.2.1 Morphology of the Cerebellum

The cerebellum is the largest part of the hindbrain. It is dorsal to the pons and medulla, and its median region is separated from them by the fourth ventricle (Gray, 2000). It is joined to the brain stem by three pairs of cerebellar peduncles, which contain afferent and efferent fibres. The cerebellum occupies the posterior cranial fossa, where it is covered by the tantrum cerebelli. It is roughly spherical, but somewhat constricted in its median region, and flattened its greatest diameter is transverse (Clerke, 1996). In other words, the cerebellum is located at the bottom of the brain, with the large mass of the cerebral cortex above and the portion of the brainstem called the pons in front of it as shown in figure 2.5 (Clerke, 1996). It is separated from the overlying cerebrum by a layer of leathery dura mater. All of its connections with the other parts of the brain travel through the pons.

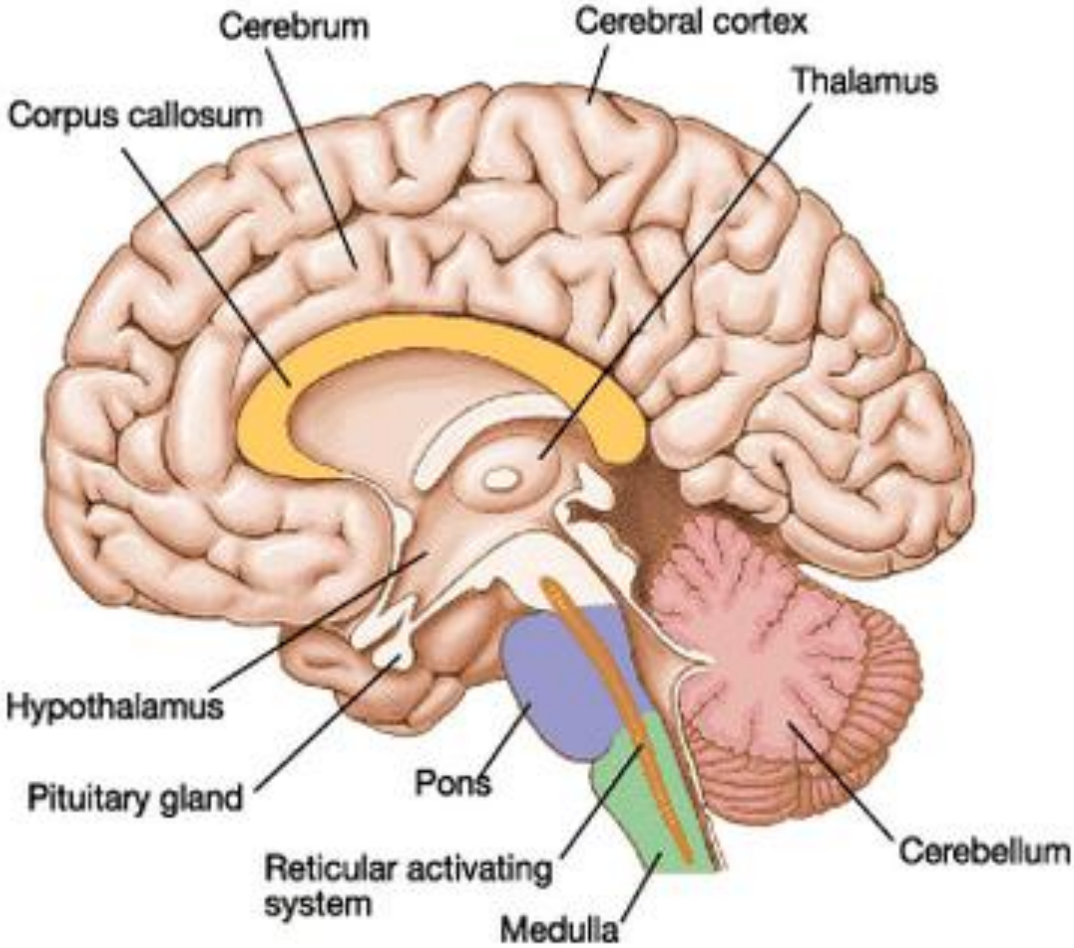

Figure 2.5: location of the human cerebellum (Clerke, 1996).

2.7.2.2 Cellular Layers of the Cerebellum

The cerebellar cortex is divided into three layers. The layers from the lower to the upper include the followings; granular, Purkinje and molecular layers as shown in figure 2.6.

The thick granular layer: is densely packed with granule cells, along with interneurons, mainly Golgi cells and unipolar brush cells (Ghez, 1985; Bell, 2008). Their cell bodies are packed into a thick layer at the lower part of the cerebellar cortex. A granule cell emits only four to five dendrites, each of which ends in an enlargement called a dendritic claw (Linas *et al.,* 2004). These enlargements are sites of excitatory input from mossy fibers and inhibitory input from Golgi cells (Linas *et al.*, 2004).

The Purkinje layer: lies in the middle, forming a narrow zone that contains only the cell bodies of Purkinje cells (Linas *et al.,* 2004). They are distinguished by the shape of the dendritic tree. The dendrites branch very profusely, but are severely flattened in a plane perpendicular to the cerebellar folds. Thus, the dendrites of a Purkinje cell form a dense planar net, through which parallel fibers pass at right angles (Linas *et al.*, 2004; Bell, 2008). The dendrites are covered with dendritic spines, each of which receives synaptic input from a parallel fiber. Purkinje cells receive more synaptic inputs than any other type of cell in the brain. The large, spherical cell bodies of Purkinje cells are packed into a narrow layer, one cell thick of the cerebellar cortex, called the Purkinje layer (Linas, *et al.,* 2004).

The molecular layer: is thick and it contains a sparse population of neurones, dendritic arborizations, non-myelinated axons and radial fibres of the neuroglial cells (Clerke, 1996; Gray, 2000). Purkinje cell dendritic trees extend towards the surface and spread out in a plane perpendicular to the long axis of the cerebellar folia. Purkinje cell dendrites are flattened. This outermost layer of the cerebellar cortex also contains two types of inhibitory interneurons, stellate cells and basket cells (Linas *et al.,* 2004).

2.7.2.3 Functions of the Cerebellum

The cerebellum performs the following functions:

i. Coordination of movements; the cerebellum controls the timing and pattern of muscle activation during movement,
ii. Maintenance of equilibrium in conjunction with the vestibular system.
iii. Regulation of muscle tone and modulation of spinal cord and brain stem mechanisms involved in postural control (Linas *et al.,* 2004).

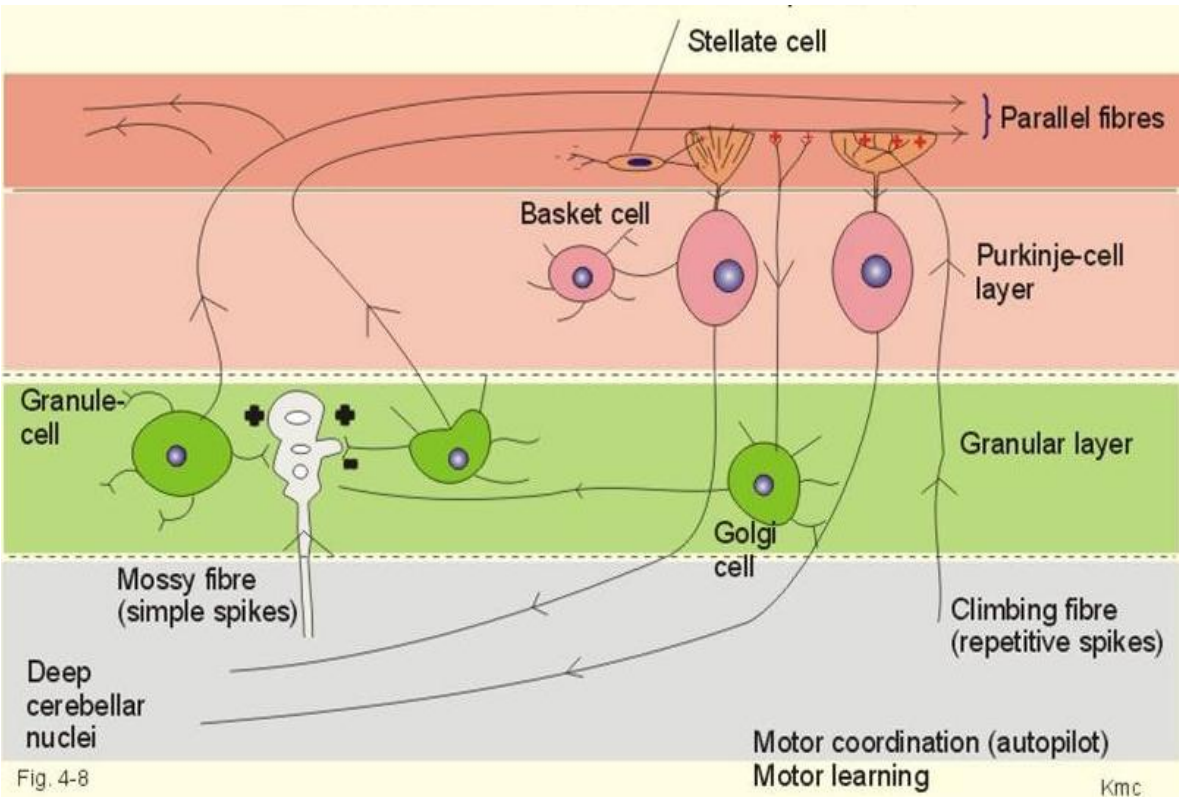

Figure 2.6: Schematic diagram of the cellular layers of the cerebellum showing the outer molecular layer (upper layer), the Purkinje cell layer and the granular layer (Linas *et al.,* 2004).

2.7.3 The Cerebrum

2.7.3.1 The morphology of the cerebrum

The cerebrum comprises a large portion of the brain as shown in figure 2.7. It lies in front or on top of the brainstem and in humans is the largest and best-developed of the five major divisions of the brain. The cerebral cortex is the largest part of the brain; it is formed by grey matter and cerebral hemisphere on the surface. The cortex is convoluted with projections called sulci and gyri. The cerebral cortex contains nerve cells, fibers, neuroglia and blood vessels. The cerebral hemisphere is supplied by cerebral branches of polygon of Willis (formerly cycle of Willis) i.e. the anterior, middle and posterior cerebral arteries (Kandel *et al.*, 2000).

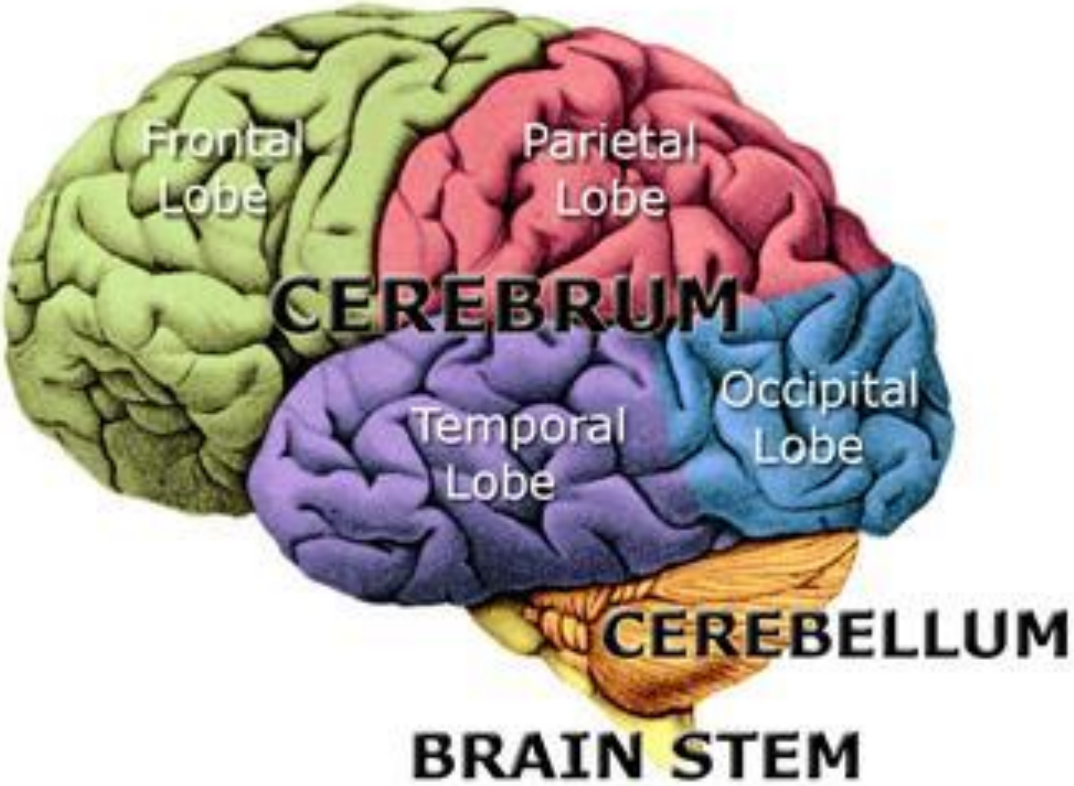

Figure 2.7: The structure of the brain showing lateral view of human cerebrum (Kandel *et al.,* 2000).

2.7.3.2 Cells of the cerebral cortex

The neurons of the cerebral cortex vary in size, shapes, lengths, branching patterns and orientation of their processes. These neuronal cells are named based on their specific characteristics features which demarcate them from each other. The neurons include the following:

The pyramidal cells: are most abundant cervical neurons in the cortex. All others are referred to as non-pyramidal neurons. About 2/3 (two third) of the cerebral cortex cells are pyramidal cells. Their cell bodies are triangular in shape; apex is directed towards the cortex. Large dendrites arise from the apex while others arise from basal angles. The axis arises from the base of the pyramidal cells (Kandel *et al.*, 2000). The process of pyramidal cells extends vertically through the entire thickness of the cortex and established numerous synapses. The axons of pyramidal cells may terminate in different ways. They may travel to the other regions like the basal ganglia, the brain stem or the spinal cord. Also they may give collateral fibers that terminate within the cortex and may be short and terminate within the same area in the cortex (Kandel *et al.*, 2000).

The stellate cells: are small and multipolar and form 1/3 (one third) of the total population of neurons of the cortex under low magnification. They look like granular cells. They are therefore been termed granular neurons (cells) by earlier workers. Stellate cells are of various types depending on their bodies and on the pattern of their ramification processes. Their axons are short and end within the cortex and their processes extend chiefly in radical direction within the cortex in some case horizontally. Some Stellate cells may be uniform rather than Stellate, with one process arising at either end. Stellate neurons are classified as spiny and non-spiny neurons. The spiny Stellate cells use glutamate as neurotransmitter, while in most non-spiny Stellate cells it is Gamma ammo Butyric Acid (GABA). The neurons of the cerebral cortex are grouped into six main layers (Kandel *et al.,* 2000; Shipp, 2007; Saladin 2010).

2.7.3.3 Layers of the cerebral cortex

Layer I: is the molecular layer, 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 as shown in figure 2.8. Some Cajal-Retzius and spiny stellate cells can be found in the molecular layer. Inputs to the apical tufts are thought to be crucial for the “feedback” interactions in the cerebral cortex involved in associative learning and attention (Saladin, 2010). While it was once thought that die input to layer I came from the cortex itself, it is now realized that layer I across the cerebral cortex mantle receives substantial input from “matrix” or M-type thalamus cells in contrast to “core” or C-type that go to layer IV.

Layer II: is the External granular layer, contains small pyramidal neurons and numerous stellate neurons.

Layer III: is the External Pyramidal layer, contains predominantly small and medium-size pyramidal neurons, as well as non-pyramidal neurons with vertically-oriented intracortical axons. Layers I through III are the main target of Intel-hemispheric corticocortical afferents, and layer III is the principal source of corticocortical efferents (Shipp, 2007).

Layer IV: is the Internal Granular layer, contains different types of stellate and pyramidal neurons, and is the main target of thalamocortical afferents from thalamus type C neurons as well as intra-hemispheric corticocortical afferents (Saladin, 2010).

Layer V: is the Internal Pyramidal layer, contains large pyramidal neurons such as the Betz cells in the primary motor cortex. It is the principal source of subcortical efferents, as such there are large pyramidal cells which give rise to axons leaving the cortex and running down through the basal ganglia, the brain stem and the spinal cord (Saladin, 2010).

Layer VI: is the Polymorphic or Multiform layer 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 other ones via the thalamic reticular nucleus that inhibit these thalamus neurons or ones adjacent to them. 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" (Saladin, 2010).

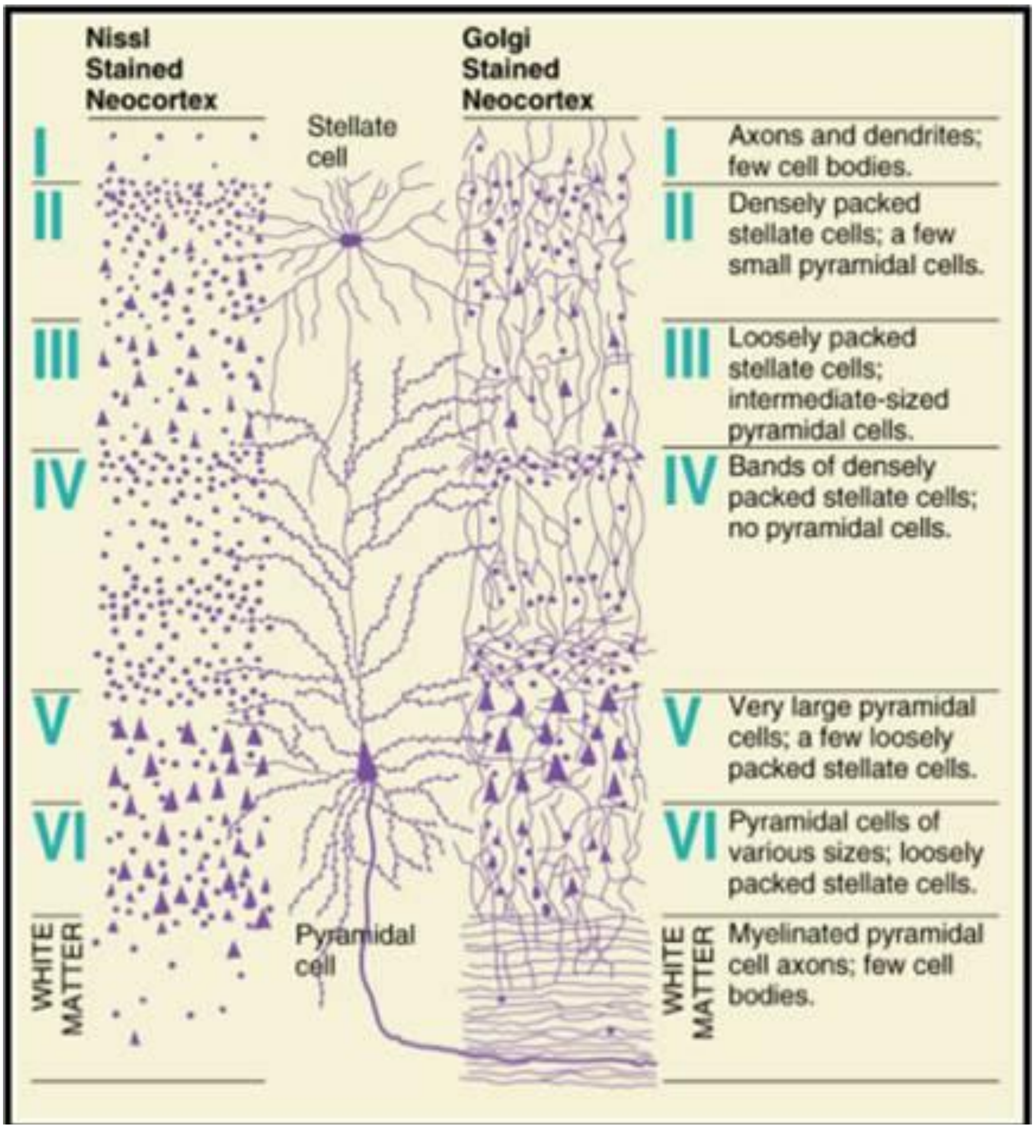

Figure 2.8: The cellular layers of cerebral cortex of human (Kandel *et al.,* 2000).

2.7.3.4 Variations of cortical structure

The structure of the cerebral cortex shows considerable variations in the region, both in terms of thickness and in prominence of the various laminae. These variations are as follow:

i. In the agranular cortex, the external and internal granular Laminae are inconspicuous. This type of cortex is referred to as precentral gyrus (area 4) and is therefore to be of typical area.
ii. In the granular cortex, the granular cortex is highly developed while the pyramidal and ganglion layer are poorly developed or absent. This type of cortex is mostly present in sensory area including the post-central gyrus (Saladin, 2010).

2.7.3.5 Functions associated with cerebrum

The cerebrum is the most highly developed part of the human brain and is responsible for determining intelligence, motor function, planning and organization, touch sensation, thinking, perceiving, producing and understanding language. Most information processing occurs in the cerebral cortex. The cerebral cortex is divided into lobes of which each has a specific function (Saladin, 2010).

Motor Function: the cerebrum directs the conscious or volitional motor functions of the body. These functions originate within the primary motor cortex and other frontal lobe motor areas where actions are planned. Upper motor neurons in the primary motor cortex send their axons to the brainstem and spinal cord to synapse on the lower motor neurons, which innervate the muscles. Damage to motor areas of cortex can lead to certain types of motor neuron diseases (Shipp, 2007). This kind of damage results in loss of muscular power and precision rather than total paralysis. The motor area of classical description is located in the precentral gyrus on the superior lateral surface of the hemisphere and in the anterior part of the precentral lobule; the medial surface. This corresponds to area 4 of Brodman and possibly part of area 6, which lies in the pre-central gyrus, the specific region in the area responsible for movement of specific part of the body (Shipp, 2007).

Sensory processing: the primary sensory areas of the cerebral cortex receive and process visual, auditory, somato-sensory, gustatory, and olfactory information. Together with association cortical areas, these brain regions synthesize sensory information into our perceptions of the world around us.

Olfaction: the olfactory bulb in most vertebrates is the most anterior portion of the cerebrum, and makes up a relatively large proportion of the telencephaton. However, in humans, this part of the brain is much smaller, and lies underneath the frontal lobe. The olfactory sensory system is unique in the sense that neurons in the olfactory bulb send their axons directly to the olfactory cortex, rather than to the thalamus first. Damage to fee olfactory bulb results in the loss of the sense of smell (Kandel *et al.,* 2000).

2.8 MEMORY

Memory is an organism’s ability to store, retain and recall information and experience (Hacking, 1996 Mark, 2007; Michael, 2009). During the late nineteenth and early twentieth centuries, scientists have put memory within the paradigm of cognitive psychology. In recent decades, it has become one of the principal pillars of a branch of science called cognitive neuroscience, an interdisciplinary link between cognitive psychology and neuroscience (Hacking, 1996; Mark, 2007).

2.8.1 Types of Memory

2.8.1.1 Sensory memory

Sensory memory corresponds approximately to the initial 200-500 milliseconds after an item is perceived (Mandler, 1967; Bruce *et al.,* 2006). The ability to look at an item, and remember what it looks like within just a second of observation, or memorization, is known as sensory memory. With very short presentations, participants often report that they seem to see more than they can actually report (Hacking, 1996). Sensory memory cannot be prolonged via rehearsal (Alberimi, 2001).

2.8.1.2 Short-term memory

Short-term memory involves the hippocampus and allows recall for a period of several seconds to a minute without rehearsal. Its capacity is also very limited (Mandler, 1967). Memory capacity can be increased through a process called chunking. For example, in recalling a ten-digit telephone number, a person could drank the digits into three groups: first, the area code such as 215, then a three-digit chunk 123 and lastly a four-digit chunk 4567 (Brockmeier, 2010). This method of remembering telephone numbers is far more effective than attempting to remember a string of 10 digits. This is because of the ability to chunk the information into meaningful groups of numbers. Short-term memory is believed to rely mostly on an acoustic code for storing information, and to a lesser extent a visual code (Alberimi, 2001).

2.8.1.3 Long-term memory

Long-term memory involves the posterior cinglate gyrus and can store much larger quantities of information for potentially unlimited duration, sometimes a whole life span

(Alberimi, 2001). Its capacity is immeasurably large. For example, given a random seven-digit number we may remember it for only a few seconds before forgetting, suggesting it was stored in our short-term memory. On the other hand, a person can remember telephone numbers for many years through repetition, this information is said to be stored in long-term memory (Tyler *et al.,* 1979). This type of memories, are maintained by more stable and permanent changes in neural connections widely spread throughout the brain. The hippocampus is essential for learning new information and the consolidation of information from short-term to long-term memory, although it does not seem to store information itself (Gross, 1993). Without the hippocampus, new memories are unable to be stored into long-term memory, and there will be a very short attention span (Gross, 1993; Mark, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

Materials used in the study included the followings; experimental animals, lead acetate, fresh leaves of *Psidium guajava,* digital weighing balance, cages, water bottles, dissecting set, orogastric tubes, beakers, syringes, plastic pipette, glass slides, normal saline, etc.

3.1.1 Experimental Rats

Thirty five (35) adult male Wistar rats were obtained from Animal House of the Department of Human Anatomy, Ahmadu Bello University, Zaria-Nigeria. The rats were acclimatized to experimental condition for a period of two weeks and fed with rat chow and water was allowed *ad libitum*.

3.1.2 Obtaining and identification of *Psidium guajava* leaves

Fresh leaves of *Psidium guajava* were obtained from the premises of the Faculty of Medicine, Ahmadu Bello University, Zaria. The leaves were identified and authenticated with a voucher number of 3253 by Mallam Namadi Sanusi at Department of Biological Science Herbarium, Ahmadu Bello University, Zaria.

3.1.3 Obtaining of lead acetate

Laboratory grade of Lead acetate of 99% to 103% purity manufactured by BDH Chemical Ltd England was purchased from Cardinal Scientific, No.11/12 Sokoto Road, Opposite Longman Nig. Plc. Samaru-Zaria. The chemical was taken to the Department of Chemistry, Ahmadu Bello University Zaria for identification.

3.2 METHODS

3.2.1 Preparation of Extract

Extraction of plant (guava leaf) was done in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Aqueous extraction of guava leaf was prepared according to the procedure of Ofodile *et al.* (2013). In this procedure, fresh leaves of guava were collected, washed thoroughly, shade-dried and pulverized using a mechanical grinder. The powder obtained was macerated with distilled water for 72 hours at 30±4°C. The mixture was filtered and the filtrate was concentrated in water bath at 50°C. Finally, the concentrated crude aqueous extract was subjected to drying to solid mass. The testing samples were prepared by dissolving the solid mass with distilled water to obtain a final concentration of 500 mg and 1000 mg/ml used for the experiment.

3.3 EXPERIMENTAL PROCEDURE

A previous study had established the LD_{50} of *Psidium guajava* leaves aqueous extract to be 5000 mg/kg bwt (Shubhangi, *et al.*, 2013) following the procedure of Organisation for Economic Co-operation and Development (OECD) guidelines (OECD, 2001). Therefore, doses of 10% (500 mg/kg bwt) and 20% (1000 mg/kg bwt) of the LD_{50} were used in this study.

Based on the reported oral LD_{50} of Lead acetate which was 600 mg/kg bwt for Wistar rats (Sujatha *et al.*, 2011), 20% of the LD_{50} (120 mg of lead acetate/kg bwt) was used in this study.

The dose of Succimer used was 10 mg/kg bwt according to Alan and Miller, (1998).

3.4 EXPERIMENTAL DESIGN

Thirty five (35) adult male Wistar rats were divided into 7 groups of 5 rats per group as shown in table 3.1.

Group 1 (Control) received 1 ml of distilled water for 35 days.

Group 2 was administered with 1000 mg/kg body weight (bwt) of aqueous extract of *Psidium gaujava* leaves only, for 35 days.

Group 3 was administered with 120 mg/kg bwt of lead acetate for 21 days and then the rats were sacrificed on day 22 of the administration to study the direct effect of lead acetate.

Group 4 was administered with 120 mg/kg bwt of lead acetate for 21 days and then administered with distilled water from day 22 to day 35 of the administration. This was to study if there will be any natural recovery that would take place after the administration of lead acetate when compared to Group 3.

Group 5 was administered with 120 mg/kg bwt of lead acetate for 21 days and then treated with 500 mg/kg bwt of aqueous extract of *Psidium gaujava* leaves from day 22 to day 35 of the administration.

Group 6 was administered with 120 mg/kg bwt of lead acetate for 21 days and then treated with 1000 mg/kg bwt of aqueous extract of *Psidium gaujava* leaves from day 22 to day 35 of the administration.

Group 7 was administered with 120mg/kg bwt of lead acetate for 21 days and treated with 10 mg/kg bwt of Succimer as standard treatment drug from day 22 to day 35 of the administration.

All the administrations were carried out orally once per day for 35 days.

Table 3.1: Grouping of animals and their treatment

Groups	No of rats	Administration (dosage/kg bwt)	Duration
1	5	Distilled water (Control)	Day 1 to day 35
2	5	1000mg *Psidium gaujava*	Day 1 to day 35
3	5	120mg Lead acetate and then Sacrificed on day 22	Day 1 to day 21
4	5	120mg Lead acetate + Distilled water	Day 1 to day 21 Day 22 to day 35
5	5	120mg Lead acetate + 500mg *Psidium gaujava*	Day 1 to day 21 Day 22 to day 35
6	5	120mg Lead acetate + 1000mg *Psidium gaujava*	Day 1 to day 21 Day 22 to day 35
7	5	120mg Lead acetate + 10mg Succimer	Day 1 to day 21 Day 22 to day 35

3.5 NEUROBEHAVIOURAL STUDIES

3.5.1 Morris Water Maze test

The Morris water navigation task is a behavioural procedure designed to test spatial learning and memory in the test rats according to the methods of Morris (1981), which was further modified by Monfort *et al.* (2007).

3.5.1.1 Procedure

The Morris water maze is a circular pool of water which portioned into four quadrants and an escape platform hidden a few millimeters below the water surface as shown in figure 3.1. The rats were released and allowed to swim around the pool in search of an exit and subsequent trials were performed to know if the rat was able to locate the platform in a shorter time. The rats were trained to locate the hidden platform and improvement in latency to find the platform was recorded (Monfort *et al.*, 2007) and the training lasted for one week. After learning, the latencies to locate the platform by each rat were recorded weekly during the experimental period.

Figure 3.1: Photograph of Morris water maze test pool: (A) the setup of the pool (B) dropping of the rat in the pool (C) rat swimming in searching the platform for escape (D) rat on the platform.

3.5.2 Beam Balance (Beam walking) test

The Balance beam test is a test of motor coordination (Goldstein and Davis, 1990; Goldstein, 1993; Metz, 2000; Cummings, 2007). It is also useful for sedation (Groves *et al.*, 2006) and joint pathology (Sakic *et al.*, 1996) studies.

3.5.2.1 Procedure

The beam has a starting point and an elevated end point attached with a cage as shown in figure 3.2. The rats were exposed to treats (roasted fish) in the home cage of balance beam before training to reduce neophobia. During the training, each of the animals was allowed to cross the beam, with gentle or prodding as needed, until they cross more stable and more accurately. The training lasted for seven days and the time taken to cross the beam was recorded. After the training, the above procedure was repeated weekly during the experimental period and the latencies to cross the beam were recorded.

Figure 3.2: Photograph of beam balance test; (A) setup of the beam with the rat in the home cage of balance beam (B) guiding the rat at the start point to walk along the beam to the end pound (C) the rat walking along the beam towards the cage (D) the rat enter the cage. SP: Starting pint, EP: End point, B: Beam and HC: Home cage

3.6 WISTAR RATS SACRIFICE

After the last administration, the rats were fasted for 24 hours before they were humanely anaesthesized by using ketamine. Blood samples were collected via heart puncture from each rat and the blood was divided into two (2). One half was put in Tripotassium salt of Ethylenediamine tetra acetic acid (K_3 EDTA) as anticoagulant and blood was used for haematological estimation while the other half in plain bottles, anticoagulant free blood, was allowed to coagulate and serum was harvested for biochemical estimation.

Incision was made through the skin and muscles of the skull and the skull was opened through the mid sagittal suture and the brain tissues were removed and fixed in Bouin's fluid. The tissues were taken to the histology laboratory of Human Anatomy Department, Ahmadu Bello University, Zaria for tissue processing.

3.7 TISSUE PROCESSING

The tissues were fixed in Bouin's fluid for 48 hours for proper fixation. The tissues were processed routinely, sectioned at 5µm thick, stained using haematoxylin and eosin (H and E) technique and special stain using Cresyl Echt Violet method for the identification of neurons in tissue sections or the demonstration of both Nissl substance and nuclei or the loss of Nissl substance (Freida, 2007).

3.7.1 Haematoxylin and Eosin (H and E) Staining Method

The methods of H and E staining were carried out by de-waxing the tissue in two changes of xylene for three (3) minutes each, hydrated by passing them through descending grades of alcohol (100%, 95%, 90% and 70%) for three minutes each, then stained in Harris haematoxylin for ten minutes, and washed in tap water to remove excess stain. The slides were then flooded with acid alcohol for few seconds for differentiation and then washed in tap water again. The slides were then blued in Scott's tap water for five minutes and counter stained with Eosin for three minutes. The sections were rinsed in tap water, and then dehydrated in ascending grades of alcohol and cleared in xylene. The sections were then cover-sliped using a media (Freida, 2007). Sections of the tissues were viewed under light microscope and photomicrographs were made using digital Am scope (MD 900) microscope.

3.7.2 Special Stain Using Cresyl Echt Violet Method

Frozen sections were deparaffinized and hydrated to distilled water and then stain for 3 to 5 minutes in cresyl echt violet solution. The stained sections were rinsed in two changes of distilled water and placed in 95% alcohol for 30 seconds. Sections were transferred to absolute alcohol for 30 seconds and then placed in xylene and balsam-xylene mixture for 1 minute and 2 minutes respectively. Differentiations were made in absolute alcohol, two changes for 10 to 30 seconds each. The sections were taken through several changes of xylene and mounted with synthetic resin. Digital photomicrographs at the magnification of X 250 were taken from all the experimental groups with the aid of MD900 Am scope microscope digital camera (Freida, 2007).

3.8 MICROSCOPIC CELL COUNT

Brain cells such as Purkinje cells of the cerebellum, pyramidal cells of the cerebrum and hippocampus were counted using Digimizer image analysis software. Specific fields of the brain slides were snapped at X 100 and X 250 magnifications with the aid of MD900 am microscope digital camera and uploaded into the image area of the software and marker tool was used to click in the image on the cells to mark and count the number of each cell and the sum were automatically displayed in the statistics window and subjected to statistically analysis.

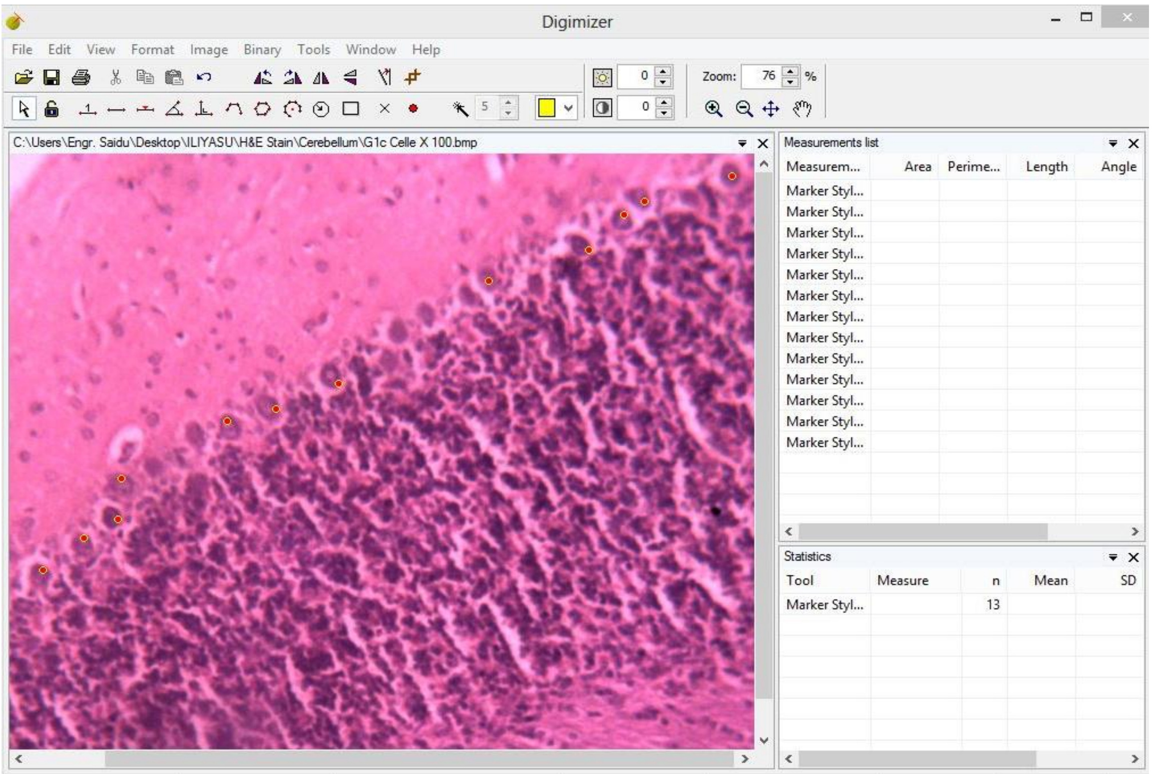

Figure 3.3: Window of Digimizer image analysis software with the uploaded cerebellar section in image area, statistic area and measurement area.

3.9 DETERMINATION OF HAEMATOLOGICAL PARAMETERS

The whole blood samples collected into anticoagulated (EDTA) specimen bottle were analysed within 6 hours of collection using systemex XE-2100 Automated Haematology Blood Analyser according to the method of Monica, (2000). Each blood sample was well mixed at room temperature using a blood rotor machine for five minutes. The authomated analyser was put on about 30 minutes for the system to warm. A 10 µl of blood sample was aspirated through tube of the machine. Haematological parameters such as differential leucocytes count (DLC), white blood cell (WBC) count red blood cell (RBC) count, packed cell volume (PCV), haemoglobin concentration (Hb) and Platelets count were analysed.

3.10 DETERMINATION OF OXIDATIVE STRESS PARAMETERS

3.10.1 Determination of catalase activity

Catalase activity was determined using the method described by Sinha (1972). In this procedure, 5 % Potassium heptaoxochromate (VI) $K_2Cr_2O_7$ was mixed with glacial acetic acid in the ratio 1:3, and stored in brown bottle at room temperature, after which 0.9 ml of distilled water was added to 0.1 ml of sera and mixed thoroughly. A 2.5 ml of phosphate buffer was put in a small conical flask; 0.5 ml of sera was added; and 2.0 ml of H_2O_2 added as well, starting the stop watch. The reaction mixture was thoroughly mixed and the reaction was stopped after every 60 seconds for 3 minutes with Dichromate/Acetic acid solution. This was heated in water bath for 10 minutes at 80°C. Absorbance was read at 570 nm. Standard curve was obtained using absorbance obtained at various H_2O_2.

Calculations: the quantity of H_2O_2 consumed was obtained from the graph of the catalase standard curve, this determined the catalase activity.

3.10.2 Determination of superoxide dismutase activity

Superoxide Dismutase (SOD) activity was determined by a method described by Fridovich, (1989). Based on this method, 0.1 ml of sera was diluted in 0.9 ml of distilled water to make 1:10 dilution of sera. An aliquant mixture of 0.20 ml of the diluted sera was added to 2.5 ml of 0.05 M Carbonate buffer. The reaction started with the addition of 0.3m1 of 0.3 mM Adrenaline. The reference mixture contains *2.5* ml of 0.05 M Carbonate buffer. A 0.3 m1 of 0.3 mM Adrenaline and 0.20 ml of distilled water were used. Absorbance was measured over 30 s up to 150 s at 480 nm.

Calculations: Increase in absorbance per minute = $(A5 - A_1)/2.5$

$$\% \text{ inhibition} = 100 - \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance of blank}} \times 100$$

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

3.10.3 Assessment of lipid peroxidation

Lipid peroxidation as evidenced by the formation of TBARS was measured by the modified method of Niehaus and Samuelson (1968). In this method, to 150 µl of sera, (O.25M sucrose solution) were treated with 2m1 of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.37%, 0.25N HCL and 15% TBA) and place in water bath for l hr at 90°C. The mixture was cooled and centrifuged at 3000 rpm for 5 mm at 4°C. The absorbance of the pink supernatant 2.0 ml was measured against a reference blank using spectrophotometer at 535 nm.

Calculations: The MDA was calculated using the molar extinction coefficient of 1.56 x 10 cmM

MDA conc. = absorbance/ 1.56 $x10^5 cm-^1 M^{-1}$ x l

3.10.4 Assay of reduced Glutathione concentration

Reduced glutathione (GSH) concentration measurements were done according to the method of Ellman, (1959). In this procedure, 0.2M phosphate buffer: 8.40 g of NaH_2PO_4 and 9.94 of Na_2HPO_4 was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask; the buffer was then adjusted to pH 8.

To 150 µl of serum (in phosphate -saline PH 7.4), 1.5 m1 of 10% TCA was added and centrifuged at 1 500 g for 5mm. A 1 ml of supernatant was treated with 0.5 ml of Ellman’s reagent (19.8 mg of 5, 5’-dithiobis (nitro benzoic acid) (DNTB) in 100 ml of 0.1% sodium nitrate) and 3 ml of phosphate buffer (0.2 M, p1-I 8). The absorbance was read at 412 nm.

3.11 DATA ANALYSIS

Data obtained were expressed as mean ± SEM (standard error of mean). One-way analysis of variance (ANOVA) was used to compare the mean differences followed by Tukey’s post-hoc test. P-value less than to 0.05 was considered to be statistically significant. All the results were analysed using the Statistical Package for Social Sciences (SPSS *version 16*).

CHAPTER FOUR

RESULTS

4.1 GENERAL OBSERVATION

4.1.1 Physical changes in the Rats

There were no visible physical changes observed in the Control Group (Group 1) and Group 2 after administration of distilled water and extract, respectively, all the rats were very active. Scratching of the nose was observed in rats from Groups 3, 4, 5 6 and 7 immediately after administration of lead acetate from the1st to the 3rd weeks of the experiment. Five hours after administration, there were restlessness, reduction in physical activities, gnawing, exophthalmus, frequent urination and watery faeces in the rats administered with lead acetate.

4.1.2 Movement of Rats on Balance Beam test

At the end of the training, all the rats were able to cross the beam with gentle guide within five seconds with fine movement and no tremor observed. Throughout the administration, groups one and two administered distilled water and aqueous extract of *Psidium guajava*, respectively, were able to cross the beam within five seconds with fine movement and no tremor. There were increased in time, serious tremor, poor movement and stability in crossing the beam of rats administered lead acetate (groups 3 to 7) from the 1st to the 3rd week of administration. From the 4th to 5th week of administration of extract groups 5, 6 and Succimer group 7, the rats were able to cross the beam with shorter latencies as shown in figure 4.1.

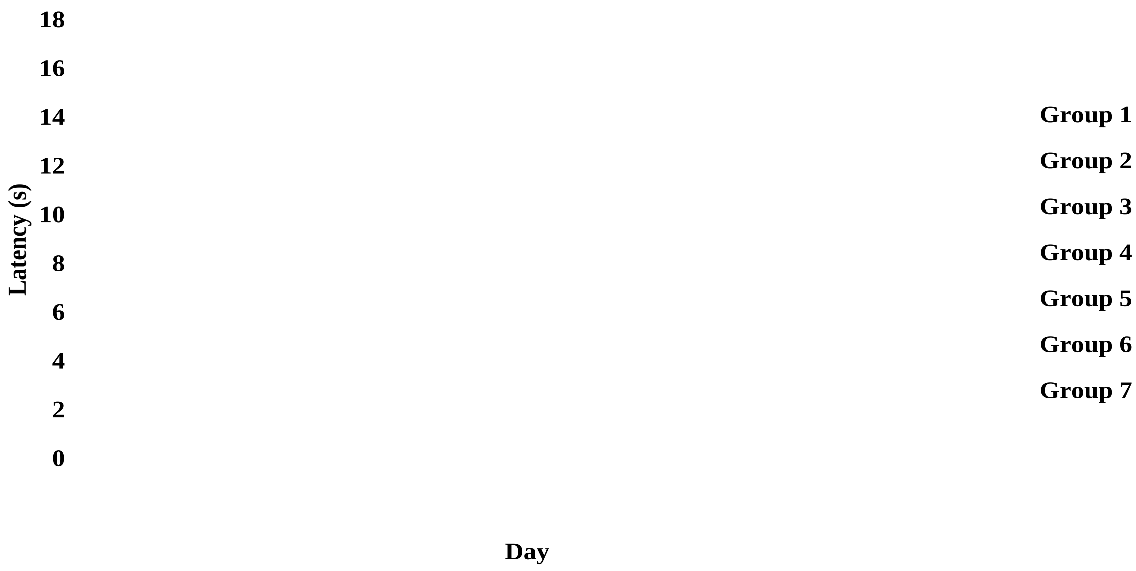

Figure 4.1: Mean latencies of all the groups in crossing the beam balance during the training and the administration periods of the experiment. The increased in mean of Groups 3 to 7 during weeks 1, 2 and 3 were significant when compared with groups 1 and 2 at $p < 0.05$.

4.1.3 Spatial Learning and Memory of the Rats

At the end of the training, all the rats were able to locate the platform in the pool within six seconds. Throughout the administration, rats in Groups 1 and 2 administered with distilled water and aqueous extract of *Psidium guajava*, respectively, were able to locate the platform within six seconds. While there was increase in time taken to locate the platform in Groups 3 to 7 administered with 120 mg/kg of lead acetate from the 1st to the 3rd weeks of administration, there was decrease in time taken for the rats to locate the platform during 4th to 5th weeks of administration of aqueous extract of *Psidium guajava* to Groups 5, 6 and Succimer Group 7 as shown in figure 4.2.

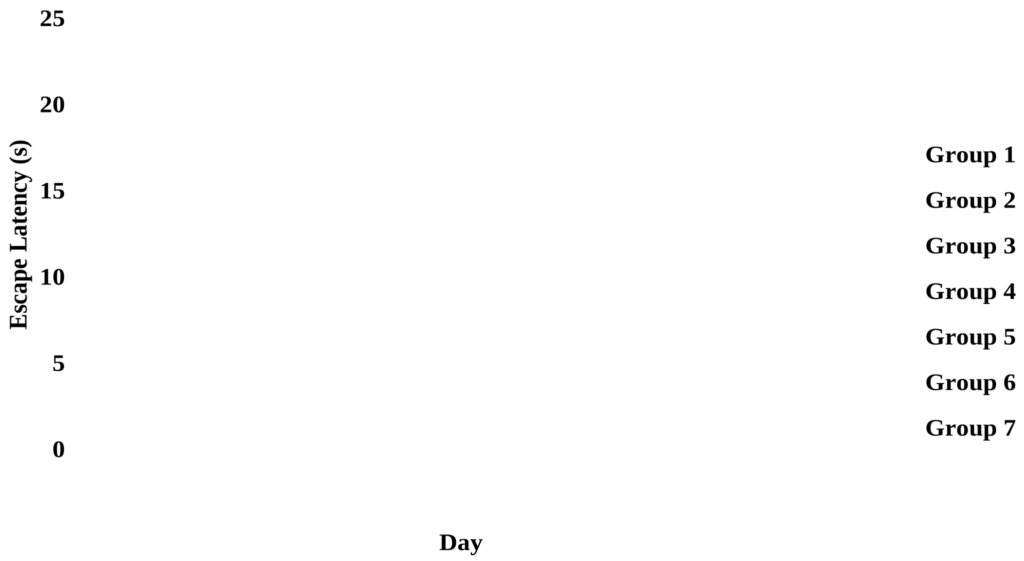

Figure 4.2: Mean latencies of all the groups in Morris water maze test during the training and the administration periods of the experiment. The increased in mean of Groups 3 to 7 during weeks 1, 2 and 3 were significant when compared with groups 1 and 2 at $p < 0.05$.

4.2 ANALYSIS OF NEUROBEHAVIOURAL STUDY

4.2.1 Motor coordination in the Rats

The results of balance and coordination using balance beam test (Table 4.1) showed that there were no significant differences in the mean latencies across the groups at the last day of the training. There were also no significant differences in the mean latencies between Group 1 (Control) and Group 2 (1000 mg/kg of extract) throughout the experimental period. There was no significant increase in the mean latency in Group 3 (120 mg/kg of lead acetate) for the first three weeks of administration when compared with Groups 1 and 2. On the other hand, there was a significant increase in the mean latency in Group 4 (120 mg/kg of lead acetate) from the 1st week to the 5th week except week 4 of the experiment when compared with Groups 1 and 2. There was an insignificant increase in the mean latency in Group 5 for the first three weeks (120 mg/kg of lead acetate) and an insignificant change for the last 2 weeks (500 mg/kg of extract) of administration when compared with Groups 1 and 2 ($p > 0.05$). The increased in the mean latency in Group 6 for the first three weeks (120 mg/kg of lead acetate) was significant in the 2nd and the 3rd weeks of administration when compared with Groups one and two and this later decreased in the last two weeks (1000 mg/kg of extract) of administration with an insignificant changes when compared with Groups one and two ($p > 0.05$). There was also an insignificant increase in the mean latency in Group 7 for the first 3 weeks of administration when compared with Groups 1 and 2 and later decreased in the last two weeks (10 mg/kg of Succimer) of administration with insignificant changes when compared with Groups 1 and 2 ($p > 0.05$) as shown in Table 4.1.

Table 4.1: Motor activity measure in time (s) taken to cross the beam balance

Group	Administration	Training	1st Week	2nd Week	3rd Week	4th Week	5th Week
		Mean±SEM(s)	Mean±SEM(s)	Mean±SEM(s)	Mean±SEM(s)	Mean±SEM(s)	Mean±SEM(s)
1	(Distilled Water)	4.25 ± 0.44	4.11 ± 0.23	4.44 ± 0.46	4.39 ± 0.16	4.39 ± 0.37	3.06 ± 0.17
2	($AEPG_{1000}$)	4.12 ± 0.74	3.97 ± 0.58	3.72 ± 0.96	3.79 ± 0.90	3.67± 0.84	3.09± 0.60
3	(Pb^{2+}) 1st – 3rd wks	3.06 ± 0.39	5.61 ± 0.55	7.20 ± 0.84	8.93 ± 1.60	-	-
4	(Pb^{2+} + Dist. H_2O)	4.25 ± 0.28	8.83 ± 0.88*	11.03 ± 0.92*	12.79 ± 1.27*	7.97 ± 0.98	7.96 ± 2.33*
5	(Pb^{2+} + $AEPG_{500}$)	2.61 ± 0.48	3.71 ± 0.68	6.42 ± 0.43	10.21 ± 3.29	4.17 ± 0.23	4.57± 0.34
6	(Pb^{2+} + $AEPG_{1000}$)	2.62 ± 0.33	5.66 ± 0.84	11.21 ± 2.81*	12.90 ± 1.14*	6.12 ± 1.53	4.26 ± 0.34
7	(Pb^{2+} + SUC_{10})	2.94 ± 0.50	4.26 ± 0.39	9.03 ± 0.83	9.71 ± 0.92	4.92 ± 1.35	3.13± 0.40

Pb^{2+}: Lead acetate, $AEPG_{1000}$: 1000 mg/kg bwt of aqueous extract of *Psidium guajava*, $AEPG_{500}$: 500 mg/kg bw of aqueous extract of *Psidium guajava*, SUC_{10}: 10 mg/kg bwt of Succimer (standard drug).
*: Significantly different from the control group and group 2 ($p < 0.05$)

4.2.2 Test for spatial learning and memory using Morris Water Maze test

Spatial learning and memory was assessed by the reduction in latency to find the hidden platform in Morris Water Maze test (Table 4.2) and this was clearly evident in all the groups during the training. Rats learnt the task, and every day required less time to find the hidden platform. During the learning phase of the spatial learning and memory, no significant difference in the mean latencies was observed in all the Groups at the 7^{th} day (last day). However, at 1^{st}, 2^{nd} and, 3^{rd} weeks in lead acetate administered groups (Groups 3 to 7), the rats significantly used more time to locate the hidden platform compared to Groups 1 and 2 ($p < 0.05$). At weeks 4 and 5, Groups 1 and 2 used almost the same time to locate the hidden platform as compared to initial Pb exposed rats (Groups 5 to 7) which were later administered with aqueous extract of *Psidium guajava* (Groups 5 and 6) and Succimer (Group 7). But Group 4 (untreated group with neither extract nor Succimer) used significantly more time to find the hidden platform compared with the control, extract and Succimer administered rats ($p < 0.05$). Pb exposure significantly decreased spatial learning and memory in lead acetate administered rats as compared to the Control and extract administered rats ($p < 0.05$) as shown in Table 4.2.

Table 4.2: Mean latencies for spatial learning and memory using Morris water maze test

		Training	1st Week	2nd Week	3rd Week	4th Week	5th Week
Group	**Administration**	**Mean±SEM(s)**	**Mean±SEM(s)**	**Mean±SEM(s)**	**Mean±SEM(s)**	**Mean±SEM(s)**	**Mean±SEM(s)**
1	(Distilled Water)	4.45 ± 0.06	4.39 ± 0.10	4.13 ± 0.89	4.26 ± 0.23	4.13 ± 0.24	4.31 ± 0.32
2	($AEPG_{1000}$)	5.56 ± 0.46	5.22 ± 0.22	5.06 ± 0.43	4.56 ± 0.21	4.02 ± 0.04	3.95 ± 0.07
3	(Pb^{2+}) 1st – 3rd wks	4.81 ± 0.23	7.35 ± 0.27*	7.99 ± 0.57*	8.94 ± 1.22*	-	-
4	(Pb^{2+} + Dist. H_2O)	5.07 ± 0.22	7.51 ± 0.18*	8.18 ± 0.38*	8.18 ± 0.18*	6.23 ± 0.13*	6.47 ± 0.25*
5	(Pb^{2+} + $AEPG_{500}$)	4.72 ± 0.15	7.22 ± 0.18*	9.63 ± 2.05*	9.48 ± 0.73*	4.54 ± 0.39	4.75 ± 0.35
6	(Pb^{2+} + $AEPG_{1000}$)	5.08 ± 0.37	8.10 ± 0.19*	9.74 ± 0.94*	10.91 ± 1.50*	3.95 ± 0.36	3.84 ± 0.36
7	(Pb^{2+} + SUC_{10})	5.54 ± 0.56	8.60 ± 1.05*	8.40 ± 0.58*	8.14 ± 0.23*	4.02 ± 0.05	3.83 ± 0.05

Pb^{2+}: Lead acetate, $AEPG_{1000}$: 1000 mg/kg bwt of aqueous extract of *Psidium guajava*, $AEPG_{500}$: 500 mg/kg bwt of aqueous extract of *Psidium guajava*, SUC_{10}: 10 mg/kg bwt of Succimer (standard drug).

*: Significantly different from the control Group and Group 2 ($P < 0.05$)

4.3 ANALYSIS OF OXIDATIVE STRESS MARKERS

The study revealed a significant increase in lipid peroxidation levels among rats in groups 3, 4 and 5 (Table 4.3) when compared with those in group 1 ($p < 0.05$). On the other hand, there was a significant decrease in catalase levels in rats of groups 3 and 4 ($p < 0.05$) compared to the value obtained in group 1. While there were insignificant differences in the levels of superoxide dismutase in all the groups and the mean glutathione levels were significantly lowered in groups 3 ($p < 0.05$) when compared with group 1.

Table 4.3: Oxidative stress markers of the Wistar rats

Group	Administration	LPO (μM)	CAT (U/mL)	SOD (U/mL)	GSH(U/ml)
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
1	(Distilled Water)	2.23 ± 0.09	44.67 ± 0.88	1.80 ± 0.15	2.40 ± 0.06
2	($AEPG_{1000}$)	2.15 ± 0.06	45.00 ± 1.68	1.90 ± 0.07	2.33 ± 0.13
3	(Pb^{2+}) 1^{st} – 3^{rd} wks	4.08 ± 0.05*	38.75 ± 1.31*	1.50 ± 0.13	1.93 ± 0.09*
4	(Pb^{2+} + Dist. H_2O)	4.00 ± 0.06*	39.67 ± 0.88*	1.73 ± 0.09	1.97 ± 0.03
5	(Pb^{2+} + $AEPG_{500}$)	2.73 ± 0.11*	42.75 ± 0.25	1.80 ± 0.18	2.30 ± 0.09
6	(Pb^{2+} + $AEPG_{1000}$)	2.45 ± 0.10	43.00 ± 0.41	1.75 ± 0.16	2.35 ± 0.06
7	(Pb^{2+} + SUC_{10})	2.30 ± 0.20	43.67 ± 0.33	1.83 ± 0.15	2.50 ± 0.06

LPO: Lipid Peroxidation, CAT: Catalase, SOD: Superoxide Dismutase and GSH: Reduced Glutathione. Pb^{2+}: Lead acetate, $AEPG_{1000}$: 1000mg/kg bwt of Aqueous extract of *Psidium guajava*, $AEPG_{500}$: 500 mg/kg bwt of aqueous extract of *Psidium guajava*, SUC_{10}: 10 mg/kg bwt of Succimer (standard drug).

*: Significantly different from Control Group ($P < 0.05$)

4.4 HAEMATOLOGICAL ANALYSIS

The results showed that the mean Red blood cell (RBC) count, Haemoglobin concentration (Hb) and Packed cell volume (PCV) values were reduced in Groups 3 and 4, though the differences were not statistically significant when compared with Group 1 ($p > 0.05$). However, the mean RBC and Hb values increased insignificantly in Groups 2, 5, 6 and 7 compared to group 1. It was observed that the mean RBC was significantly decreased in Groups 3 and 4 when compared with Group 2 ($p < 0.05$). The mean Platelet count (PLT) increased insignificantly in Groups 3, 4, 5, 6 and 7 when compared with Group 1 ($p < 0.05$). The mean total White blood cells (WBC) counts were found to have increased in Groups 3 and 4. However, these increase observed were not statistically significant ($p > 0.05$). The mean values of differential leucocytes counts with respect to lymphocytes, neutrophils, eosinophil and monocytes also varied insignificantly among the groups (Table 4.4).

Table 4.4: Haematological indices of the Wistar rats

Parameters	Group 1 (Distilled H_2O)	Group 2 ($AEPG_{1000}$)	Group 3 (Pb^{2+}) 1st–3rd wk	Group 4 (Pb^{2+} + Dist.H_2O)	Group 5 (Pb^{2+} + $AEPG_{500}$)	Group 6 (Pb^{2+} + $AEPG_{1000}$)	Group 7 (Pb^{2+} + SUC_{10})
RBC (X 10^{12}/L)	6.36 ± 0.63	6.54 ± 0.37	4.12 ± 0.79*[a]	4.57 ± 0.23*	6.40 ± 0.42	6.56 ± 0.51	6.69 ± 0.26
Hb (g/L)	11.97 ± 0.75	12.18 ± 0.28	9.58 ± 0.35	10.27 ± 0.27	12.02 ± 0.73	12.24 ± 0.60	11.93 ± 0.37
PCV (%)	41.67 ± 1.20	40.60 ± 1.96	36.00 ± 2.00	38.60 ± 1.03	39.00 ± 1.41	40.40 ± 0.98	39.00 ± 0.58
PLT (X 10^9/L)	447.0 ± 8.02	433.2 ± 63.90	507.0 ± 75.00	477.4 ± 42.35	466.0 ± 15.34	459.8 ± 32.30	449.3 ± 14.40
WBC (X 10^9/L)	8.23 ± 0.58	7.80 ± 0.27	9.10 ± 0.80	8.43 ± 0.28	7.94 ± 0.09	7.86 ± 0.28	7.44 ± 0.64
Lymp (%)	83.47 ± 4.57	82.38 ± 1.35	77.80 ± 1.50	79.82 ± 2.53	79.81 ± 3.10	82.70 ± 3.99	78.10 ± 4.84
Neut (%)	13.30 ± 2.81	15.62 ± 1.74	19.45 ± 1.15	18.70 ± 2.26	18.54 ± 2.90	15.30 ± 3.22	19.33 ± 3.48
Eos (%)	2.60 ± 0.60	1.30 ± 0.61	2.30 ± 0.00	0.98 ± 0.44	1.72 ± 0.52	1.40 ± 0.60	1.43 ± 0.43
Mono (%)	0.30 ± 0.30	0.66 ± 0.35	0.45 ± 0.35	0.10 ± 0.06	0.56 ± 0.35	0.60 ± 0.40	1.03 ± 1.03
Baso (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

RBC: Red blood cell, Hb: Haemoglobin concentration, PCV Packed cell volume, PLT: Platelet count, WBC: White blood cells, Nuet: Percent neutrophil count, Lymp: Percent lymphocyte count, Mono: Percentage monocytes, Baso: Percentage Basophil Pb^{2+}: Lead acetate, $AEPG_{1000}$: 1000 mg/kg bwt of aqueous extract of *Psidium guajava*, $AEPG_{500}$: 500 mg/kg bwt of aqueous extract of *Psidium guajava*, SUC_{10}: 10 mg/kg bwt of Succimer (standard drug).

*: significantly different from group 2 ($p < 0.05$)

[a]: significantly different from group 6 and 7 ($p < 0.05$)

4.5 HISTOLOGICAL OBSERVATIONS

4.5.1 Observation in number of cells in Hippocampus, Cerebellum and Cerebrum

The results from the study showed a significant decrease in pyramidal cells of the hippocampus among the rats in Groups 3 and 4 when compared to those in Groups 1, 2, 5, 6 and 7. There was also a significant difference in pyramidal cells among rats in Groups 3 and 4. The Purkinje cells decreased significantly among the rats in Groups 3, 4, 5, 6 and 7 compared to those in groups 1 and 2. However, there was significant decreased in Purkinje cells in Group 3 compared to Groups 5, 6 and 7 while in Group 4 the decrease was significant when compared with Group 7 only. There was decrease in pyramidal cells of the cerebrum among the rats in Groups 3, 4, 5, 6 and 7 when compared with those in Groups 1 and 2. On the other hand, there was significant decrease in pyramidal cells among the rats in Groups 3 and 4 when compared with those in Group 7.

Table 4.5: Number of cells counts in hippocampus, cerebellar and cerebral sections of the Wistar rats

Group	Administration	Hippocampus (Pyramidal cell)	Cerebellum (Purkinje Cell)	Cerebrum (Pyramidal cell)
1	(Distilled Water)	30.00 ± 0.86	13.00 ± 0.71	14.00 ± 1.21
2	($AEPG_{1000}$)	29.00 ± 0.51	13.00 ± 1.07	12.00 ± 1.21
3	(Pb^{2+}) 1st – 3rd wks	14.00 ± 1.22*[a]	3.00 ± 0.71*[b]	4.00 ± 0.71*[d]
4	(Pb^{2+} + Dist. H_2O)	23.00 ± 0.93*	4.00 ± 0.45*[c]	5.00 ± 0.75*[d]
5	(Pb^{2+} + $AEPG_{500}$)	29.00 ± 1.23	6.00 ± 0.37*	7.00 ± 0.55*
6	(Pb^{2+} + $AEPG_{1000}$)	28.00 ± 1.40	6.00 ± 0.58*	7.00 ± 0.58*
7	(Pb^{2+} + SUC_{10})	28.00 ± 0.81	8.00 ± 0.49*	9.00 ± 0.32*

Pb^{2+}: Lead acetate, $AEPG_{1000}$: 1000 mg/kg bwt of aqueous extract of *Psidium guajava*, $AEPG_{500}$: 500mg/kg bwt of aqueous extract of *Psidium guajava*, SUC_{10}: 10 mg/kg bwt of Succimer (standard drug).

* = Significantly different from groups 1 and 2 ($p < 0.05$)
[a] = significantly different from group 4 ($p < 0.05$)
[b] = significantly different from group 6 and 7 ($p < 0.05$)
[c] = significantly different from group 7 ($p < 0.05$)
[d] = significantly different from group 7 ($p < 0.05$)

4.5.2 Histopathology of cerebellum of the rats

The cerebellar cortex examined had three layers: an outer molecular layer (ML), middle Purkinje layer (PL), and inner granular layer (GL). The Purkinje layer was made up of one layer of Purkinje cells (PC) which are formed by large pyriform or flask in sharp with large vesicular nucleus and granular cytoplasm. The cerebellar cortex of the rats in Group 1 (Plates I & II) and Group 2 (Plates III & IV) showed cytoarchitecture of the molecular, Purkinje and granular layers which appeared normal. The cerebellar cortex of the rats in Group 3 (Plate V) revealed the Purkinje layer as the most affected area. There was increase in the sparsity of Purkinje cells in the Purkinje layer compared to Control Group and Group 2. Some of the Purkinje cells were necrotized or pyknotic (Plate VI). In plate VI, the Purkinje layer was highly distorted but little distortion of the granular layer, and no distortion of the molecular layer was observed. The cerebellar cortex of the rats in Group 4 (Plate VII) revealed similar features as in Group 3. The cytoarchitecture of the molecular, granular and Purkinje layers were irregular in outline of Purkinje cells (Plate VIII). The cerebellar cortex of the rats in Group 5 showed normal cytoarchitecture of the molecular and granular layers, while the Purkinje layer was irregular outline of some Purkinje cells (Plates IX & X). The cerebellar cortex of the rats in Group 6 showed normal orientation of the Molecular and Granular layers but cells of the Purkinje layer were irregular outline (Plates XI & XII). The cerebellar cortex of the rats in Group 7 showed normal orientation of the molecular, granular, and Purkinje layers were irregular in outline of some Purkinje cells (Plates XIII & XIV).

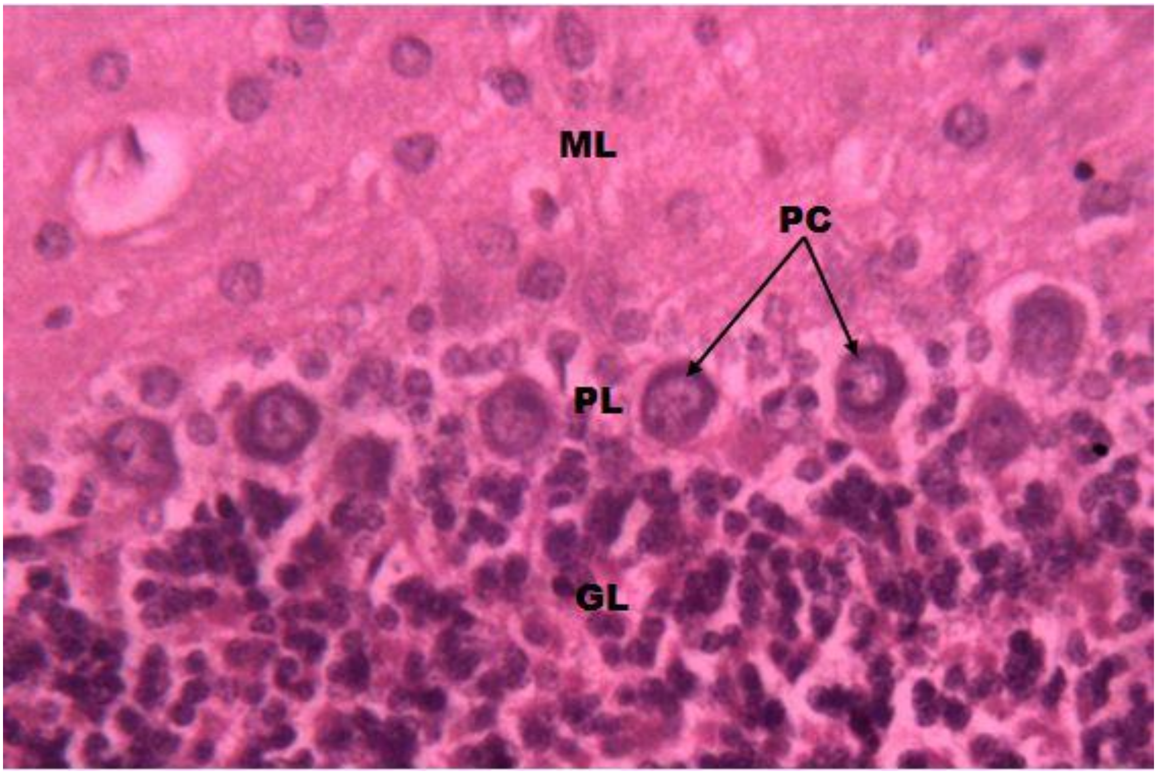

Plate I: A section of the cerebellum in Group 1 (Control group) showing normal orientation of the molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with normal Purkinje cell (PC) (H & E Stain Mg X 250).

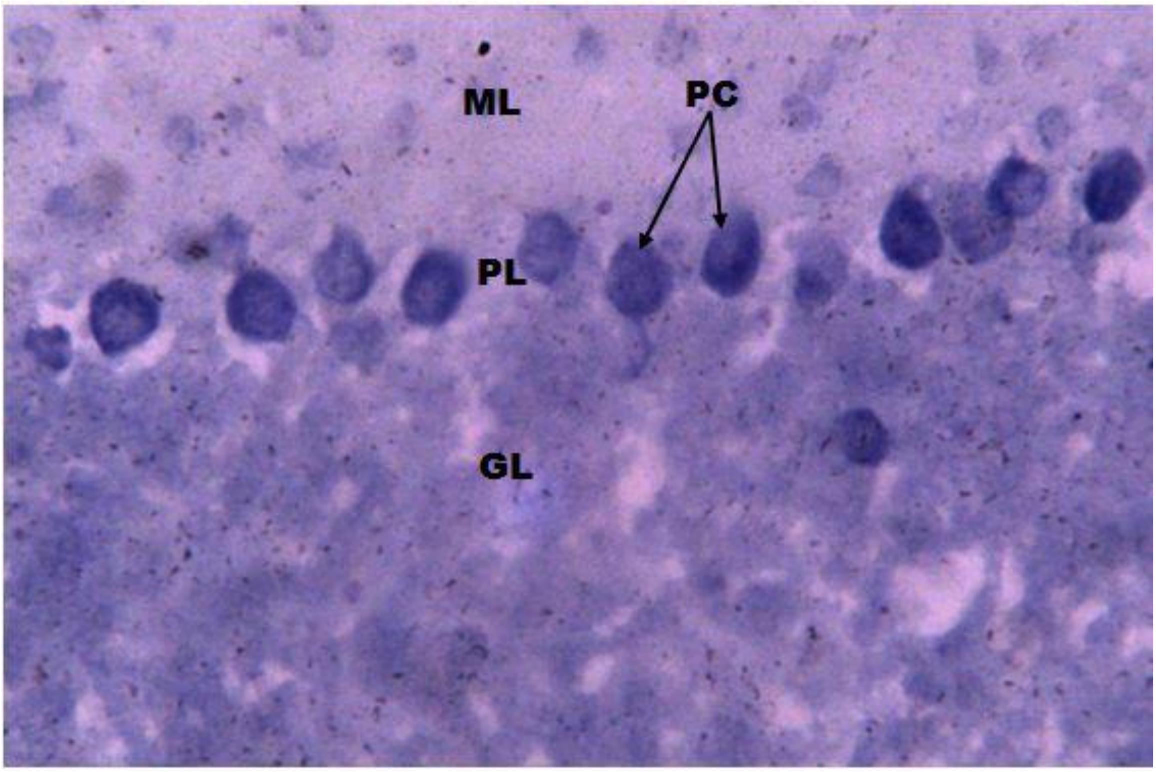

Plate II: A section of the cerebellum in Group 1 (control group) showing normal orientation of the molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with normal Purkinje cells (PC) (Cresyl Echt Violet Stain Mg X 250).

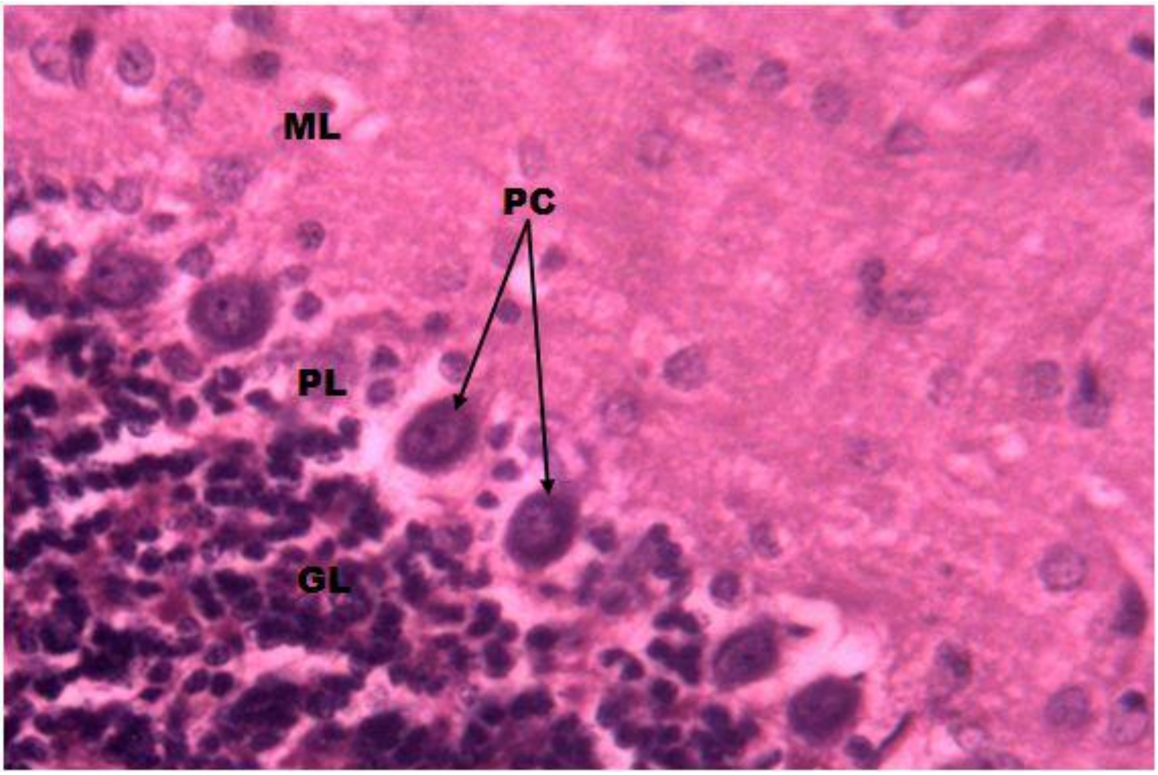

Plate III: A section of the cerebellum in group 2 (1000 mg/kg of extract treated rats) showing normal orientation of the molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with normal Purkinje cells (PC) (H & E Stain Mg X 250).

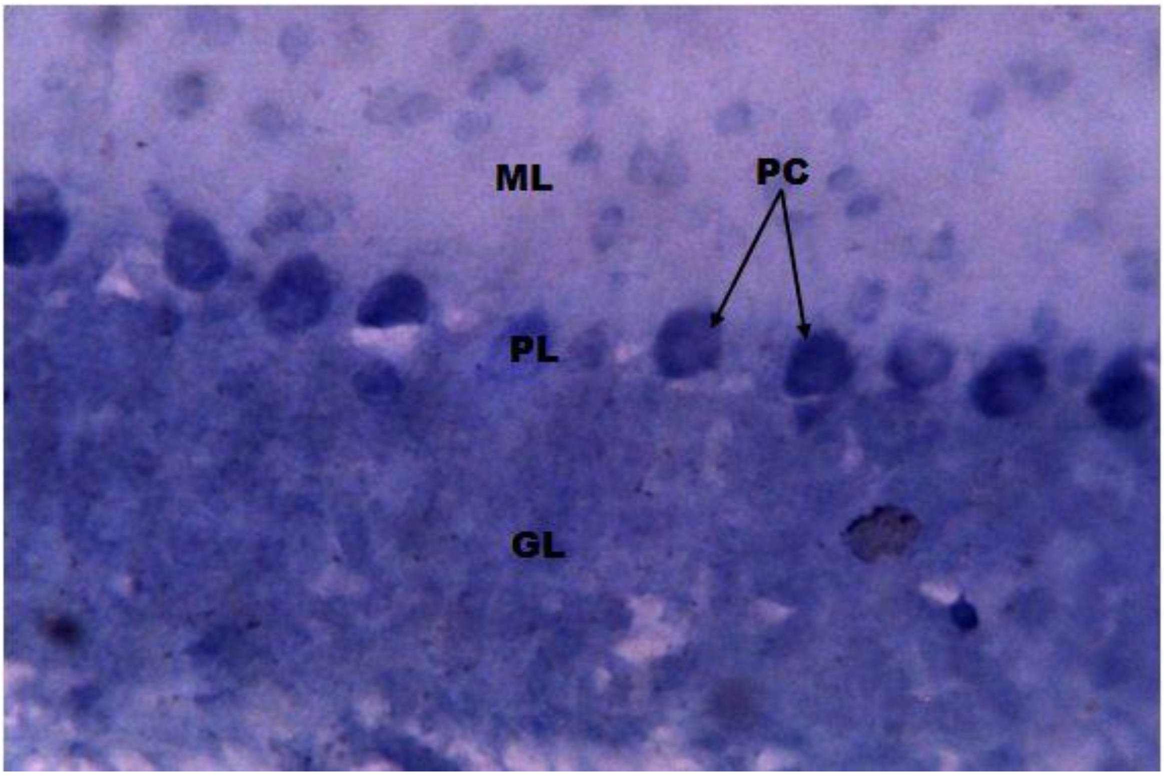

Plate IV: A section of the cerebellum in Group 2 (1000 mg/kg of extract treated rats only) showing normal orientation of the molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with normal Purkinje cells (PC) (Cresyl Echt Violet Stain Mg X 250).

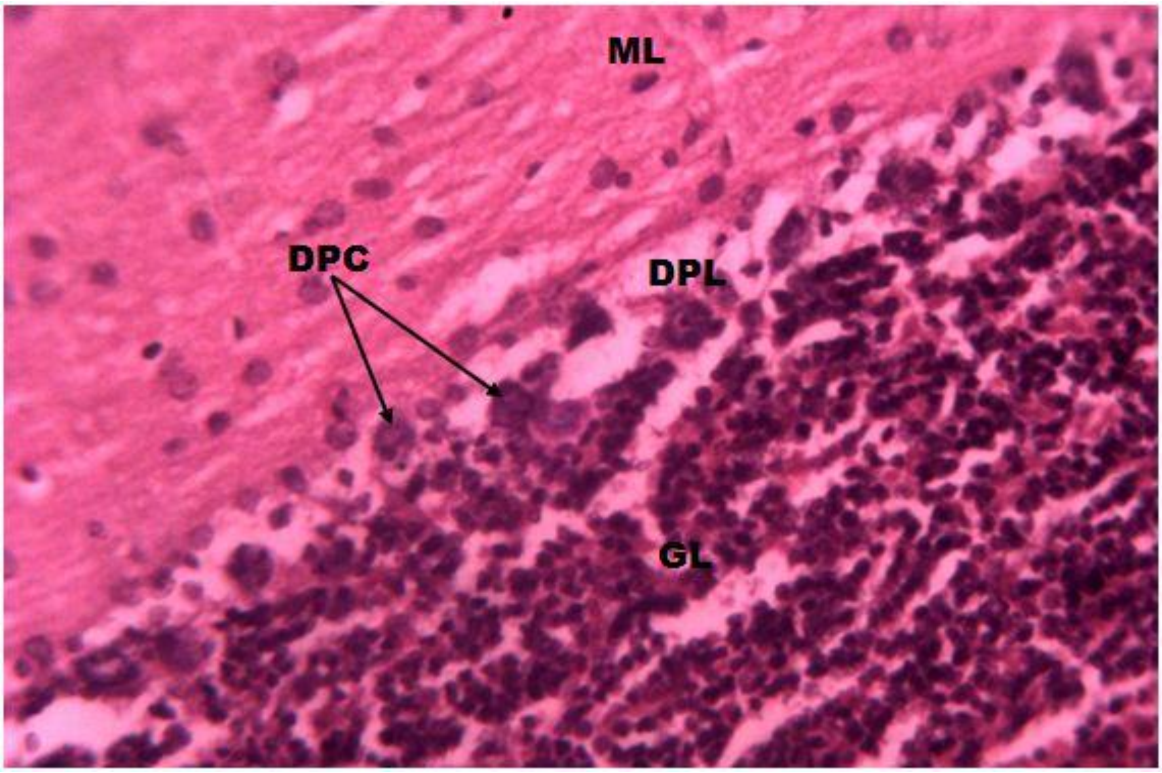

Plate V: A section of the cerebellum in Group 3 (administered with 120 mg/kg bwt of lead acetate) showing normal orientation of the molecular layer (ML), clumping of granular cells in Granular layer (GL) and Distorted Purkinje layer (DPL) with degenerated Purkinje cells (DPC). Some of the DPC are fallen off leaving empty spaces while some of the PC are irregular in outline (H & E Stain Mg X 250).

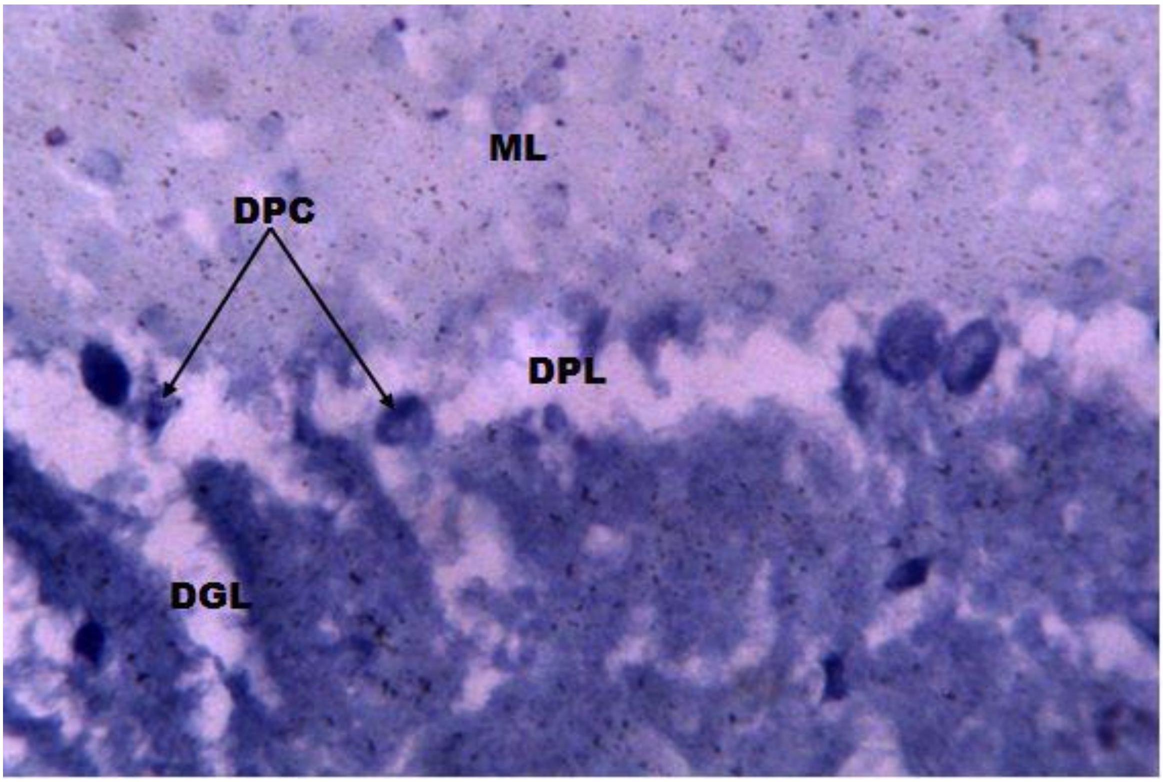

Plate VI: A section of the cerebellum in Group 3 (administered with 120 mg/kg bwt of lead acetate) showing orientation of the molecular layer (ML), distorted granular layer (DGL) and Purkinje layer (DPL) with more degenerated Purkinje cells (DPC). Some of the DPC are fallen off leaving empty spaces while some are irregular in outline (Cresyl Echt Violet Stain Mg X 250).

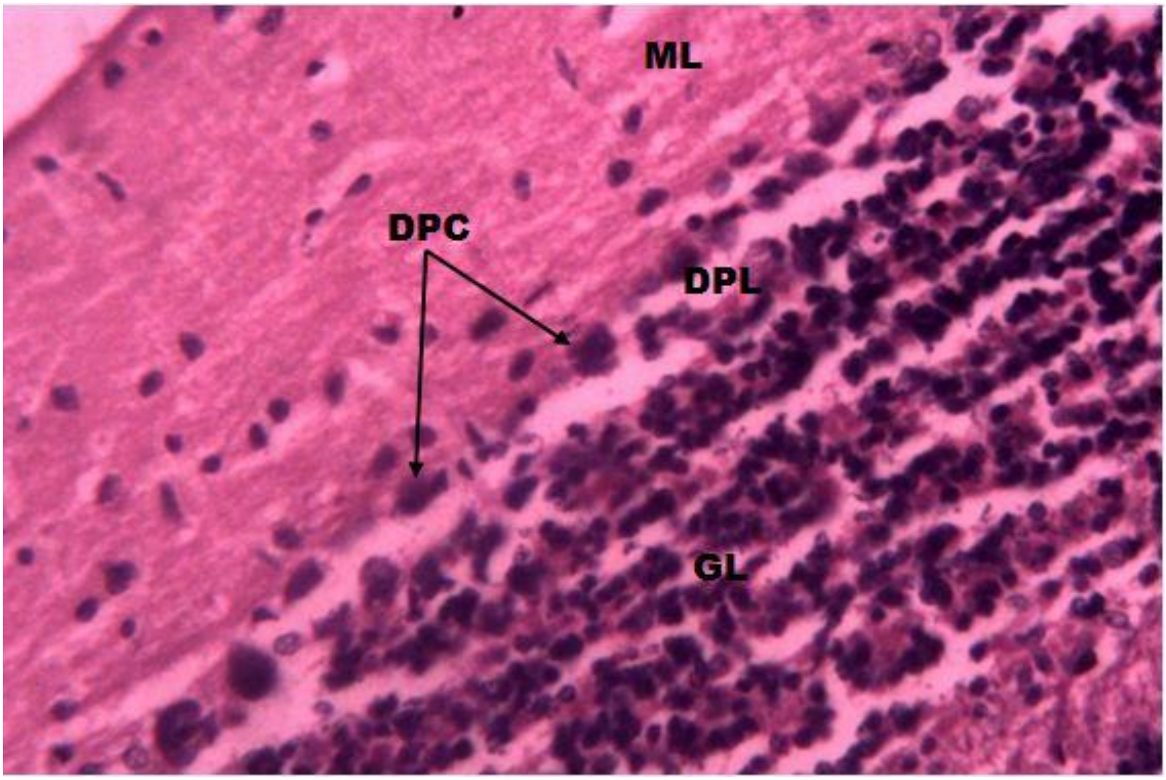

Plate VII: A section of the cerebellum in group 4 (administered with 120 mg/kg bwt of lead acetate and then withdrawn) showing normal orientation of the molecular layer (ML), clumping of granular cells in Granular layer (GL) and distorted Purkinje layer (DPL) with more sparse degenerated Purkinje cells (DPC). Some DGC were fallen off leaving empty spaces while some were irregular in outline (H & E Stain Mg X 250).

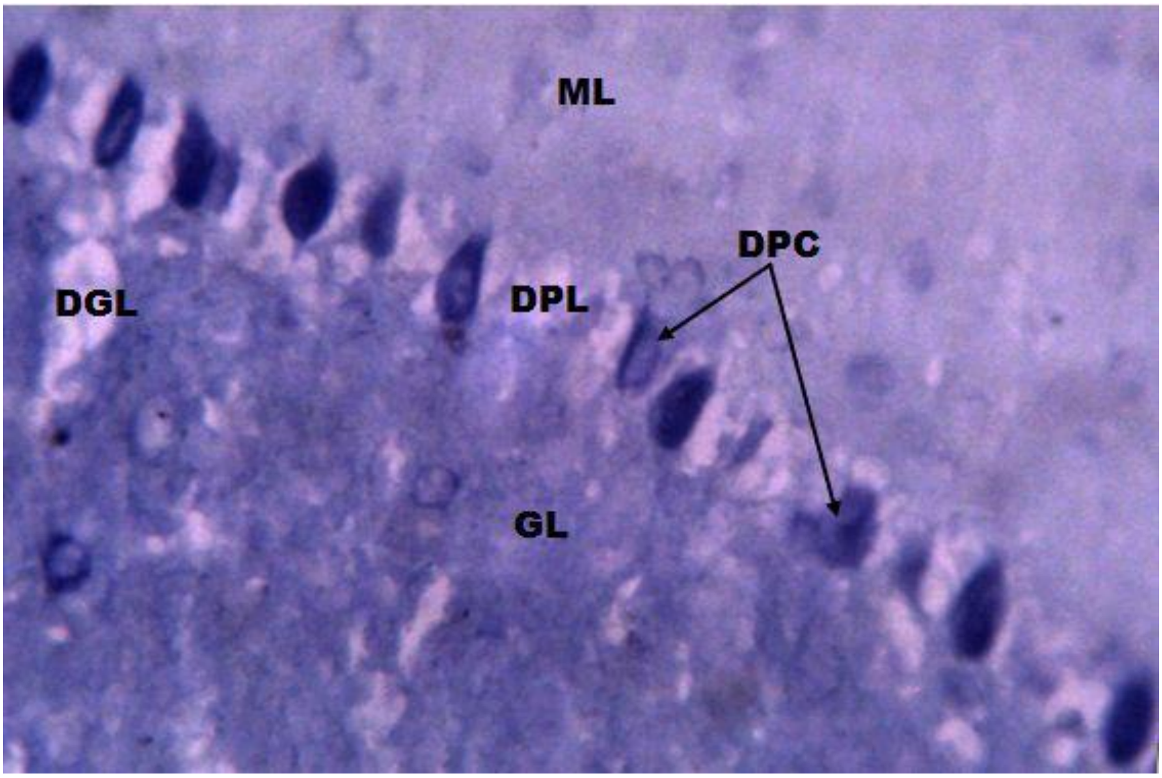

Plate VIII: A section of the cerebellum in Group 4 (administered with 120 mg/kg bwt of lead acetate and then withdrawn) showing orientation of the molecular layer (ML), distorted granular layer (DGL) and Purkinje layer (DPL) with irregular outline of some Purkinje cells (DPC) (Cresyl Echt Violet Stain Mg X 250).

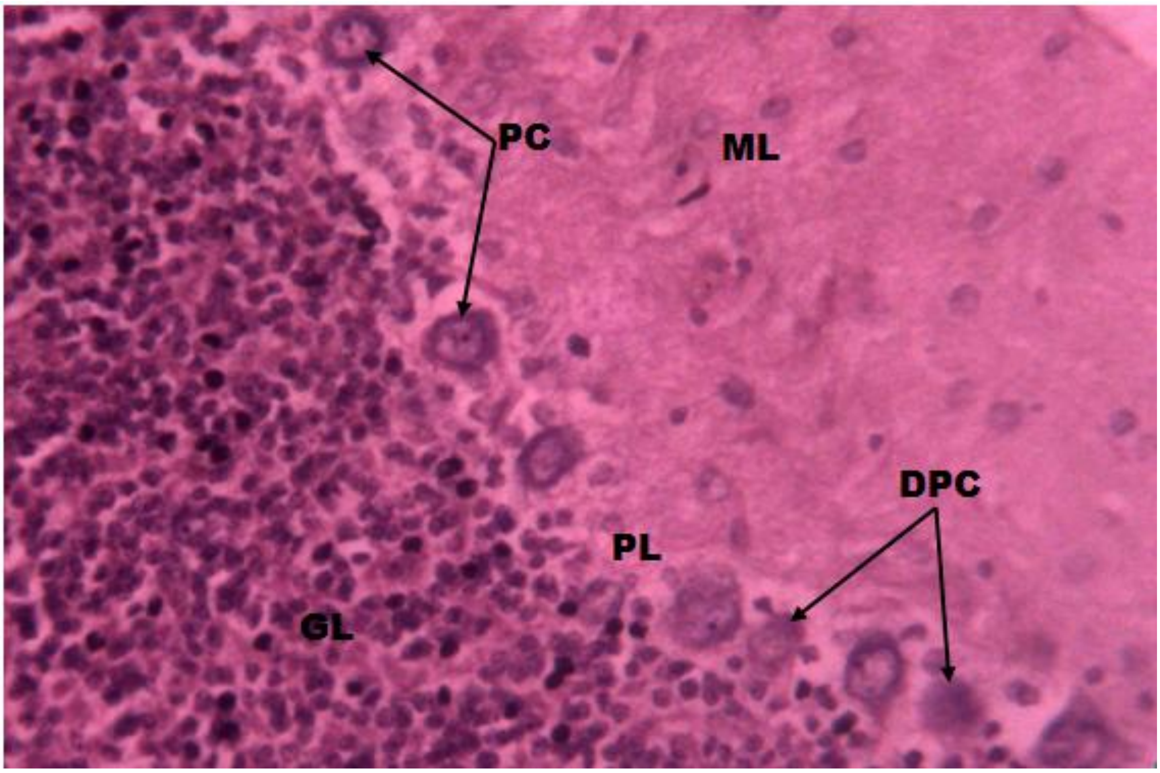

Plate IX: A section of the cerebellum in Group 5 (administered with 120 mg/kg bwt of lead acetate and then treated with 500mg/kg of extract) showing normal orientation of the molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with some normal and distorted Purkinje cells (DPC) (H & E Stain Mg X 250).

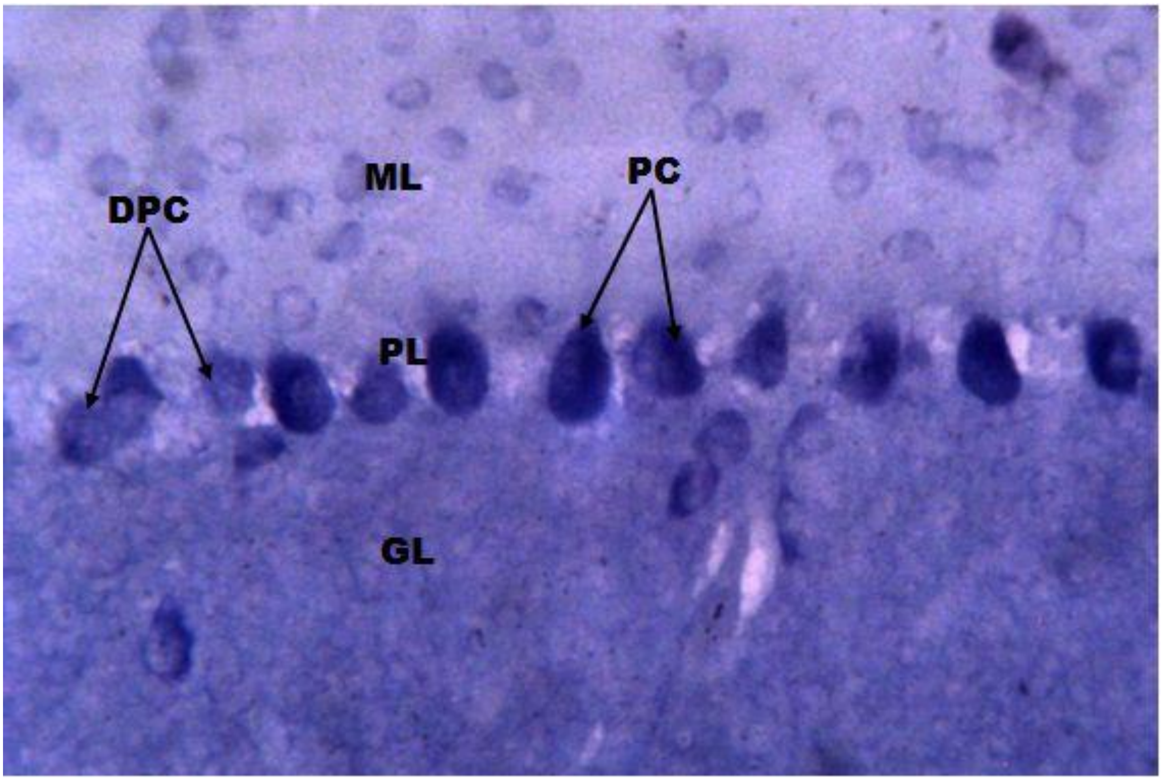

Plate X: A section of the cerebellum in Group 5 (administered with 120 mg/kg bwt of lead acetate and then treated with 500mg/kg of the extract) showing normal orientation of the molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with normal Purkinje cells and few degenerated Purkinje cells (DPC) (Cresyl Echt Violet Stain Mg X 250).

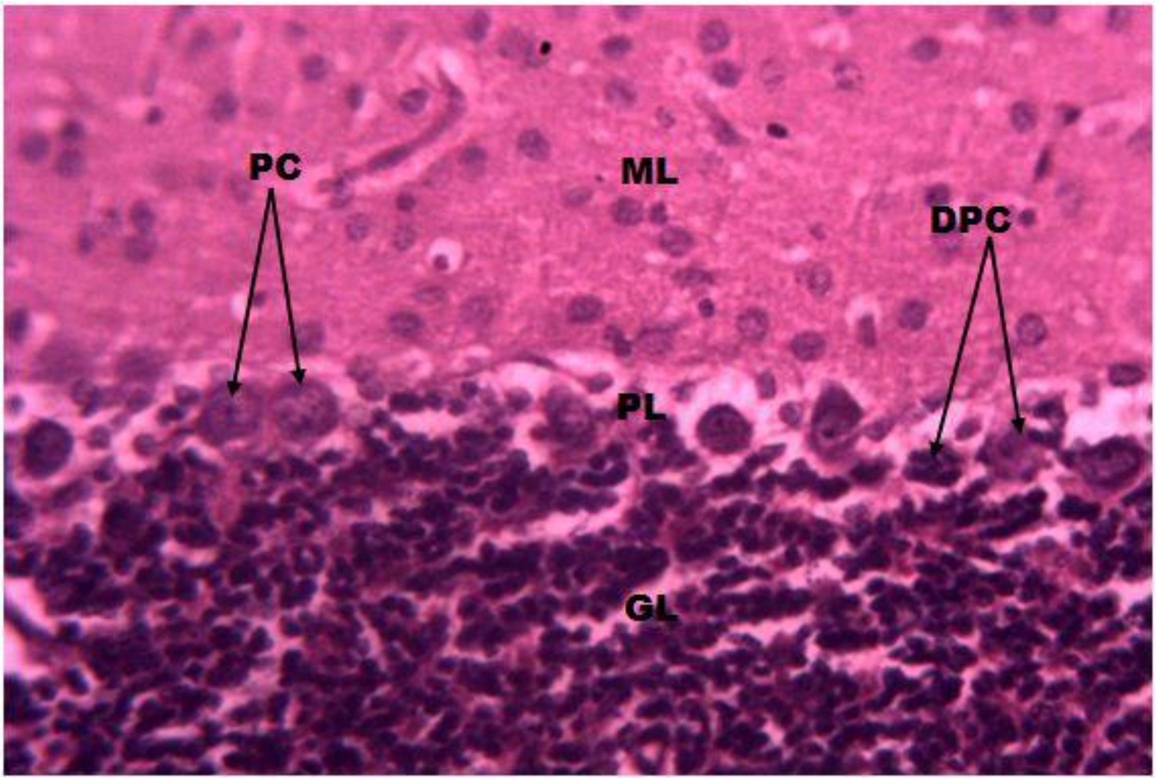

Plate XI: A section of the cerebellum in Group 6 (administered with 120 mg/kg bwt of lead acetate and then treated with 1000mg/kg of extract) showing normal orientation of the molecular layer (ML), clumping of granular cells in granular layer (GL) and Purkinje layer (PL) with normal Purkinje cells (PC) and Degenerated Purkinje cells (DPC) (H & E Stain Mg X 250).

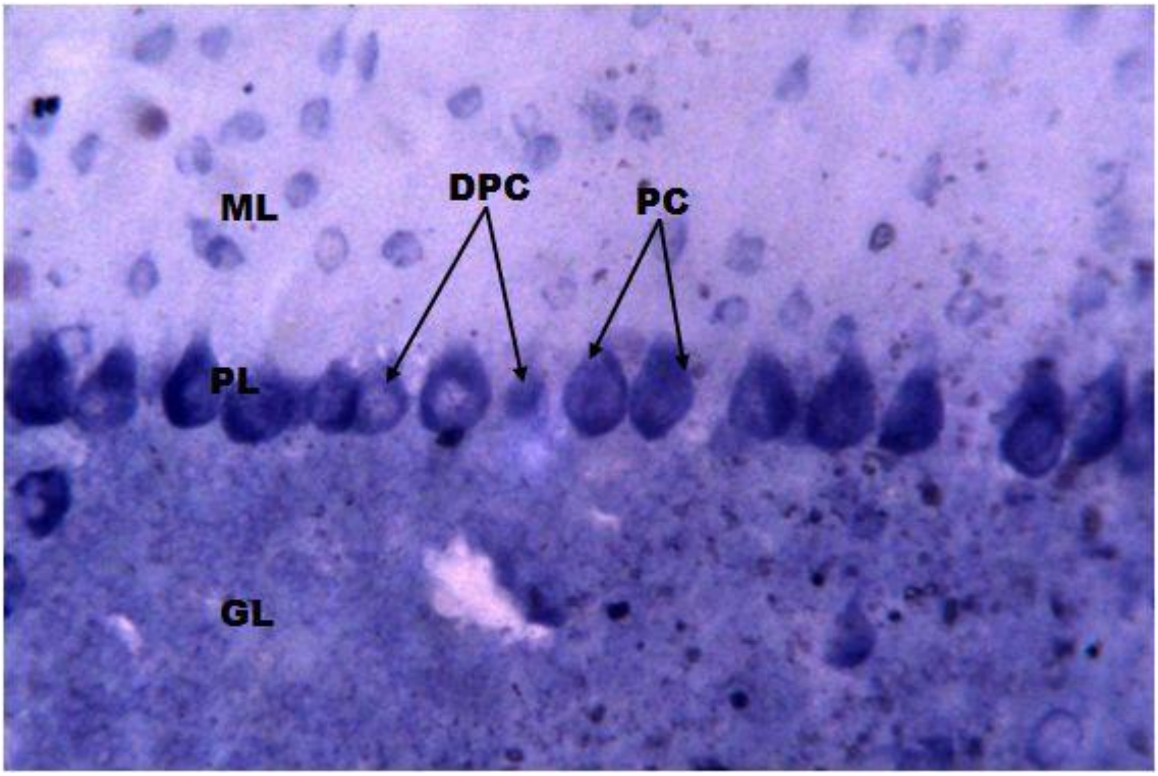

Plate XII: A section of the cerebellum in Group 6 (administered with 120 mg/kg bwt of lead acetate and then treated with 1000 mg/kg of extract) showing normal orientation of the molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with normal Purkinje cells and few degenerated Purkinje cells (DPC) (Cresyl Echt Violet Stain Mg X 250).

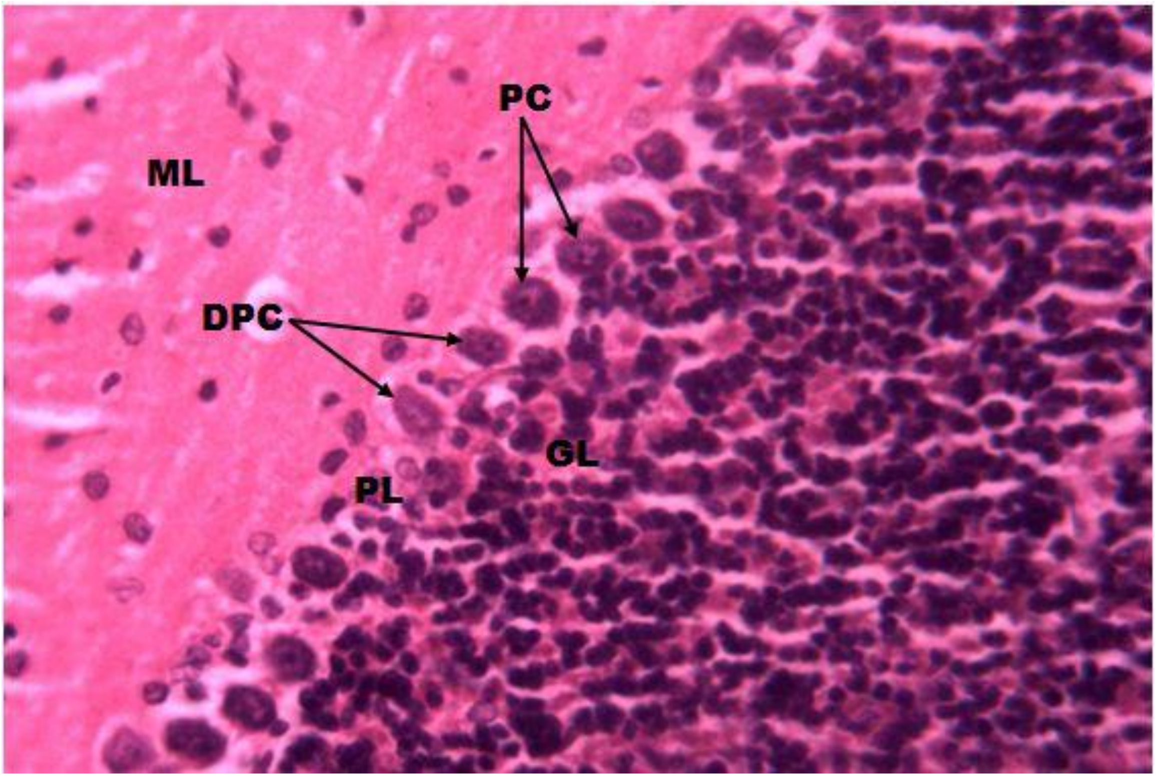

Plate XIII: A section of the cerebellum in Group 7 (administered with 120 mg/kg bwt of lead acetate and then treated with 10 mg/kg of Succimer) showing normal orientation of the molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with normal Purkinje cells (PC) and degenerated Purkinje cells (DPC) (H & E Stain Mg X 250).

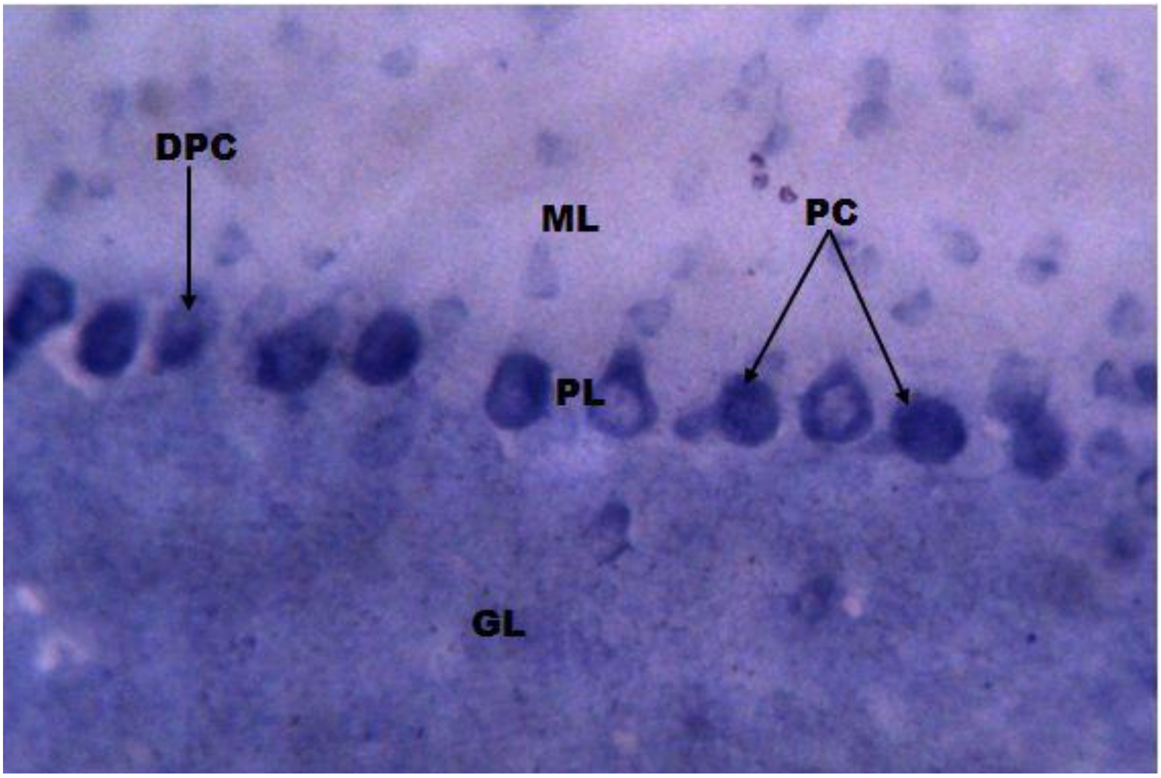

Plate XIV: A section of the cerebellum in Group 7 (administered with 120 mg/kg bwt of lead acetate and then treated with 10 mg/kg of Succimer) showing normal orientation of the molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with normal Purkinje cells (PC) and few degenerated Purkinje cells (DPC) (Cresyl Echt Violet Stain Mg X 250).

4.5.3 Histopathology of cerebrum

The cerebral cortex of the rats in Groups 1 and 2 showed normal architecture with normal pyramidal, Stallete and Cortical cells (Plates XV, XVI, XVII and XX). The cerebral cortex of the rats in Group 3 showed some degenerated pyramidal, Stallete and Cortical cells (Plates XIX and XX). The cerebral cortex of the rats in Group 4 showed some degenerated pyramidal, Stallete and Cortical cells and some normal Cortical and Stallete cells (Plates XXI and XXII). The cerebral cortex of the rats in Group 5 showed normal pyramidal, Stallete and Cortical cells with some degenerated Stallete and Cortical cells (Plates XXIII and XIV). The cerebrum of the rats in Groups 6 and 7 showed normal architecture with normal pyramidal, Stallete and Cortical cells (Plates XXV, XXVI, XXVII and XXVIII).

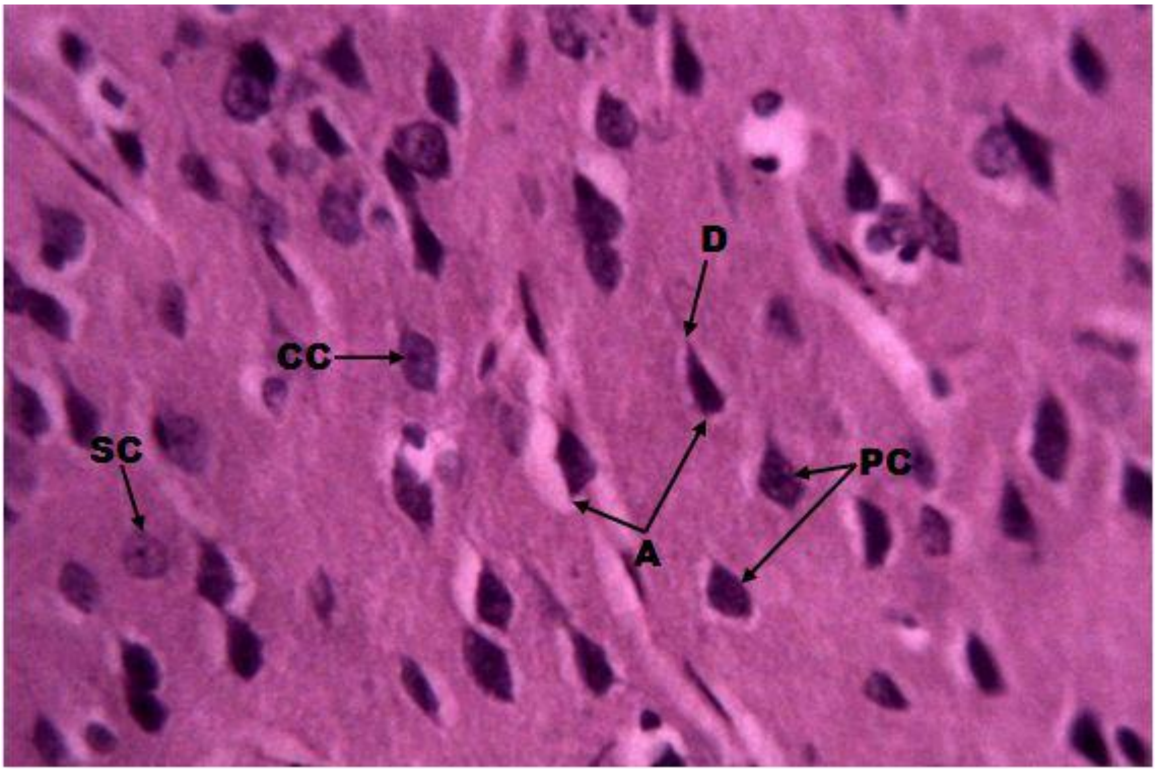

Plate XV: A section of the cerebral cortex of Group 1 (Control group) showing normal architecture with Pyramidal cells (PC), Stellate cells (SC), Cortical cells (CC), Axon (A) dendrites (D) of Pyramidal cell (H & E Stain Mg X 250).

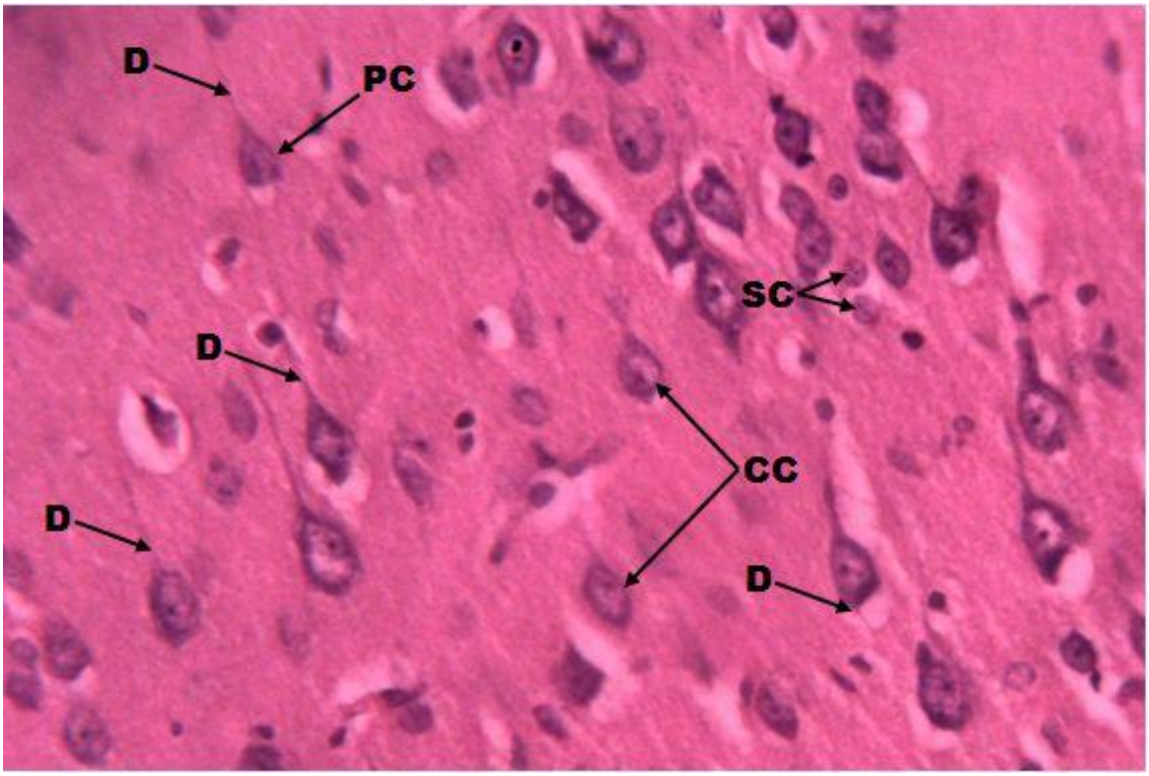

Plate XVI: A section of the cerebral cortex of Group 2 (1000 mg/kg of extract treated rats) showing normal architecture with pyramidal cell (PC), Cortical cells (CC), Stellate cells (SC), Dendrite (D) of Pyramidal cell (H & E Stain Mg X 250).

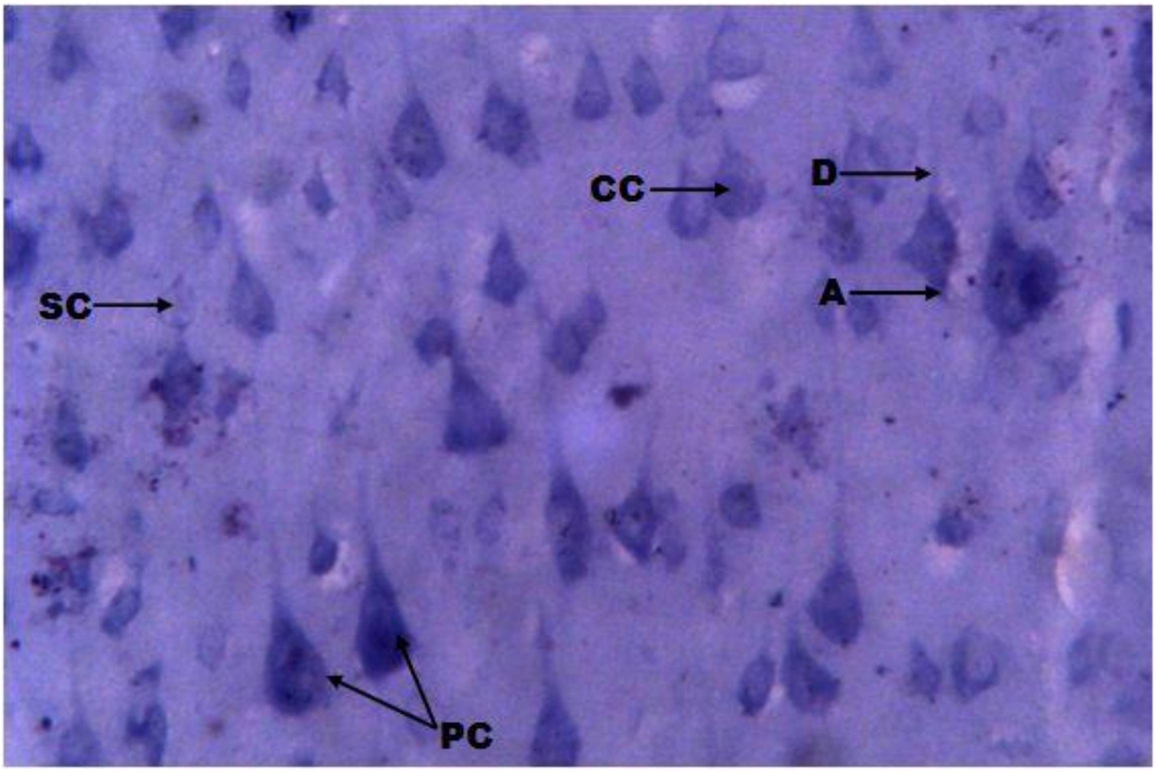

Plate XVII: A section of the cerebral cortex of Group 1 (Control group) showing normal architecture of Pyramidal cells (PC), Stellate cells (SC), Cortical cells, Axon (A) and Dendrite (D) of Pyramidal cell (Cresyl Echt Violet Stain Mg X 250).

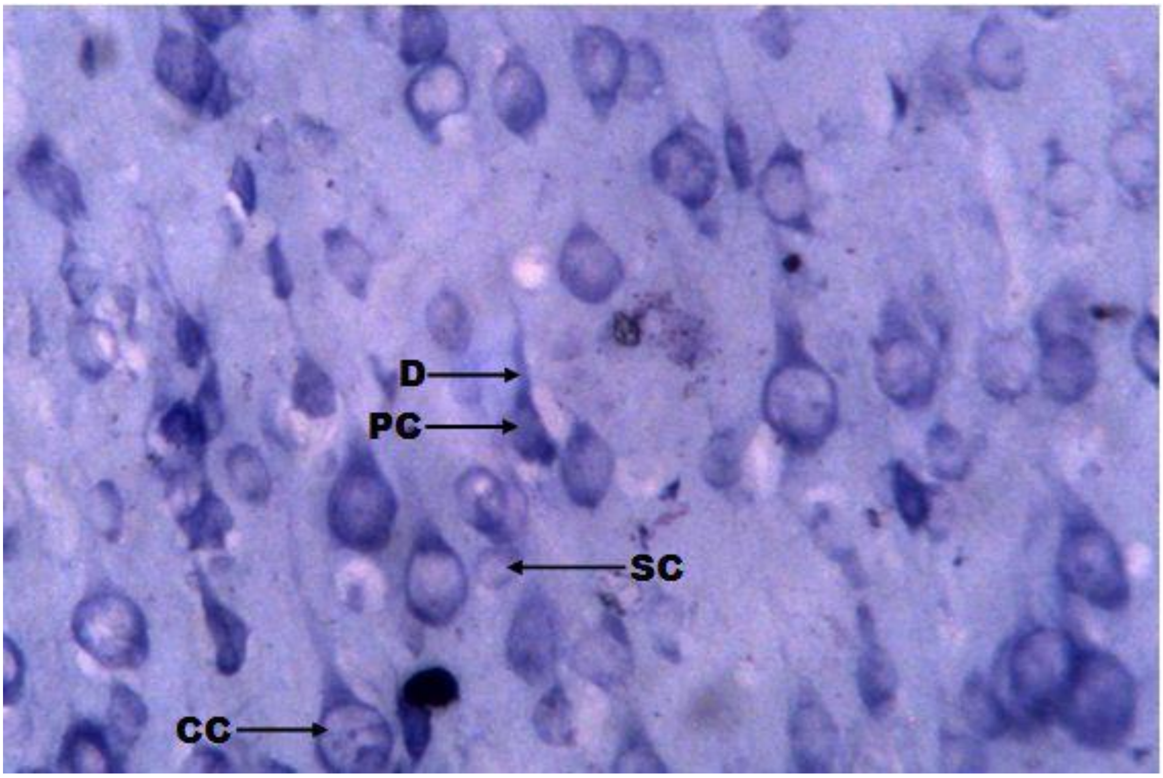

Plate XVIII: A section of the cerebral cortex of group 2 (1000 mg/kg of extract treated rats only) showing normal architecture of Pyramidal cells (PC), Stellate cells (SC), Cortical cells (CC) Dendrite (D) of Pyramidal cell (Cresyl Echt Violet Stain Mg X 250).

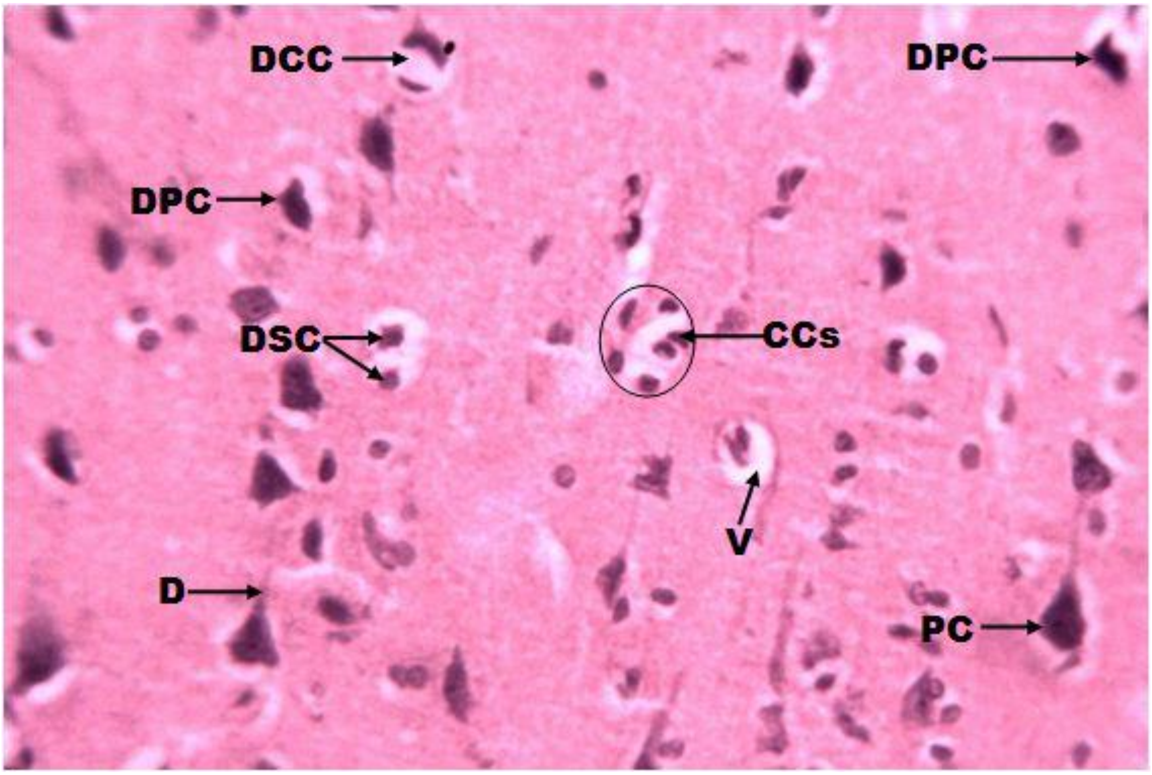

Plate XIX: A section of the cerebral cortex of Group 3 (administered with 120 mg/kg bwt of lead acetate) showing some clumping of cells (CCs), Vacuole (V) of degenerating Pyramidal cells (DPC), degenerating cortical cells (DCC) and degenerating Stellate cells (SC) with some normal Pyramidal cells (PC) and Dendrite (D) of Pyramidal cell (H & E Stain Mg X 250).

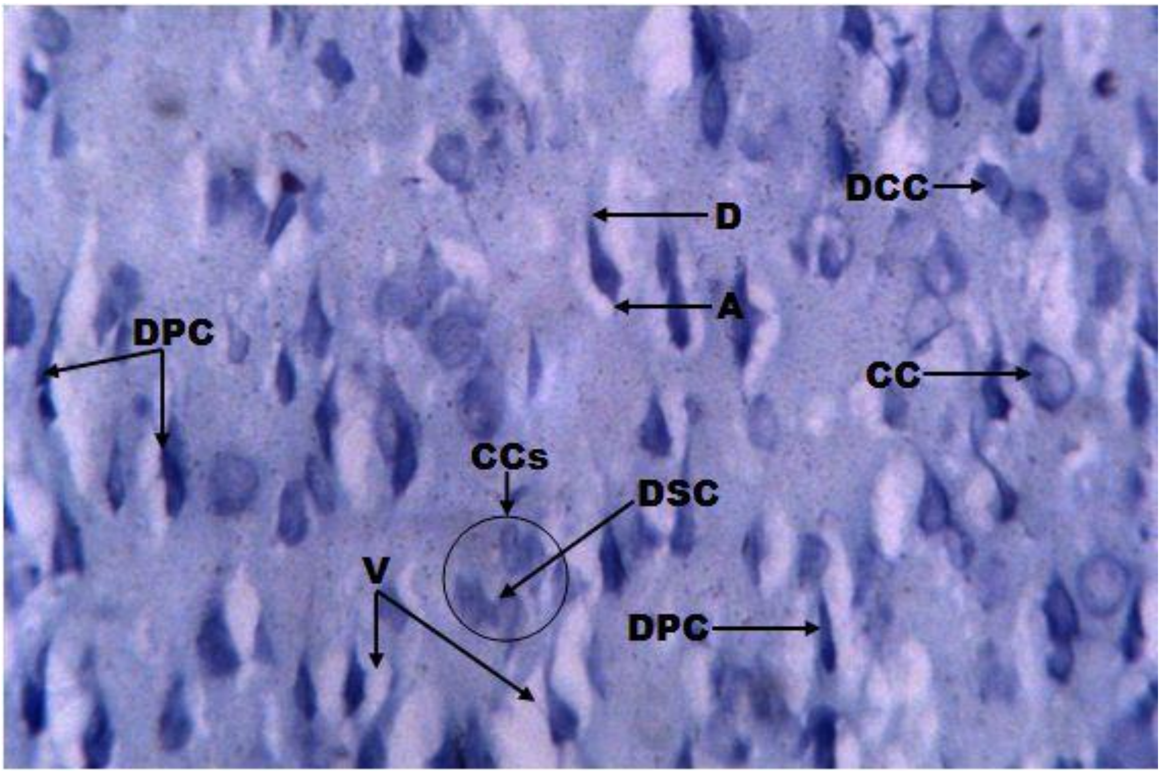

Plate XX: A section of the cerebral cortex of Group 3 (administered with 120 mg/kg bwt of lead acetate) showing some clumping of cells (CCs), Vacuoles (V) degenerating Pyramidal cells (DPC), degenerating cortical cells (DCC) and degenerating Stellate cells (DSC), Dendrite (D) and Axon (A) of Pyramidal cell (Cresyl Echt Violet Stain Mg X 250).

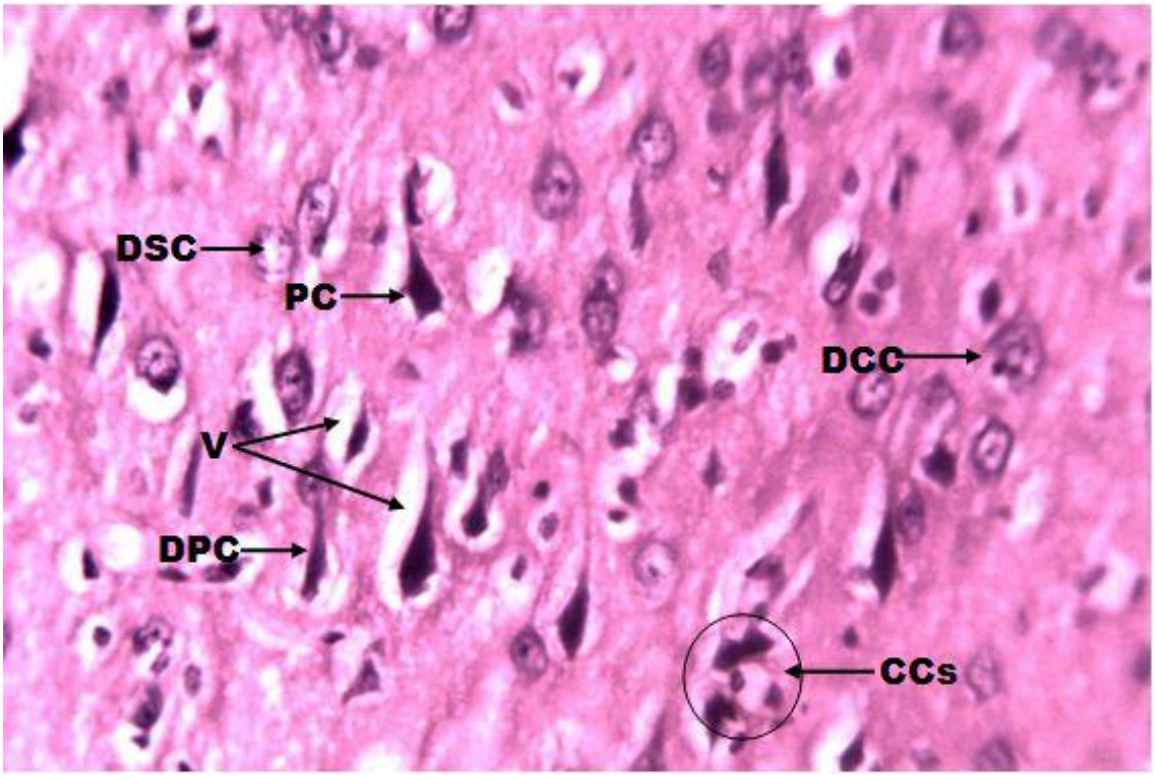

Plate XXI: A section of the cerebral cortex of Group 4 (administered with 120 mg/kg bwt of lead acetate and then withdrawn) showing some clumping of cells (CCs), Vacuoles (V) of degenerated neurons, degenerating Pyramidal cells (DPC), degenerating cortical cells (DCC) and degenerating Stellate cells (DSC) with some normal Pyramidal cells (PC) and Dendrite (D) of Pyramidal cell (H & E Stain Mg X 250).

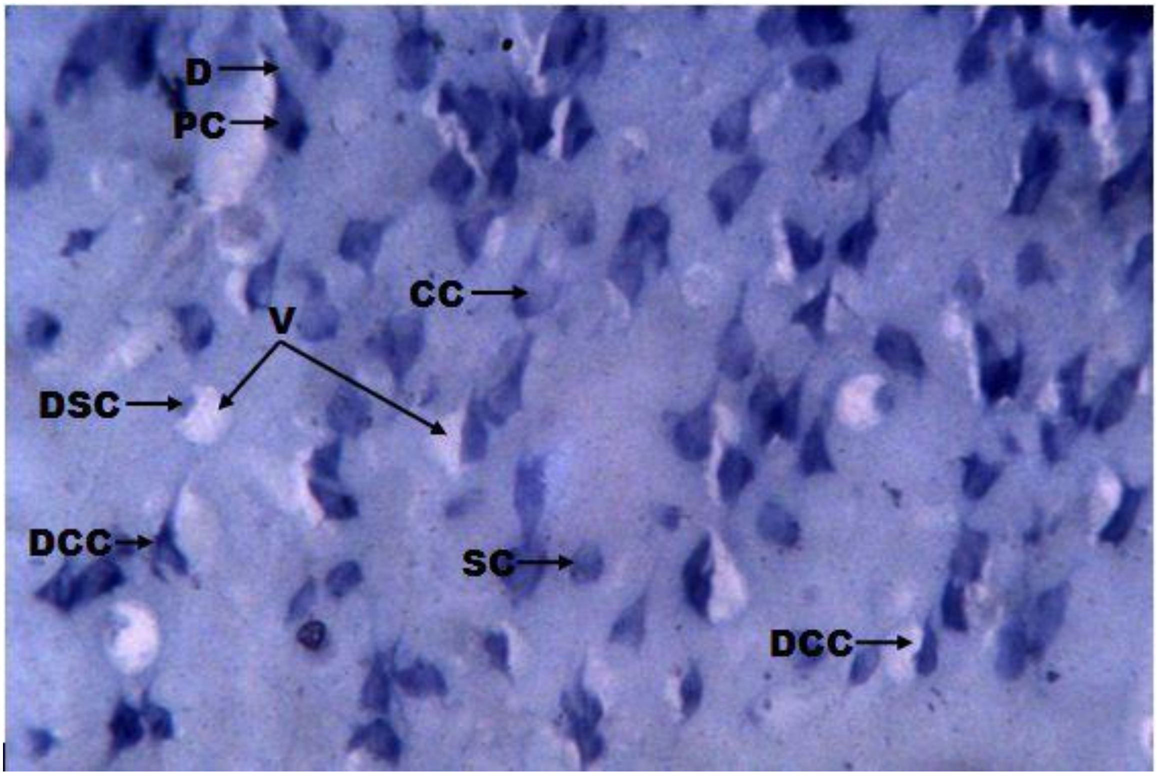

Plate XXII: A section of the cerebral cortex of Group 4 (administered with 120 mg/kg bwt of lead acetate and then withdrawn) showing degenerating Pyramidal cells (DPC), degenerating cortical cells and degenerating Stellate cells (DSC) with some normal Pyramidal cells (PC), Cortical cells (CC) and Stellate cells (SC) (Cresyl Echt Violet Stain Mg X 250).

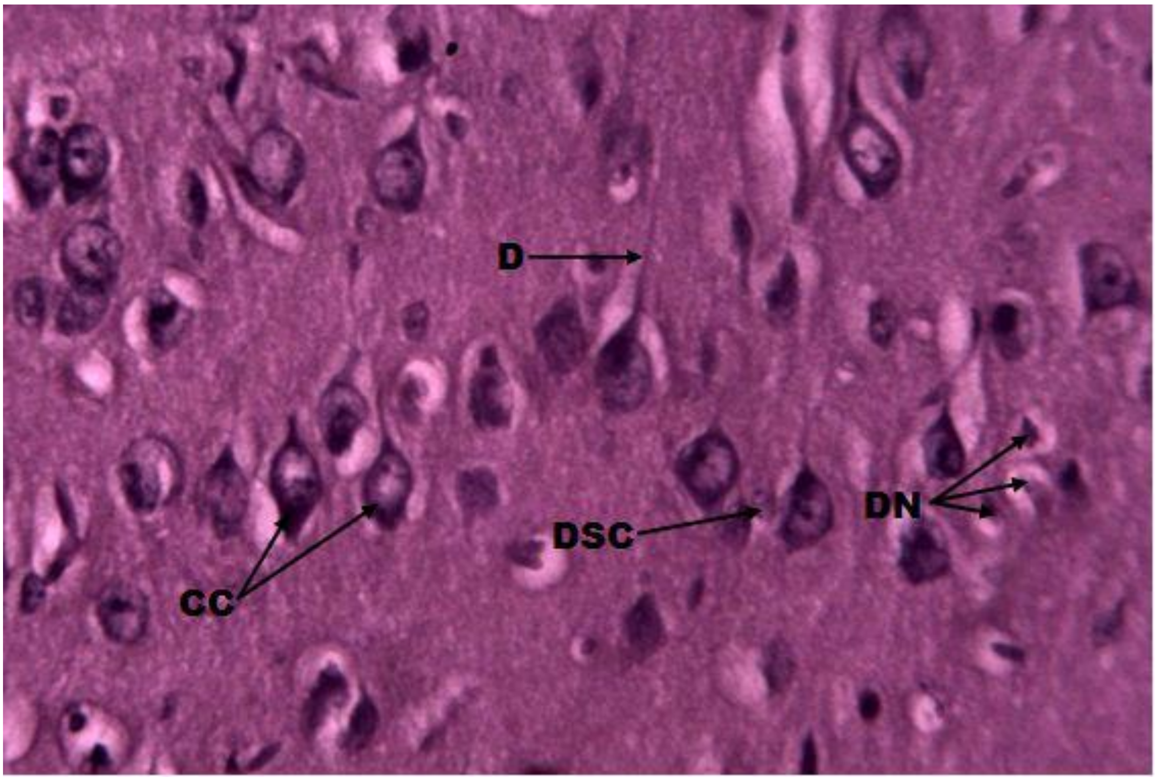

Plate XXIII: A section of the cerebral cortex of Group 5 (administered with 120 mg/kg bwt of lead acetate and then treated 500 mg/kg of extract) showing some degenerated neurons (DN) and normal Cortical cells (CC), degenerated Stellate cells (DSC), Dendrite (D) of Cortical cell (H & E Stain Mg X 250).

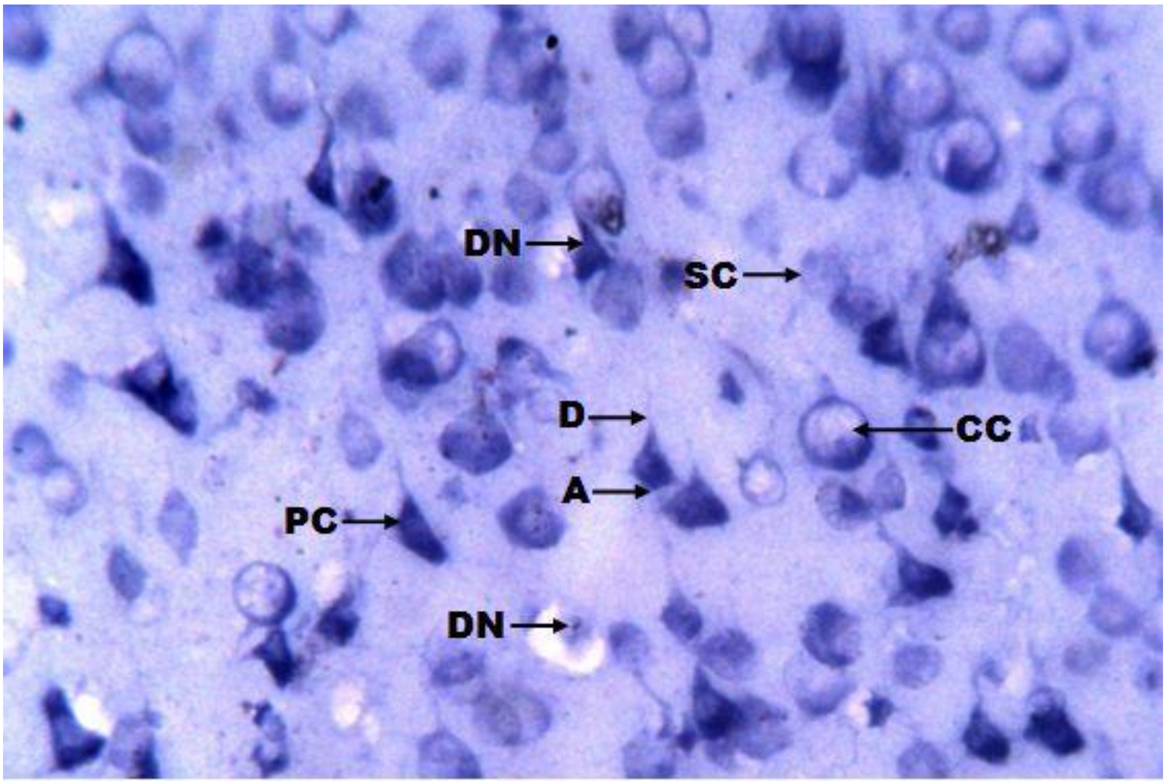

Plate XXIV: A section of the cerebral cortex of Group 5 (administered with 120 mg/kg bwt of lead acetate and then treated with 500 mg/kg of extract) showing some degenerated neurons (DN) and normal Pyramidal cells (PC), Stellate cells (SC), Cortical cells (CC) Dendrite (D) and Axon (A) of Pyramidal cell (Cresyl Echt Violet Stain Mg X 250).

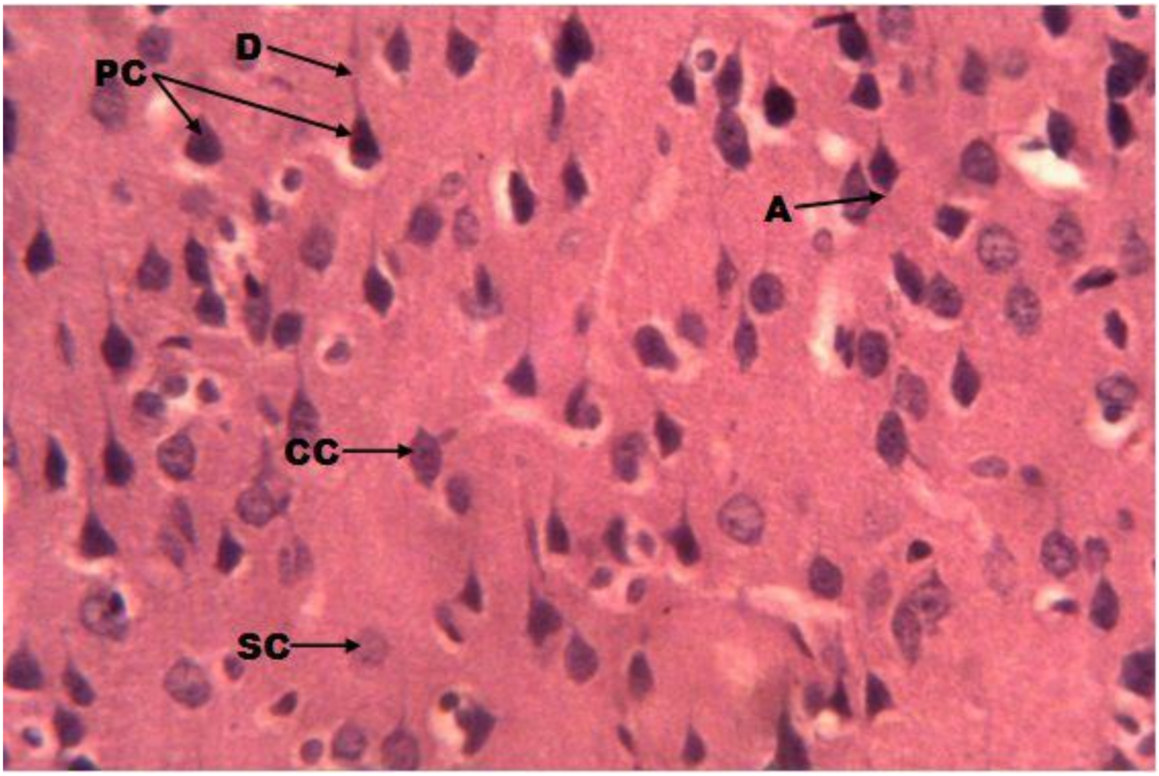

Plate XXV: A section of the cerebral cortex of Group 6 (administered with 120 mg/kg bwt of lead acetate and then treated 1000 mg/kg of extract) showing normal Pyramidal cells (PC), Cortical cells (CC) Stellate cells (SC), Axon (A) and Dendrite (D) of Pyramidal cell (H & E Stain Mg X 250).

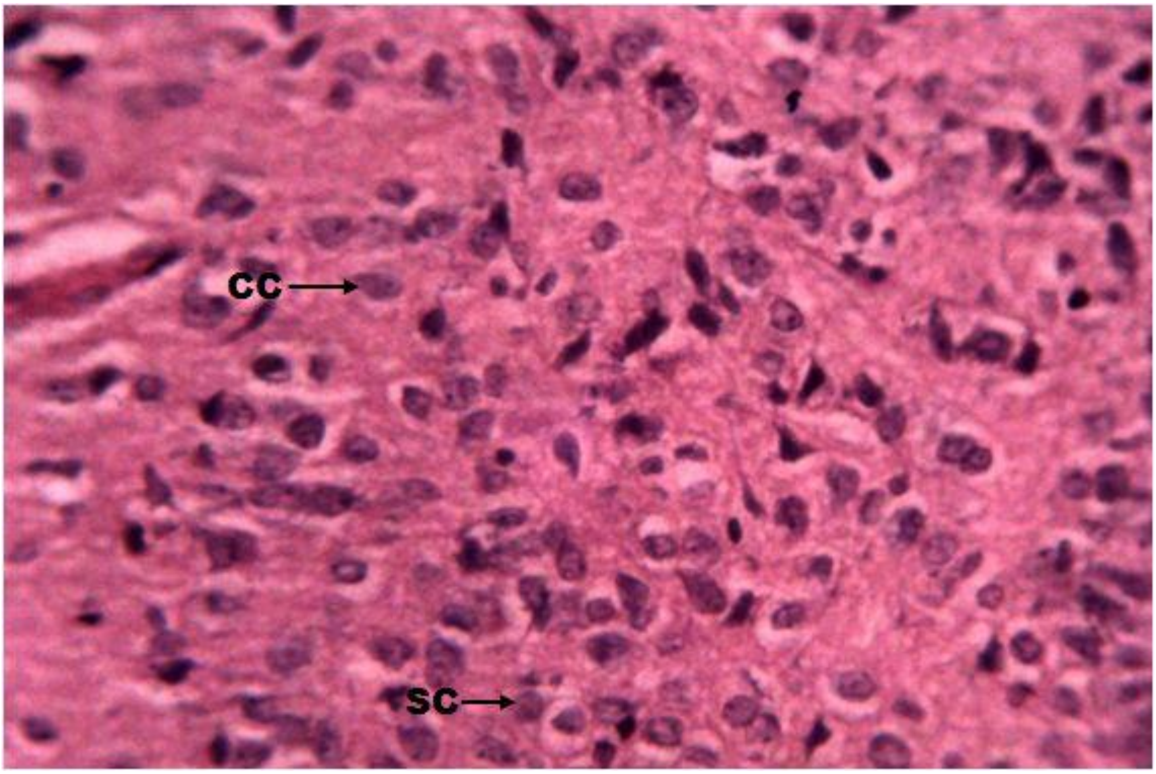

Plate XXVI: A section of the cerebral cortex of group 7 (administered with 120 mg/kg bwt of lead acetate and then treated 10 mg/kg of Succimer) showing normal Stellate cells (SC), and Cortical cells (CC), some degenerating neurons (H & E Stain Mg X 250).

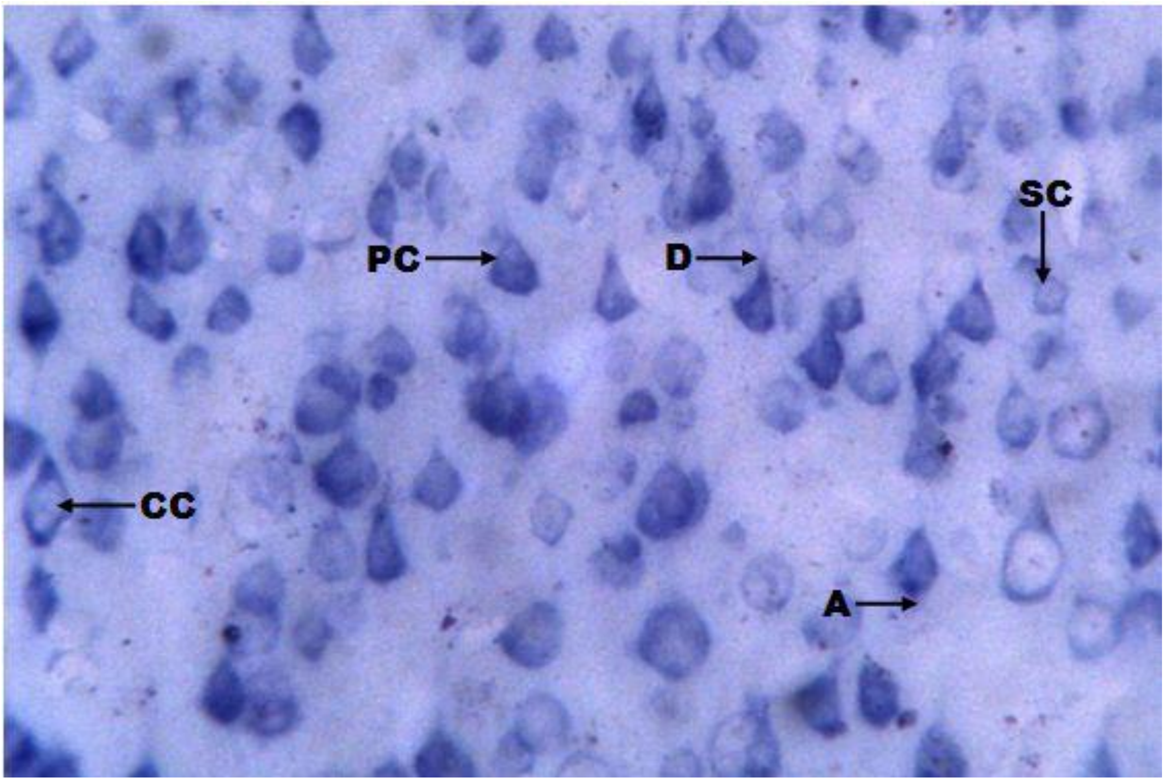

Plate XXVII: A section of the cerebral cortex of Group 6 (administered with 120 mg/kg bwt of lead acetate and then treated with 1000 mg/kg of extract) showing normal Pyramidal cells (PC), Stellate cells (SC), Cortical cells (CC) Dendrite (D) and Axon (A) of Pyramidal cell (Cresyl Echt Violet Stain Mg X 250).

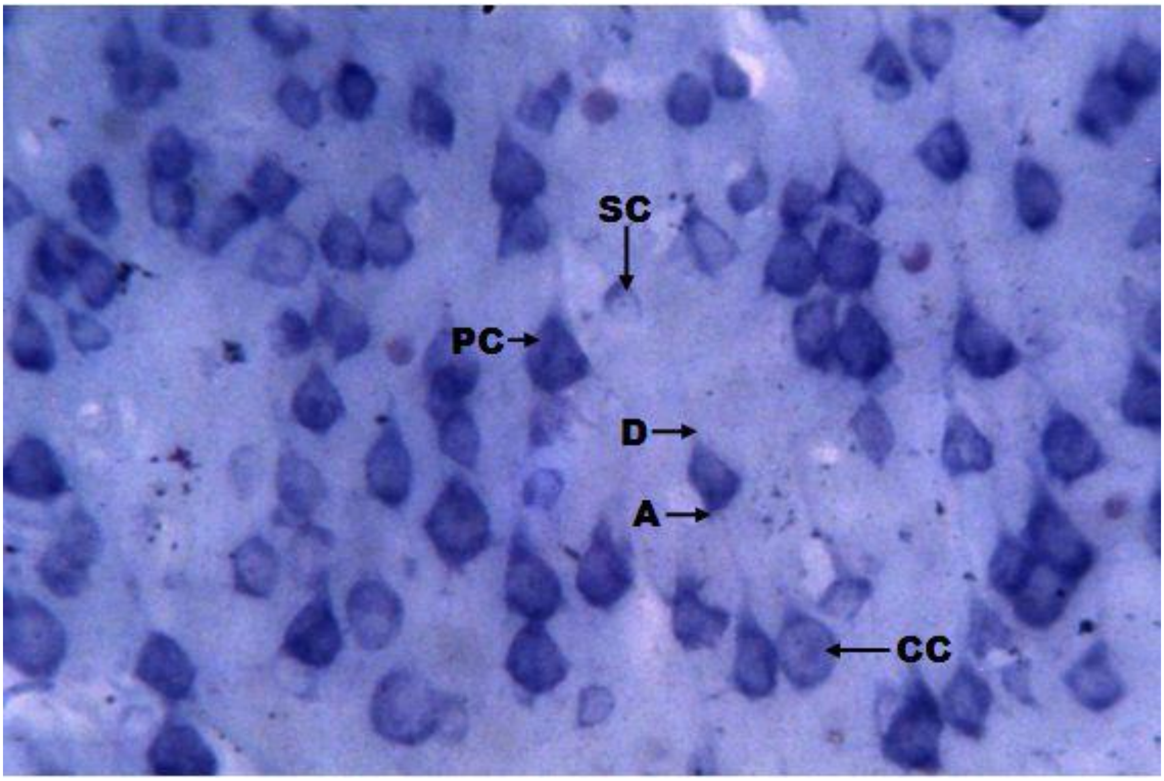

Plate XXVIII: A section of the cerebral cortex of Group 7 (administered with 120 mg/kg bwt of lead acetate and then treated 10mg/kg of Succimer) showing normal Pyramidal cells (PC), Stellate cells (SC), Cortical cells (CC), Dendrite (D) and Axon (A) of Pyramidal cell (Cresyl Echt Violet Stain Mg X 250).

4.5.4 Histopathology of the hippocampus

The hippocampus of the rats in Groups 1 and 2 showed normal appearance of pyramidal layer with normal pyramidal cells (Plates XXIX, XXX, XXXI and XXXII). The hippocampus of the rats in Groups 3 and 4 showed distortion of pyramidal layer, vacuolation, necrotic cells, no nerve cell body in some areas and some degenerated pyramidal cells (Plates XXXIII, XXXIV, XXXV and XXXVI). It was observed that the hippocampus of the rats in Group 5 showed few degenerated pyramidal cells (Plates XXXVII and XXXVII), while the same area in rats in Groups 6 and 7 showed normal appearance of pyramidal layer (Plates XXXIX, XL, XLI and XLII).

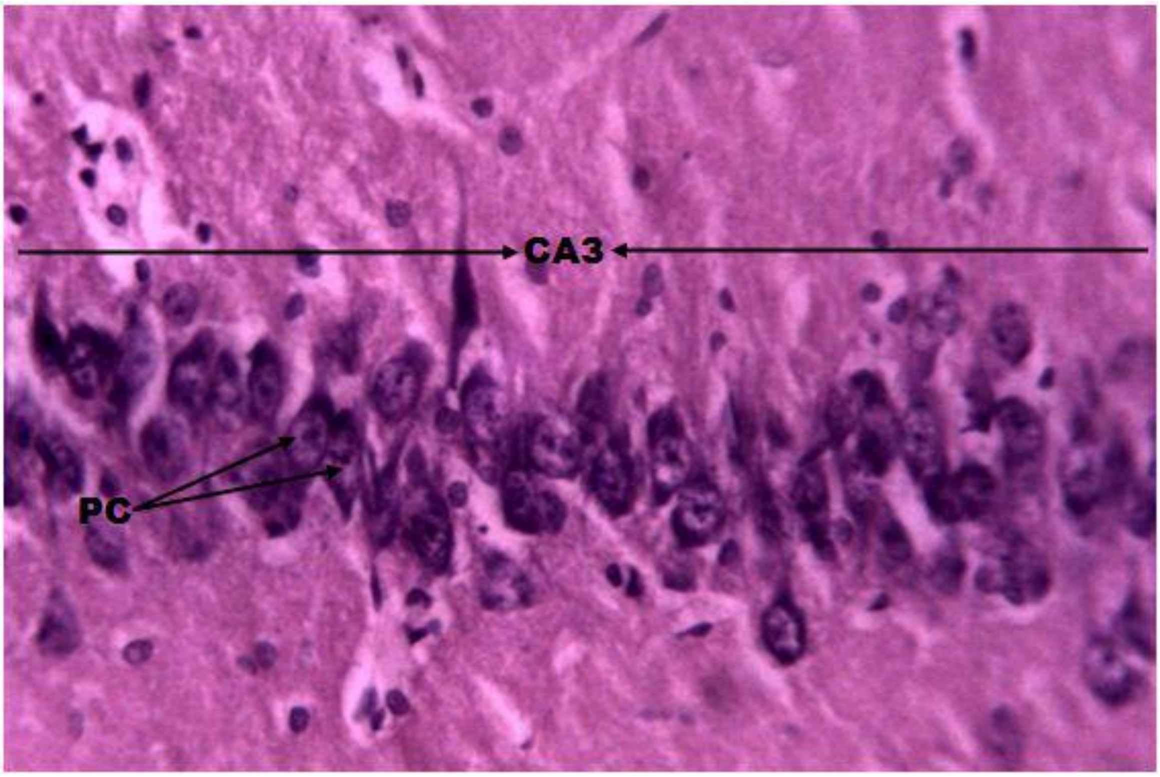

Plate XXIX: A section of the hippocampus of Group 1 (Control group) showing normal CA3 region with pyramidal cells (PC) (H & E stain Mg X 250).

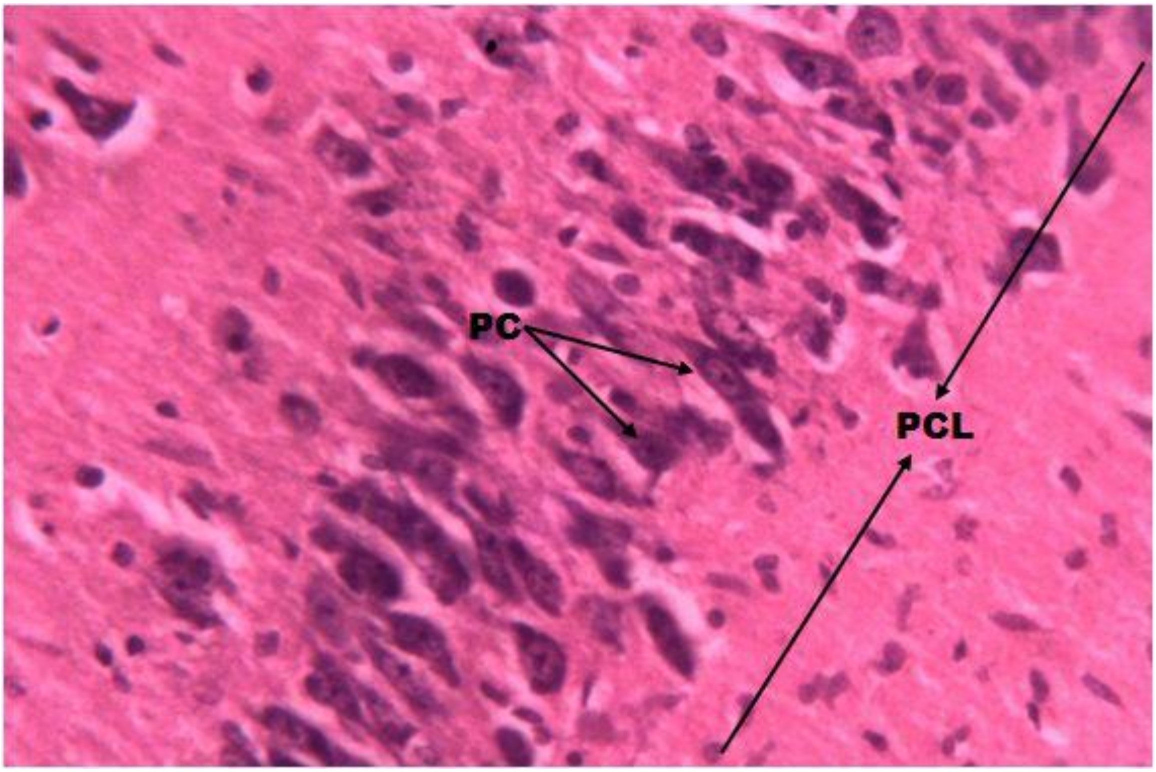

Plate XXX: A section of the hippocampus of Group 2 (1000 mg/kg of extract treated rats only) showing normal pyramidal cell layer (PCL) with pyramidal cells (PC) (H & E stain Mg X 250)

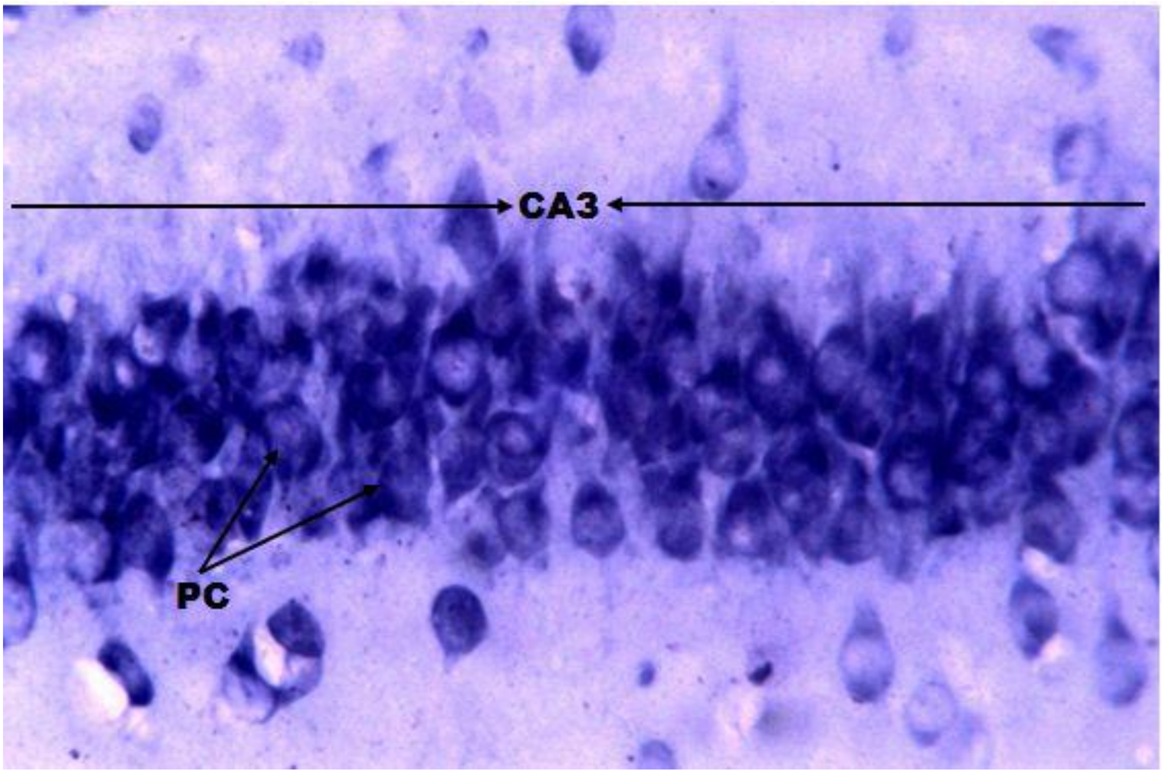

Plate XXXI: A section of the hippocampus of Group 1 (Control group) showing normal CA3 region with normal pyramidal cells (PC) (Cresyl Echt Violet Stain Mg X 250).

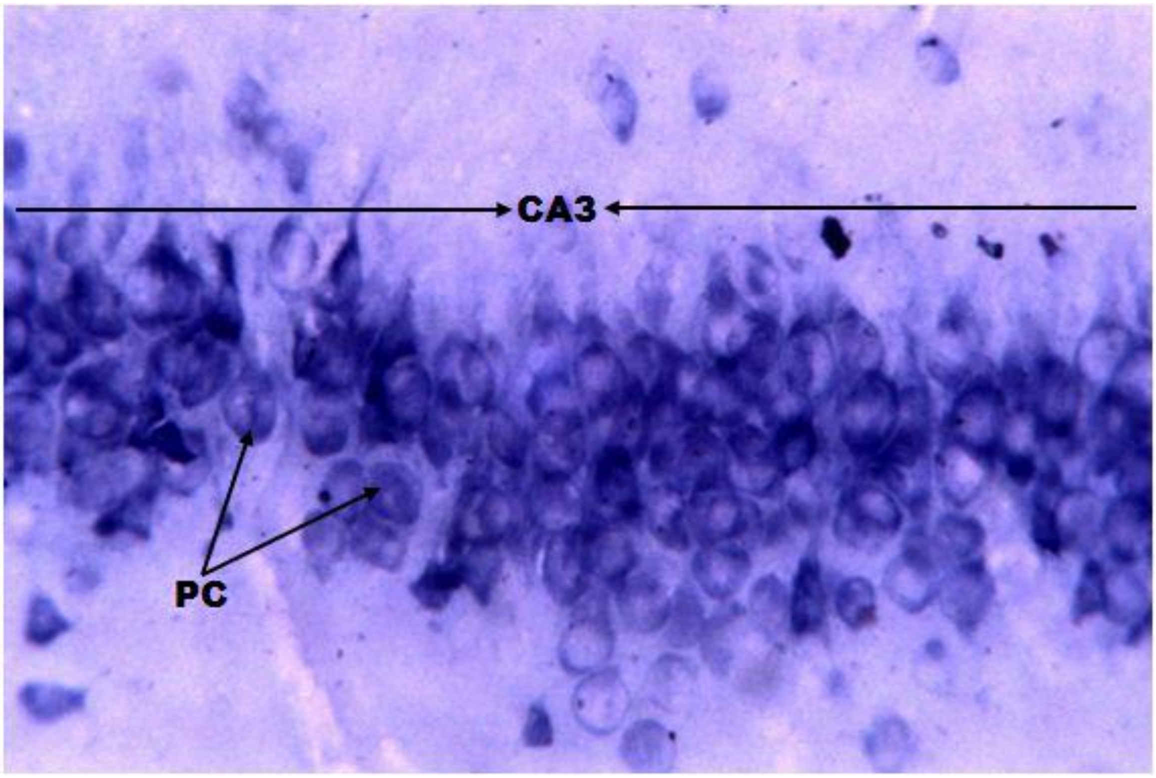

Plate XXXII: A section of the hippocampus of Group 2 (1000 mg/kg of extract treated rats only) showing normal CA3 region with normal pyramidal cells (PC) (Cresyl Echt Violet Stain Mg X 250).

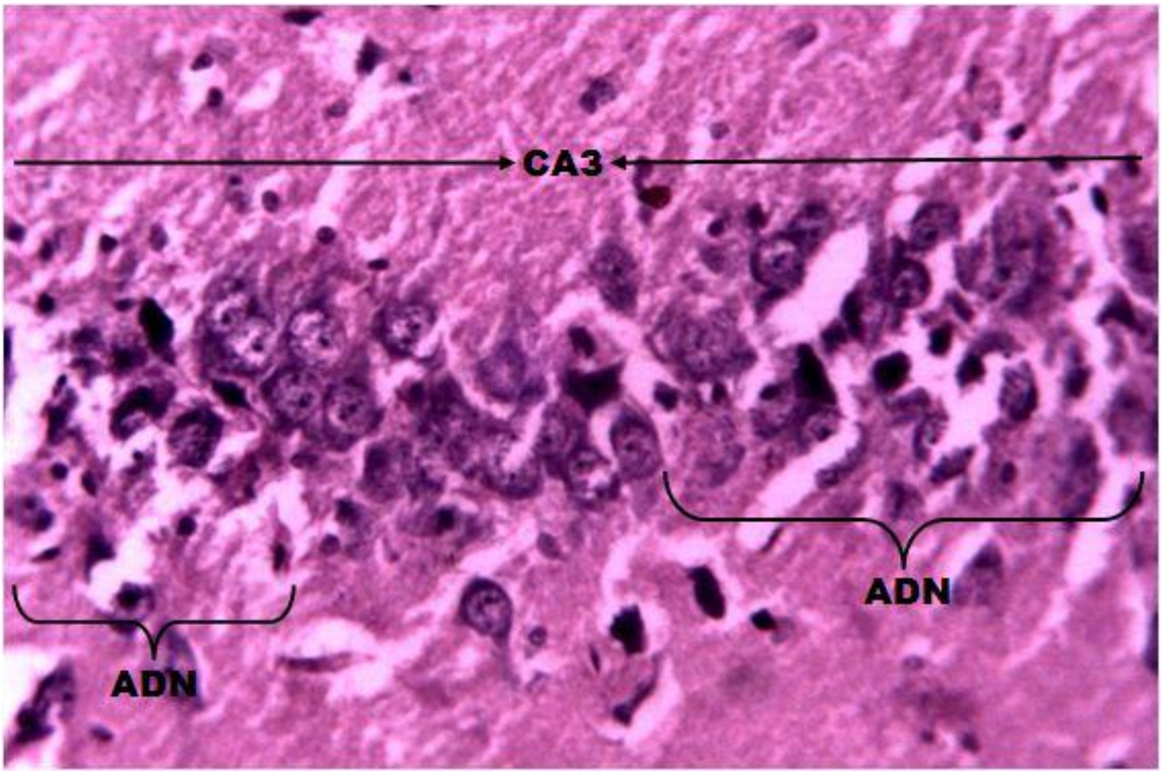

Plate XXXIII: A section of the hippocampus of Group 3 (administered with 120 mg/kg bwt of lead acetate only) showing disorientation of CA3 region with area of degenerating neurons (ADN) (H & E stain Mg X 250).

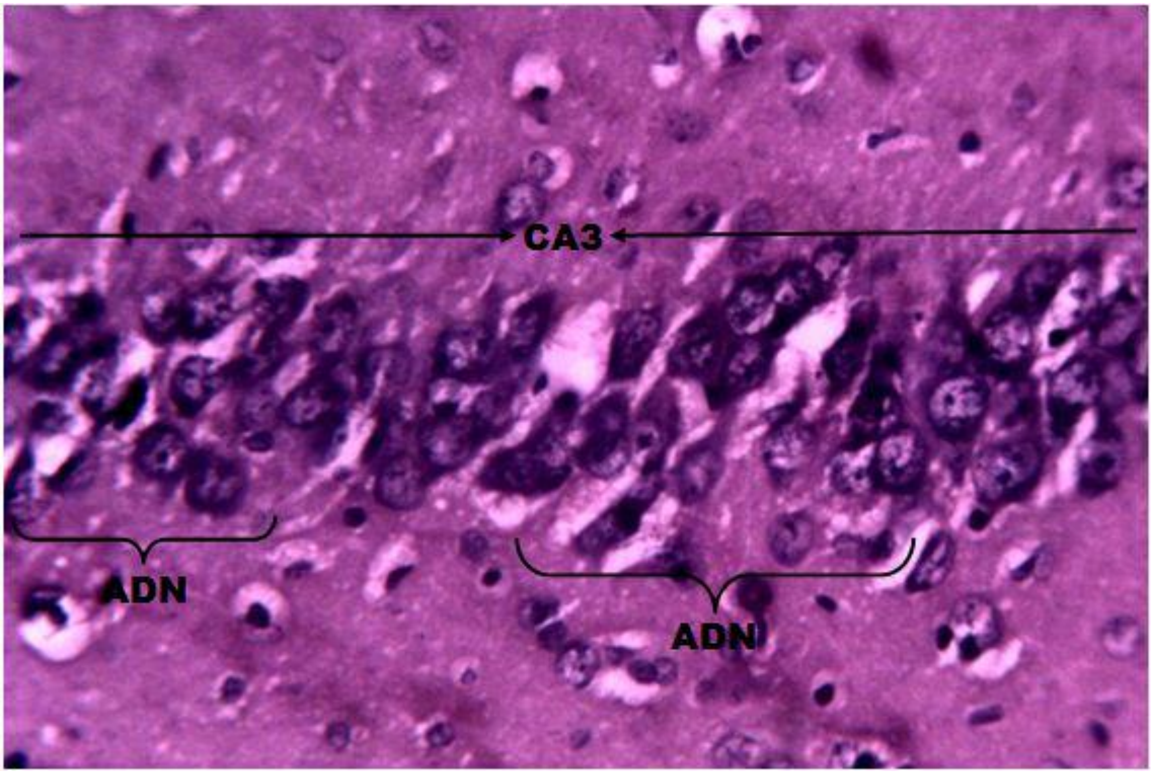

Plate XXXIV: A section of the hippocampus of Group 4 (administered with 120 mg/kg bwt of lead acetate and then withdrawn) showing disorientation of CA3 region with area of degenerating Neurons (ADN) (H & E stain Mg X 250).

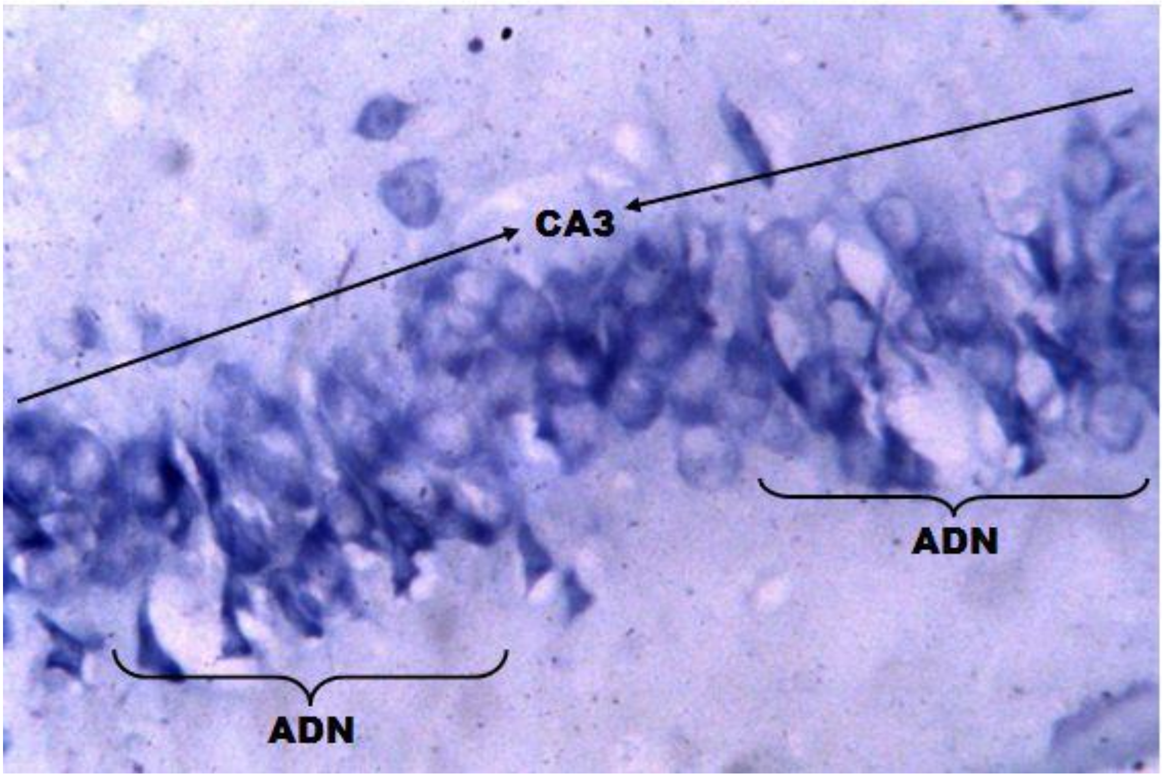

Plate XXXV: A section of the hippocampus of Group 3 (administered with 120 mg/kg bwt of lead acetate only) showing disorientation of CA3 region with area of degenerating neurons (ADN) (Cresyl Echt Violet Stain Mg X 250).

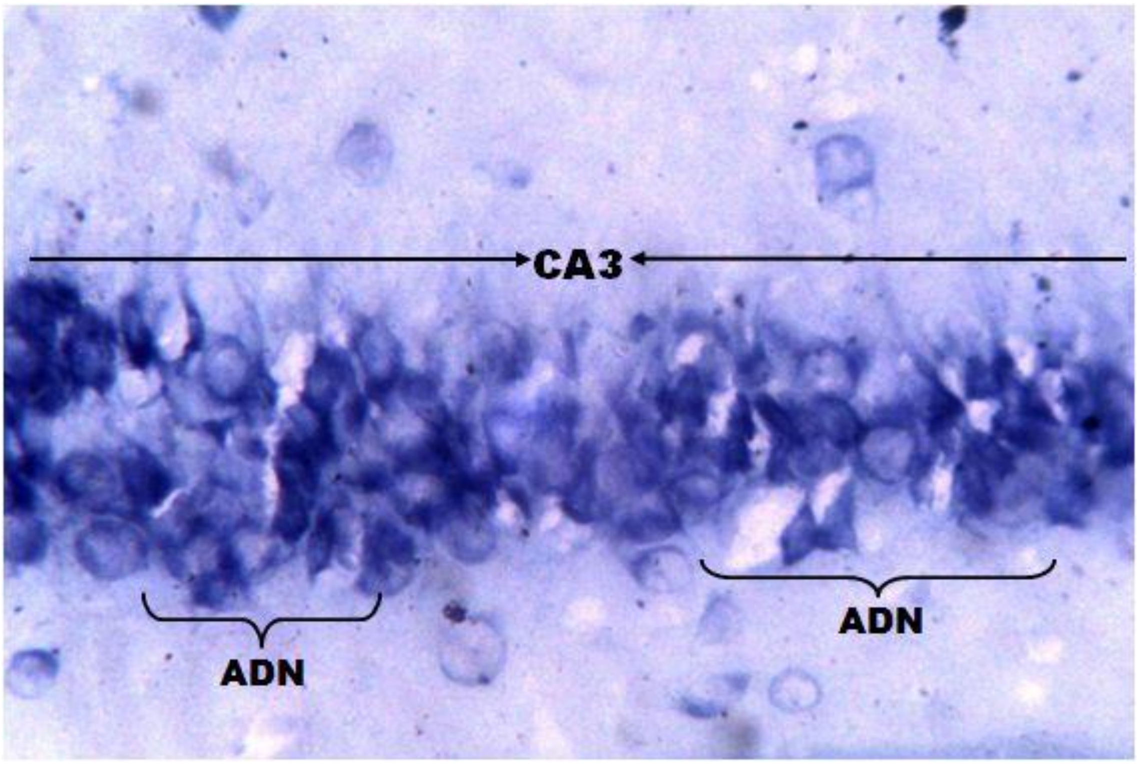

Plate XXXVI: A section of the hippocampus of Group 4 (administered with 120 mg/kg bwt of lead acetate and then withdrawn) showing disorientation of CA3 region with area of degenerating neurons (ADN) (Cresyl Echt Violet Stain Mg X 250).

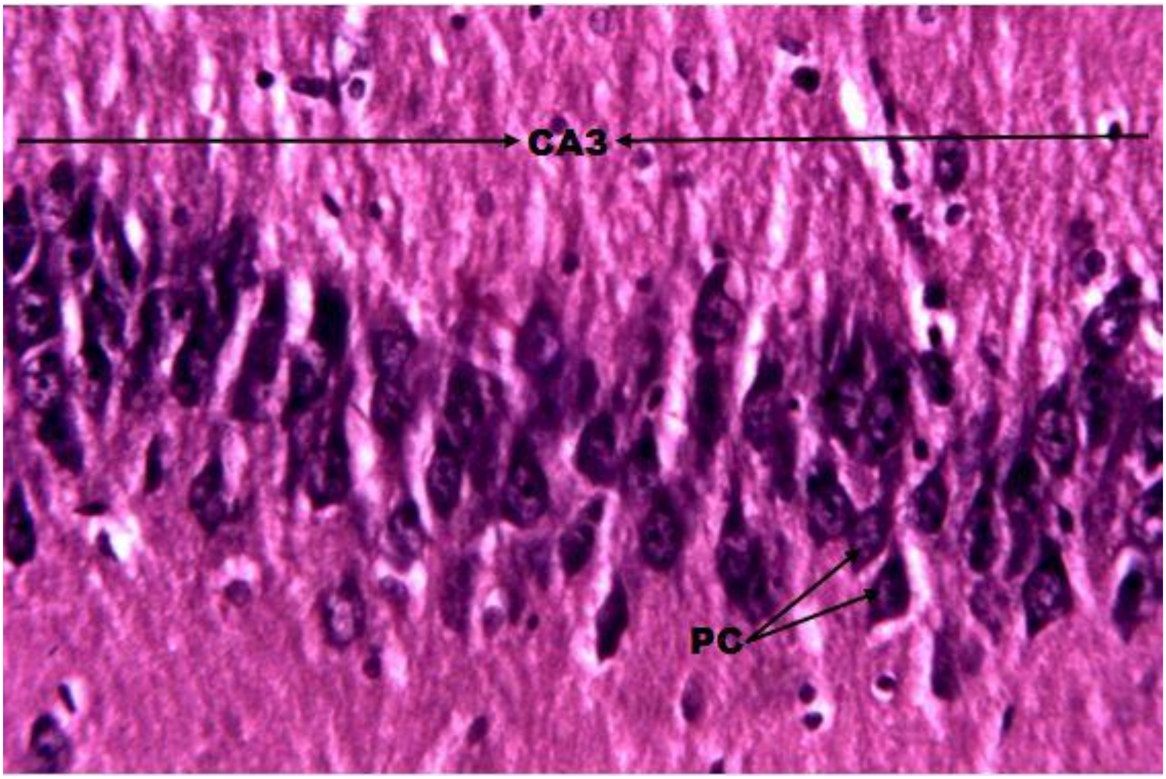

Plate XXXVII: A section of the hippocampus of the Group 5 (administered with 120 mg/kg bwt of lead acetate and then treated 500 mg/kg of extract) showing some degree of distortion in CA3 region with normal pyramidal cells (PC) some degenerated (H & E stain Mg X 250).

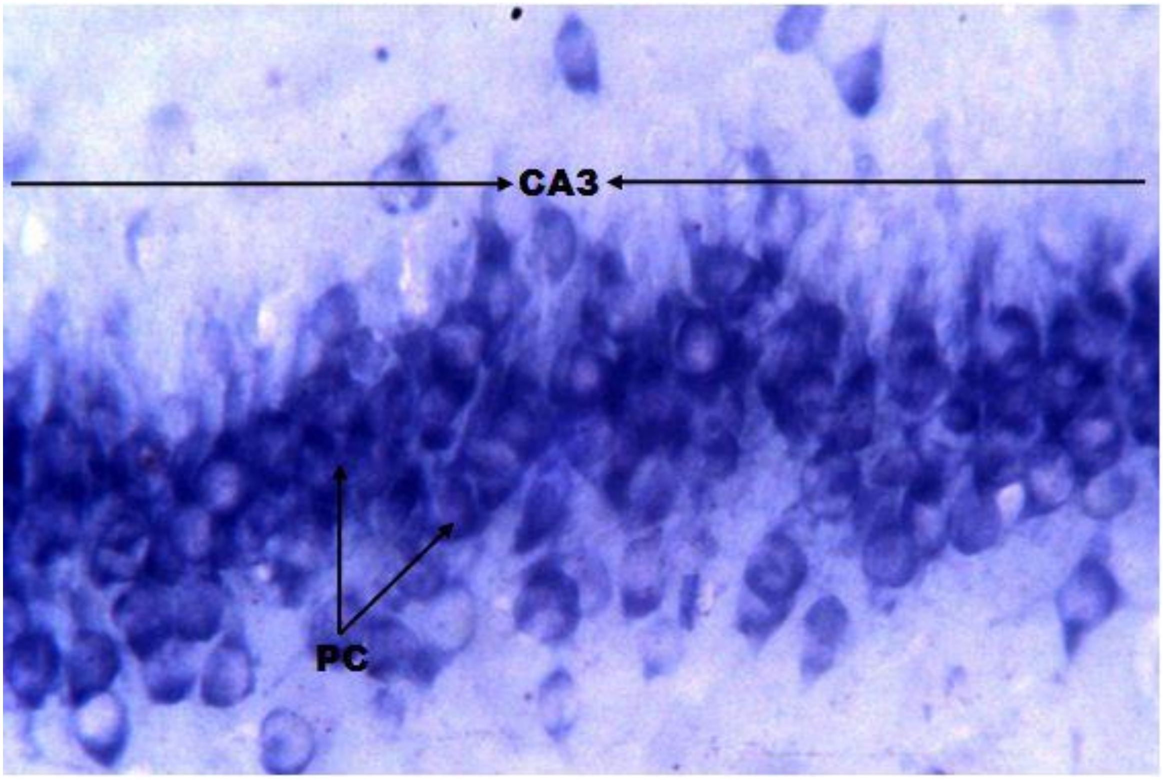

Plate XXXVIII: A section of the hippocampus of Group 5 (administered with 120 mg/kg bwt of lead acetate and then treated 500 mg/kg of extract) showing normal CA3 region with normal pyramidal cells (PC) (Cresyl Echt Violet Stain Mg X 250).

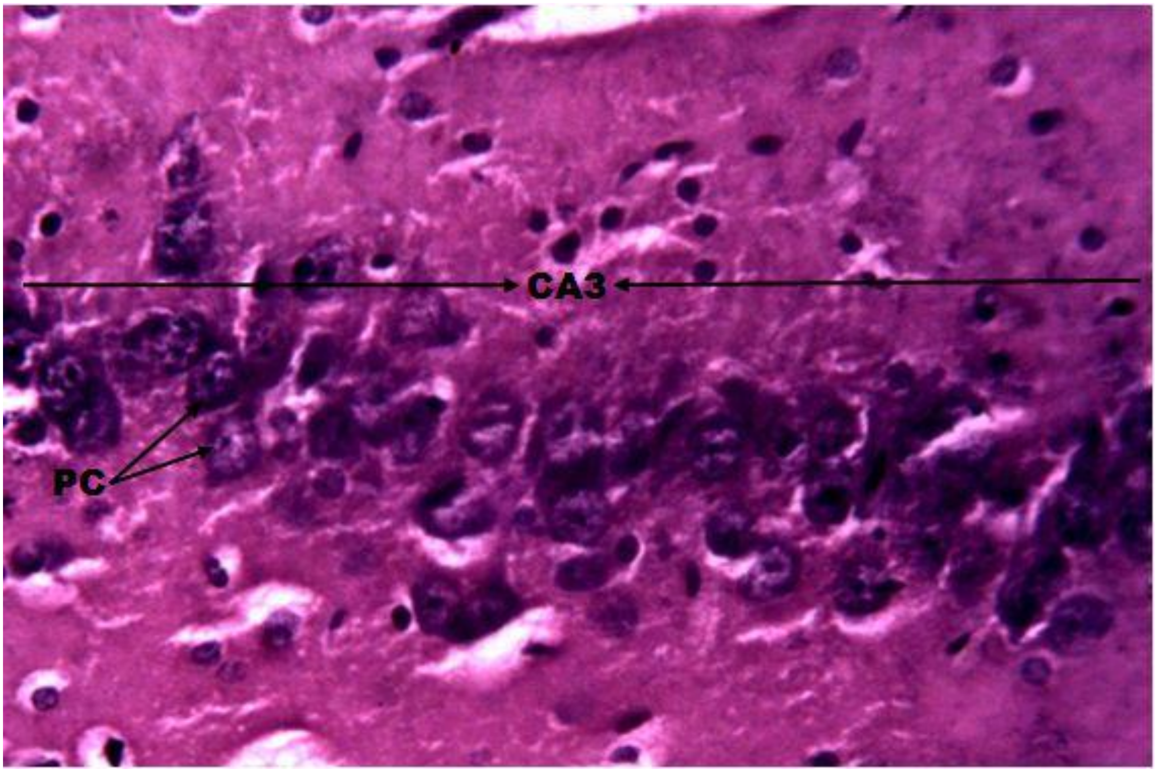

Plate XXXIX: A section of the hippocampus of group 6 (administered with 120 mg/kg bwt of lead acetate and then treated 1000 mg/kg of extract) showing some degree of distortion in CA3 region with normal pyramidal cells (PC) some degenerated (H & E stain Mg X 250).

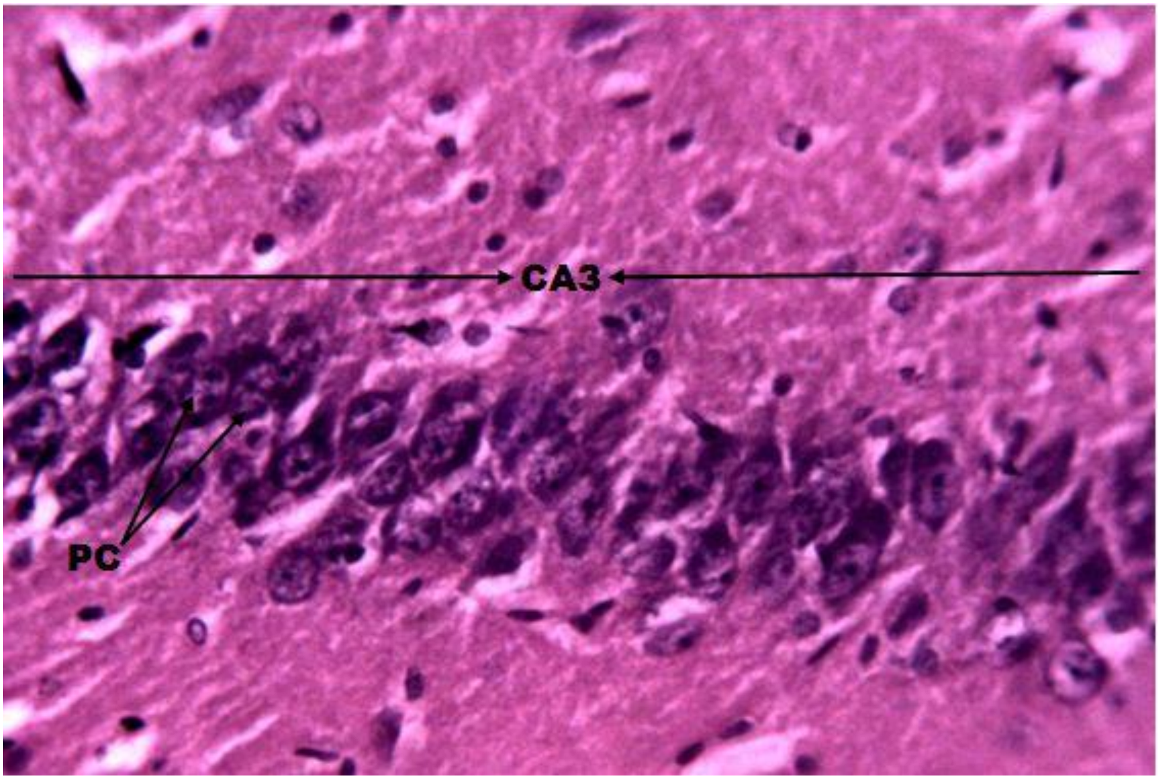

Plate XL: A section of the hippocampus of group 7 (lead administered with 120 mg/kg bwt of lead acetate and then treated 10 mg/kg of Succimer) showing some degree of distortion in CA3 region with normal pyramidal cells (PC) some degenerated (H & E stain Mg X 250).

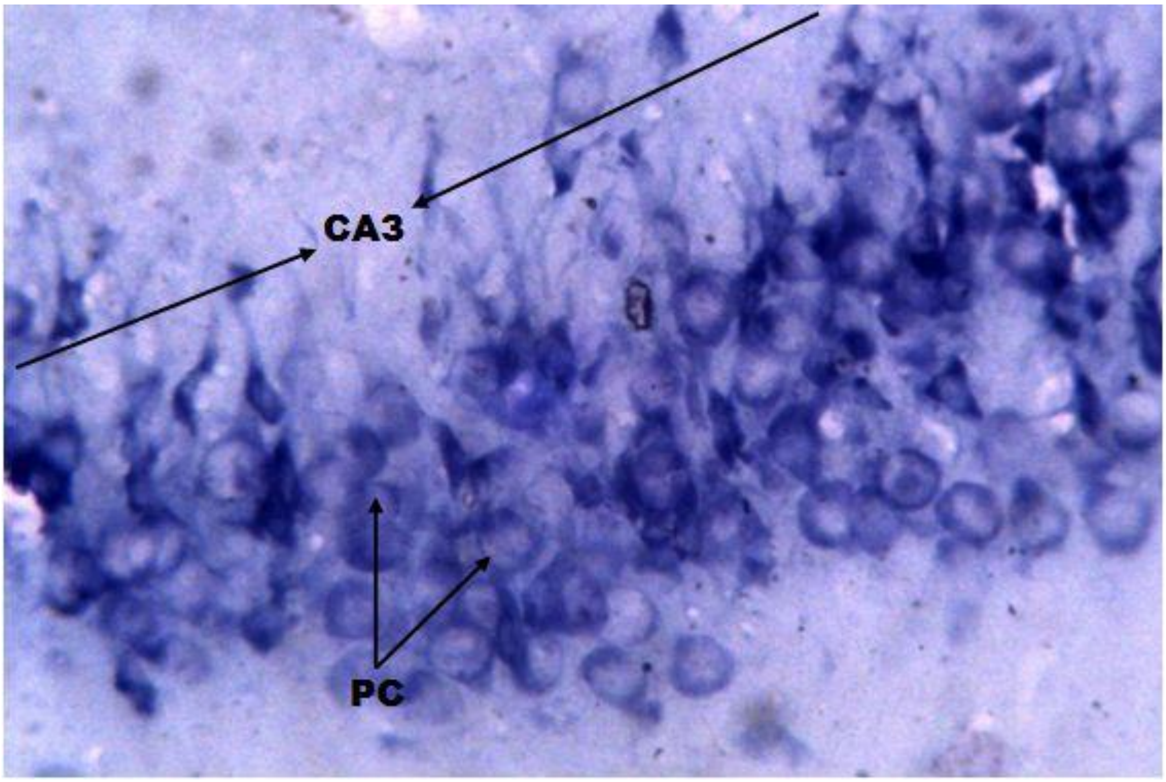

Plate XLI: A section of the hippocampus of Group 6 (administered with 120 mg/kg bwt of lead acetate and then treated 1000 mg/kg of extract) showing normal CA3 region with normal pyramidal cells (PC) (Cresyl Echt Violet Stain Mg X 250).

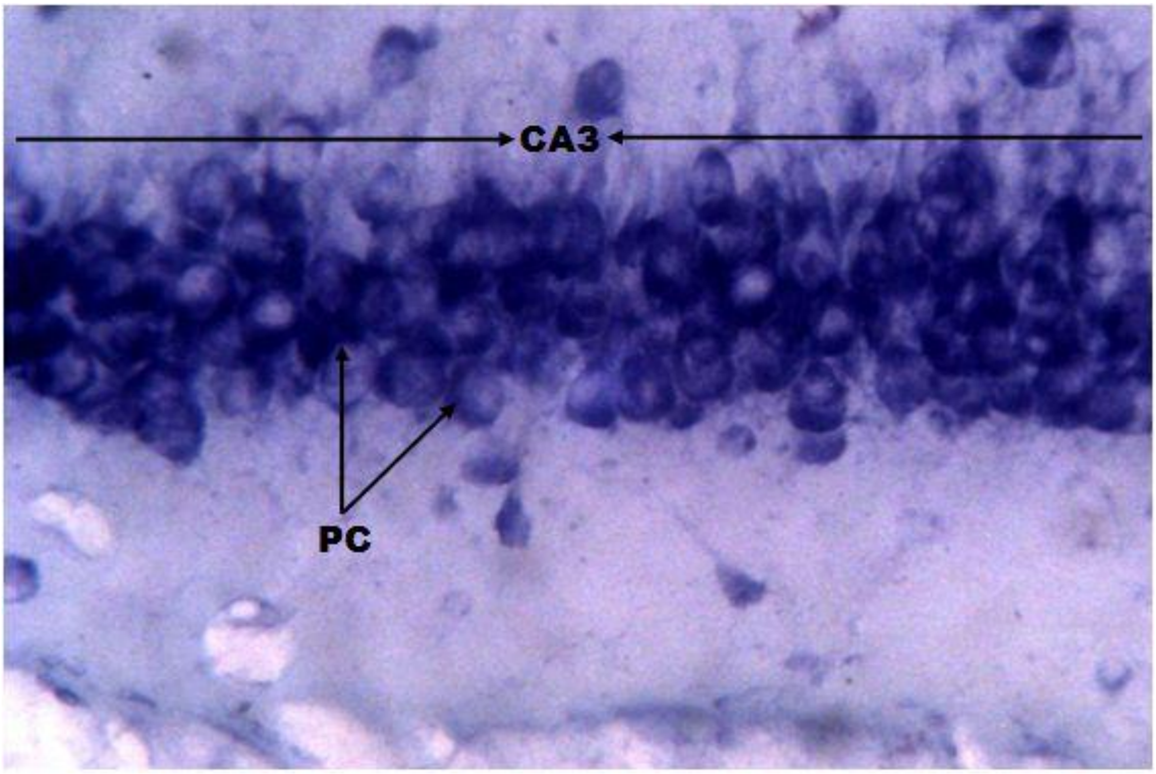

Plate XLII: A section of the hippocampus of Group 7 (administered with 120 mg/kg bwt of lead acetate and then treated 10 mg/kg of Succimer) showing normal CA3 region with normal pyramidal cells (PC) (Cresyl Echt Violet Stain Mg X 250).

CHAPTER FIVE

DISCUSSION

5.1 HISTOPATHOLOGY

The present study has shown that lead acetate induced neuronal degeneration in the Purkinje cell layer at various stages. The cerebellar degeneration in lead exposed rats resulted to decrease in number of the Purkinje cells in Purkinje cell layer. These could have caused the observed interference with the motor activity such as loss of movement, grasping, maintenance of equilibrium and regulation of muscle tone which are modulated by the spinal cord and brain stem mechanism involved in postural control. Sohair *et al.* (2010), had similarly reported marked degeneration due to chronic lead exposure at prenatal period and loss of Purkinje neurons in the adult cerebellum evidenced by decrease in the number of neurons. Such degeneration of Purkinje cells is a common feature of lead exposure (Xu *et al.,* 2005; Villeda *et al.,* 2006; Macauley *et al.,* 2008). The degeneration was characterized by abnormal mitochondria with rarified matrix and ill-defined cristae in the cytoplasm of Purkinje neurons of lead exposed rats (Sohair *et al.,* 2010), which were earlier speculated to have been due to inhibition of mitochondrial oxidative activity (Michaelson and Sauerhoff, 1974). Mitochondria are important organelles for energy production within a neuron and any change in their function may damage the cells. Thus, chronic mitochondrial alterations in cerebellum could be involved in the occurrence of depression in rats (Rezin *et al.,* 2008).

Sohair *et al.* (2010) had also reported degenerated myelin in Purkinje neurons in lead-exposed rats with the presence of vacuoles in the myelin sheaths. Morphological changes in myelin and oligodendrocytes of adult rat brains have been reported after prolonged exposure to lead (Dabrowska *et al.*, 2004). The production and maintenance of myelin is essential to normal functions of the Central Nervous System (CNS). Even small changes in myelin indices could lead to changes in conduction speed and signal timing which is crucial for the proper function of integrated neuronal circuits (Waxman, 1980; Felts *et al.*, 1997). Alterations in nerve fibers and overall myelin structures were associated with a decline in cognitive function (Peters, 1996), could influence motor function (Mattay *et al.*, 2002), and might lead to decrease propagation of nerve impulse along nerve fibers (Bondan *et al.*, 2006).

The lead exposed rats also had degeneration of sub-regions (CA1, CA3 and dentate gyrus) of the hippocampus, which manifested reduction in number of pyramidal and granular cells. This observation could explain the impaired activities of the hippocampus including storage and retrieval of information. The present findings learn credence to previous reports like Khaled *et al.* (2014) who had observed neuronal damage in brain cortex, hippocampus and cerebellum, with neurodegeneration of CA1 and CA3 regions. In this region of the brain, scar formation, demyelination and neuronal atrophy (Soltaniniejad *et al.*, 2003) could result from even low (0.2%) exposure to lead (Noor *et al.*, 2012).

The cerebral cortex showed nuclear pyknosis in the cortical cells and loss of stellate and pyramidal cells. It is known that degenerative and histomorphological changes were confirmed by decrease in number of pyramidal cells in cerebral cortex of lead exposed rats which could affect functions involved in skilled movement, ability to speak and to perceive pain and temperature (Khaled *et al.*, 2014). The extent to which the degree of neuronal degeneration and disorganization of the layers occur depends on duration of lead exposure (Nehru *et al.*, 1997). Seddik *et al.* (2011) reported that chronic lead exposure in rats caused DNA fragmentation in frontal cortex, hippocampus and cerebellum, indicating an apoptotic or necrotic underlying mechanism. Loss of cellular integrity in these three brain regions are responsible for the clinical features of lead poisoning such as disturbances in emotional response, memory and learning (Schneider *et al.,* 2012).

The administration of aqueous extract of *Psidium guajava* leaves seemed to have reduced the damages caused by lead acetate in brain tissues (cerebellum, cerebrum and hippocampus) probably due to its chelating and antioxidants properties.

5.2 NEUROBEHAVIOURAL STUDIES

The results of the present study showed decrease in motor activity observed in rats administered with lead acetate and resulted to increase in time taken to cross the balance beam. This could be related to the disorientation of the cerebellar layers and degeneration of the Purkinje cells of the Purkinje layer, which could have led to deficits in cerebellar coordination of movement, loss of grasping, maintenance of equilibrium, regulation of muscle tone and in the modulation of spinal cord. The observation is in tandem with the previous studies (Peters, 1996; Mattay *et al.*, 2002) in which altered fibers and overall myelin structure of the neurons of the cerebellum of animals exposed to lead were associated with a decline in cognitive function and could influence motor activities of the animals (Mattay *et al.*, 2002).

The study also showed that chronic lead exposure impaired learning abilities in Morris Water Maze (MWM) test in adult male Wistar rats. The impairment of spatial learning and memory being significant only suggests that exposure to lead caused damage in the hippocampus. It is observed that alteration in hippocampus and prefrontal cortex might be the basis of impairment of learning and memory abilities, in chronic lead exposure. This present observation agrees with other reports showing that Morris water maze task depend on hippocampus (Morris *et al.,* 1982) and related neuronal circuitry included prefrontal cortex which subserves attention and cognition (Silachev *et al.,* 2009; Leon *et al.*, 2010). The task of spatial learning requires involvement of hippocampal N-methyl-D-aspartate (NMDA) and muscarinic cholinergic receptors (McNamera *et al.,* 1993; Ohno *et al.,* 1992). Jett and Guilarte, (1995) had shown that developmental lead exposure causes alteration in NMDA and muscarinic cholinergic receptors in the hippocampus of rats, probably due to damage to mitochondria, microfilaments, and microtubules in hippocampal neurons and myelin sheath degeneration (Xu *et al.,* 2009). This might partly explain the reason for the spatial memory deficiency in lead treated rats in this study. It has also been reported that lead exposure causes disturbances in the aminergic system in the cerebral cortex, cerebellum and hippocampus, which contribute to cognitive and behavioural impairments in adult rats (Devi *et al.*, 2005). Inhibition in neurogenesis and alteration in process of differentiation of new cells in the dentate gyrus of hippocampus during early development (Jaako-Movits *et al.,* 2005), could be responsible for the spatial memory impairments. Decreased adult hippocampal gene expression during nerve growth factor following lead exposure after weaning (Schneider *et al.*, 2001) or during lifetime in rats (Cory-Slechta *et al.,* 2010) have also been speculated as contributing to impairment in learning and memory.

However, there was a reversal of impairment during the administration of *Psidium guajava* leaves extract. This could be due to the fact that the extract has the ability to chelate lead in brain and blood. Similarly, improvement had been reported following the administration of quercetin (Hu *et al.,* 2008) which is the main active component of

Psidium guajava serving to reduce the effect of lead (Lozoya *et al.,* 2002). The action observed may be due to synergism of quercetin with other component of the extract

5.3 OXIDATIVE STRESS MARKERS

The present study provided evidence that lead can induce oxidative stress in Wistar rats by increasing LPO and decreasing CAT, SOD and GSH. Reduced glutathione (GSH) concentrations, as well as modifications in CAT and (SOD) activities are the most frequently used markers in tissues or in blood (Khaki *et al.*, 2010). On the other hand, rats treated with lead acetate only showed a significant increase in serum LPO level and significant decrease in GSH level, CAT activity. Lead-induced oxidative stress in blood and other soft tissues has been postulated to be among the possible mechanisms of toxicity (Pande *et al.,* 2002; Auman *et al.,* 2007; Waters *et al.,* 2008). Disruption of pro-oxidant and antioxidant balance might lead to tissue injury. It was reported that lead increased the brain level of lipid peroxidation (Upasani *et al.,* 2001) and brain thiobarbituric acid-reactive substances and altered the antioxidant defense system (Adanaylo *et al.*, 1999).

This study further established that treatment with *Psidium guajava* leaves extract could lead to recovery from oxidative stress caused by lead acetate. There were no significant differences in serum LPO and GSH concentrations, CAT and SOD activities of the control and those of the animals treated with *Psidium guajava* after administration of lead acetate. This observation supports the report of Neeraj *et al.* (2012), that aqueous extract of *Psidium guajava* leaves was protective against sodium arsenite-induced toxicity in experimental rats. In that earlier study, treatment with aqueous extract of *Psidium guajava* significantly restored the level of oxidative stress markers. It was concluded that prophylactic co-administration of aqueous extract of the leaves could provide specific protection from oxidative injury.

Psidium guajava crude leaf aqueous extract, contains many flavonoids, especially quercetin (Witness *et al.*, 2008), which is a potent antioxidant has been shown to protect nitric oxide from the scavenging actions of superoxide anion (Ajay *et al.*, 2006). Quercetin chelates lead by forming a coordination bond with the lead ions through its orthophenolic groups located on the quercetin B ring (Bravo and Anacona, 2001). The hydroxyl groups of quercetin along with the carbonyl group easily donate electrons by undergoing resonance and stabilize free radicals that can initiate lipid peroxidation (Beecher, 2003). Therefore, it could be pertinent to speculate that the ability of the leaf extract of *Psidium guajava* to lower the LPO concentrations in the treatment group may be attributed to similar antioxidant properties.

5.4 HAEMATOLOGICAL INDICES

The present study showed a significant reduction of Red Blood Cell (RBC) count in rats administered with lead acetate. However, RBC increased insignificantly in rats administered with lead acetate and then treated with aqueous extract of *Psidium guajava* leaves. The observation in the present study was in agreement with other studies such as Suradkar *et al.* (2009) who observed that the reduction could be as a result of the effect of lead acetate on the activity of aminolevulinic acid dehydratase (ALAD), a key enzyme in haeme synthesis. Moreover lead also inhibit the conversion of coproporphyrinogen III to protoporphyrin IV leading to reduction in haemoglobin production and shortened life span of erythrocytes (Klassen, 2001). Progressive destruction of RBCs due to binding of lead with RBCs, leading to increased fragility and destruction could be the main reason for decrease haematological values (Rous, 2000). Helmy *et al.* (2000) reported significant decrease in Hb and PCV following exposure of rats to lead acetate.

The increase in WBC and platelet counts were not significant and were at variance with the study of Nwokocha *et al.* (2011) who observed that a significant increase in platelet and WBC count and speculated the increase could have been as a result of marrow infiltration by toxic substance and with a reactionary increase in some parameters. This was also contrary to the decrease in WBC which may directly be related with either decrease in their production from germinal centers of the lymphoid organs or increased lysis due to the presence of lead in the body (Avdheshumar *et al.*, 1998).

Significant improvement in RBC values and reduction in the WBC and platelets values in *Psidium guajava* and Succimer treated groups caused amelioration when compared to lead administered groups. This might be due to haemoprotective, immune stimulatory and anti-inflammatory effects and antioxidative property of *Psidium guajava*. The findings were in line with the results of Friday *et al.* (2010) which showed that the red blood cell counts, haematocrit and haemoglobin concentration increased significantly on the administration of guajava extract to both male and female rats but had no significant effect on the white blood cell counts. Extract of *Psidium guajava* leaves may possibly serve as an acceptable blood booster in an anaemic condition or for prophylactic purposes. Although the specific mechanism through which the extract facilitated the increase in these haematological indices was not ascertained. It is possible that the extract contains constituent(s) that can interact and stimulate the formation and secretion of erythropoietin, haematopoietic growth factors or committed stem cells (Friday *et al.*, 2010). Specifically, stimulations of haematopoietic growth factors and erythropoietin systems have been reported to enhance rapid synthesis of blood cells (Murray, 2000).

Moreover, the haematopoietic potential of the leaf extract of *Psidium guajava* may be related to its antioxidative activity (Friday *et al.*, 2010).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

From the observations in the present study, we concluded that:

i. The present study observed that there were deficits in Spatial learning, memory and motor activities in adult Wistar rats exposed to lead acetate.
ii. Lead acetate induced oxidative stress and changes in haematological indices in adult Wistar rats.
iii. There were histological changes in hippocampus, cerebellar and cerebral cortices of adult Wistar rats exposed to lead acetate.
iv. The study has shown that aqueous extract of *Psidium guajava* leaves ameliorated the changes induced by lead acetate in behaviour, oxidative stress markers, haematological indices and histopathological in adults Wistar rats.
v. Thus, aqueous extract of *Psidium guajava* leaves may be useful therapy in lead exposed patients, especially in lead poisoning endemic area

6.2 RECOMMENDATIONS

From the present study, we recommended that:

i. The consumption of *Psidium guajava* leaves should be encouraged especially the population who may be exposed to increased risk of lead poisoning.
ii. The actual underlying mechanism of *Psidium guajava* leaves extract in neuropathology is still unclear and further research is needed.

iii. Further study should be carried out using immunohistochemical methods to elucidate the deposits of lead in respective tissues of the brains exposed animals treated with *Psidium guajava* leaves extract.

iv. Phytochemical analysis of *Psidium guajava* leaves needs to be done.

v. Fractionating the extract and using the various fractions so as to determine the mechanism involved in its amelioration.

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