EVALUATION OF THE EFFECTS OF AQUEOUS *ALLIUM SATIVUM* (GARLIC) EXTRACTS ON LEAD-INDUCED CHANGES ON THE HIPPOCAMPUS AND CEREBELLUM OF WISTAR RATS

BY

Hamza Garba ADAMU

(B.Sc. A.B.U, 2010)

(P13MDHA8015)

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DECLARATION

I hereby declare that this research work reported in this dissertation entitle **"Evaluation of The Effects of Aqueous*Allium Sativum* (Garlic) Extracts On Lead Induced Changes On the Hippocampus And Cerebellum of Wistar Rats"**was carried out by me in the Department of Human Anatomy, Faculty of Medicine, ABU Zaria, under the supervision ofDr. A. A Buraimoh and Dr. A. O. Ibegbu. This dissertation work has not been presented for the award of degree elsewhere and the information derived from the literature has been dully acknowledged in the text.

Hamza GarbaAdamu **Date**

CERTIFICATION

This dissertation entitle"**Evaluation of The Effects of Aqueous *Allium Sativum* (Garlic) Extracts on Lead Induced Changes on the Hippocampus and Cerebellum of Wistar Rats"**by Adamu Hamza Garba meet the regulation governing the award of Master of Science (M.Sc.) degree in Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria under the supervision of Dr. A. A Buraimoh and Dr. A. O. Ibegbu. It is therefore approved for its contribution to knowledge and literature presentation.

Dr. A. A Buraimoh (B.Sc., M.Sc., PhD) **Date**
Chairman Supervisory Committee,
Department of Human Anatomy,
Faculty of Medicine,
Ahmadu Bello University, Zaria.

Dr. A. O. Ibegbu (B.Sc., M.Sc., PhD) **Date**
Member Supervisory Committee,
Department of Human Anatomy,
Faculty of Medicine,
Ahmadu Bello University, Zaria

Prof. S. S. Adebisi **Date**
Head of Department of Human Anatomy,
Faculty of medicine,
Ahmadu Bello University, Zaria

Prof. KabirBala **Date**
Dean School of Postgraduate Studies
Ahmadu Bello University, Zaria

DEDICATION

This research work is heartily dedicated to Almighty ALLAH (S.W.A), who spared my life to see the end of the work and to my late mother Rabi'atGarbaAdamu and also to my lovely nieces Hassana and Ussaina.

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ABSTRACT

Lead is a widespread environmental and industrial pollutant which is used in food preservation, cosmetics, pharmaceutical companies and laboratories. Despite many efforts to minimize lead poisoning, it continues to be a major health concern in many developing and developed countries.The present study was aimed at evaluating the effects of aqueous garlic extract on lead induced changes on the hippocampus and cerebellum of Wistar rats.Forty fiveWistar rats (both sexes) were used in the study and were randomly divided into nine groups with five rats in each group. The rats in Group one (G_1) served as the Control and received distilled water. The rats in Groups 2 and 3 received lead only at low (60 mg/kg) and high (120 mg/kg) respectively. The rats in Group 4 (G_4) received lead at low dose (60 mg/kg) and aqueous garlic extract at low dose (300 mg/kg) while the rats in Group 5 (G_5) received lead at high dose (120 mg/kg) and aqueous garlic extract at low dose (300 mg/kg). The rats in Group 6 (G_6) received lead at low dose (60 mg/kg) and aqueous garlic extract at high dose (500 mg/kg) while the rats in Group 7 (G_7) received lead at high dose (120 mg/kg) and aqueous garlic extract at high dose (500mg/kg). The rats in Group 8 (G_8) received lead at low dose (60 mg/kg) and Succimer (DMSA) at 30mg/kg b w while the rats in Group 9 (G_9) received lead at high dose (120 mg/kg) and Succimer (DMSA) at 30mg/kg. All the administration were carried out orally for twenty one (21) days. The rats were trained for both spartial learning and memory and beam walking before the administration and tests performed after the administration. At the end of the administration, rats were sacrificed and histological specimens of the cerebellum and hippocampus of the rats across the groups were prepared using the routine haematoxyline and eosin and cresyl violet staining

techniques.Sections of the lead treated groups revealed that lead produced deleterious effects on tissues of thehippocampal formation and cerebellum, disrupting the general histoarchitecture and cellular integrity while sections of lead and aqueous garlic extract administration revealed that aqueous garlic extract had promising positive effects on lead induced changes on the histological changes of the hippocampus and cerebellum. The result of Morris water maze and beam walking test showed that the rats in lead acetate treated Group experienced an increase in the meantime taken to complete task which when compared with the rats in Group one (Control) was found to be statistically significant (P□0.01), while the rats in the lead and aqueous garlic extract or Succimer (DMSA) treated Groups experienced a marked decrease in the meantime taken to complete task which when compared with the rats in Group one (Control) was found to be non-significant (P□0.05). The result of oxidative stress analyses of lead treated Groups rats showed decrease of superoxide dismutase, catalase and glutathione activities and increased level of malondialdehyde while there was increased superoxide dismutase, catalase and glutathione activities and decreased malondialdehyde levels in the aqueous garlic extract Groups. On the basis of the present study, it can be concluded that the promising potentials of aqueous garlic extracton lead induced toxicity were considered and it was found to be a good candidate for the therapeutic intervention of lead poisoning.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Exposure of the body organs and systems to heavy metals including lead has been found to cause adverse effects which affect their functionality (Owolabi*et al.*,2012). Although lead is a useful metal in life and is used in modern industries and agriculture, it is one of the most toxic heavy metals in the body and its poisoning is known as an important public health hazard (Patrick, 2006). General researches have demonstrated that lead can cause neurological, haematological, gastrointestinal, reproductive, circulatory and immunological disorders (Al Saleh *et al.,* 2009). Despite the strong evidences indicating the association between lead exposure and behavioral and cognitive impairments, but the mechanism by which lead causes these impairments remain poorly understood (Alireza*et al.,* 2013). Lead can enter the body mainly via eating, drinking or inhalation and transported to many tissues such as kidney, liver, bones and brain. As estimated by the World Health Organisation (WHO), the total lead intake from food by an adult is in the range of 26-28μg/dl/day in various Countries (Al-Saleh *et al.,* 2009).

Lead passes through the blood brain barrier (BBB) rapidly and causes neurotoxicity via oxidative stress molecular mechanism (El Sokkary*et al.,* 2003). The damage inflicted is usually a consequence of the ability of the lead to induce free radical generation within the cells. The elevated production of oxygen and nitrogen based radicals and related non radical products leads to the oxidation of essential macromolecules including lipids, proteins and DNA (Reiter *et al.*,2010). The resultant damage referred to as

oxidativestress and when the molecular damage is sufficiently severe, it can cause apoptosis or necrosis of neurons and glia. The brain and spinal cord are highly metabolically active organs which, even at rest, utilize an estimated 20% of the total oxygen taken by the lungs. This percentage increases substantially when the brain is active (Shukla*et al.,* 2011). Depriving the brain of its rich oxygen supply even for short intervals often has severe and irreversible detrimental morphological and physiological consequences for both neurons and glia (Shukla*et al.,* 2011). This high utilization of oxygen, however, comes at a heavy biological price. As important as oxygen is for the survival of neurons and glia, it also indirectly contributes to their destruction and death over time. The reason for this is that a small percentage of oxygen that enters the cells is metabolized to derivatives that gradually erode and destroy essential molecules(Reiter*et al.*, 2010). These destructive derivatives of oxygen are often referred to as free radicals or reactive oxygen species (ROS). Some of the notably destructive oxygen metabolites include the superoxide anion radical, hydrogen peroxide and hydroxyl radical; this latter agent is especially toxic to cells and indiscriminately harms or destroys any molecules in the vicinity of where the radical is generated (Volka*et al.*,2005).

Garlic (alliumsativum) as a phytomedicine is a specie in the onion genus, allium. Garlic is one of the most researched plants, with long history of medicinal use (Omotoso*et al.,* 2009). Garlic containssulphur, phosphorus, potassium and zinc ions, moderate amounts of selenium, vitamin A, vitamin C and smaller amounts of calcium, magnesium, sodium, iron, B complex vitamins and allicin, a compound to trap free radicals (El Demerdash*et al.*, 2005). Recently, it was found that the sulphur containing compounds of garlic have

anti mutagenic and anti-carcinogenic effects. Aqueous garlic extract exerts its antioxidant action by scavenging enzymes superoxidase dismutase, catalase and glutathione peroxidise and inhibits lipid peroxidation (Ataei*et al.*, 2014).

1.2 Significance of the Study

Lead poisoning can cause irreversible genetic and reproductive toxicity, haematological effects, neurological damage and cardiovascular effects. Despite many efforts to minimize lead poisoning, it continues to be a major health concern in many developing and developed countries (Shaik*et al.*,2014) and as such this study seeks to investigate the effects of aqueous garlic extract on lead poisoning particularly on the central nervous system.

1.3 Aim and Objectives of the study

1.3.1 Aim of the study

This study is aimed at determining the effects of aqueous garlic extract on lead induced damages on the hippocampus and cerebellum of adult Wistar rats.

1.3.2 Objectives

This study seeks to:

i. Evaluate the neurobehavioral effects of aqueous *Allium sativum*extract on lead induced changes on the hippocampus and cerebellum of adult Wistar rats using Morris water maze and beam walking methods respectively.

ii. Biochemically evaluate the effect of aqueous allium sativum extract on lead induced changes on hippocampus and cerebellum of adult Wistar rats using

oxidative stress markers namely; catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and lipid peroxidation (LPO).

iii. Evaluate the possible histological and histochemical effects of aqueous *Allium sativum* extract on lead induced changes on the hippocampus and cerebellum of adult Wistar rats using routine Hand E staining and cresylviolet methods.

1.4 Scope of the Study

This study was limited to the general histochemical, histomorphological, behavioural and cognitive changes in the hippocampus and cerebellum of the adult Wistar rats as a result of exposure to lead acetate.

1.5Study Hypothesis

Aqueous garlic extract has protective effects on lead induced changes on the antioxidants system, spatial learning and memory and cytoarchitectureof the hippocampus and cerebellum of the Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background

Metals are elements found in nature usually in the form of their respective compounds which are widely used in industries, affecting human health through occupational and environmental exposure. Most notably, are the heavy metals which have specific density greater than 5 g/cm^{3}such as mercury (Hg), cadmium (Cd), arsenic (As) and lead (Pb). Most of these heavy metals persist in the environment and produce a variety of adverse effects because they are generally not biodegradable (Alissa and Ferns, 2011). One of the leading metallic xenobiotic is lead (Pb) and because of the unique properties of lead, like softness, high malleability, ductility, low melting point and resistance to corrosion, have resulted in its widespread usage in different industries like automobiles, paint, ceramics, plastics, etc. This in turn has led to a manifold rise in the occurrence of free lead in biological systems and the inert environment (Flora *et al.,* 2012).

Lead poisoning also known as plumbism, colicapictorum, saturnism, devon colic, or painter's colic is a type of metal and a medical condition in humans and other vertebrates caused by increased levels of the heavy metal lead in the body (Karri *et al.,* 2008). Signs and symptoms may be different in adults and children. Early symptoms of lead poisoning in adults are commonly nonspecific and include depression, loss of appetite, intermittent abdominal pain, nausea, diarrhea, constipation, and muscle pain (Karri *et al.,* 2008). Other early signs in adults include malaise fatigue, decreased libido and problems with sleep. An unusual taste in the mouth and personality changes are also early signs (Patrick,

2006). Children are more at risk of lead poisoning because their smaller bodies are in a continuous state of growth and development (Landrigan, 2002). Lead is absorbed at a faster rate compared to adults, which causes more physical harm than to older people. The classic signs and symptoms in children are loss of appetite, abdominal pain, vomiting, weight loss, constipation, anemia, kidney failure, irritability, lethargy, learning disabilities, behavioral problems, and slow development of normal childhood behaviors, such as talking and use of words, and permanent intellectual disabilities are both commonly seen (Landrigan 2002). Lead poisoning has also been reported to cause oxidative damage to DNA by interfering with the incision step of DNA repair system, thus inducing carcinogenicity (Koedrith and Young, 2011). Also, a couple of proteins *in vivo* and the plasma membrane have lost their integrity and function as a result of plumbism (Abam*et al.,* 2008).

2.2 Absorption of Lead and Its Distribution

Lead is unique in that man-made sources contribute almost solely to exposure in the post-industrial era. Pre-industrial blood lead levels of Native Americans living in the United States 700-1,000 years ago are estimated to have been 0.016 μg/dL (Flegal*et al.,* 1992). These levels are 50-200 times lower than those currently reported in individuals living in remote regions of the Himalayas with no known lead exposure (0.78- 3.2 μg/dL). On the other hand, children with lead dust or soil dust exposure can have blood lead levels as high as 90 μg/dL (Flegal*et al.,* 1992). Bone levels in pre-industrial skeletal remains indicate that current body lead burdens are 500-1,000 times greater than in individuals with pre-industrial exposure to lead (Patterson *et al.,* 1991). Lead exposure occurs mainly

through the respiratory and gastrointestinal (GI) tracts. Approximately 30-40 percent of inhaled lead is absorbed into the bloodstream (Phillip *et al.,* 1994). Gastrointestinal absorption varies depending on nutritional status and age. Iron is believed to impair lead uptake in the gut, while iron deficiency is associated with increased blood lead concentrations in children (Ziegler *et al.,* 1978). Calcium supplementation studies have demonstrated that increased dietary calcium in rats, infants, and children resulted in consistent decreases in the absorption of lead (Bogden*et al.,* 1992). Increased in- takes of magnesium, phosphate, alcohol, and dietary fat have also been shown to decrease gastrointestinal absorption of lead (Barltrop*et al.,* 1979).

Gastrointestinal tract absorption of lead is greatest in infancy; infants can absorb up to 50 percent of lead ingested from food, water, contaminated dust, or soil, while adults absorb only 10-15 percent (Markowitz, 2000). Inorganic lead is minimally absorbed through the skin, but tetraethyl or alkyl-lead (leaded gasoline), which is still legally allowed in aircraft, watercraft, and farm machinery, is well absorbed through the skin (Papanikolaou*et al.,* 2005). Once absorbed, 99 percent of circulating lead is bound to erythrocytes for approximately 30-35 days (only one percent of absorbed lead is found in plasma and serum) and is dispersed into the soft tissues liver, renal cortex, aorta, brain, lungs, spleen, teeth, and bones over the following 4-6 weeks.

Due to the short half-life (35 days) in the bloodstream, blood lead levels cannot be used to diagnose or rule out evidence of exposure that occurred more than six weeks prior to testing (Rabinowitz*et al.,* 1997). In adults approximately 80-95 percent of retained lead is

stored in the bone, while in children approximately 70 percent is stored in bone, resulting in more soft tissue lead in children compared to adults. Lead is stored in bone for extended periods of time, with half-life estimates of 20-30 years. Even with the bone turnover that occurs in childhood and adolescence, there is evidence to suggest that by the seventh decade of life, more than one-third of bone mass contains lead acquired in childhood and adolescence (Kosnett*et al.,* 1994). Due to the net slow turnover and release of lead from bone, lead in bone appears to increase significantly with age. Mean tibial lead concentrations for adolescents have been measured at 3 μg/g, concentrations for adults 30-50 years at 17 μg/g, and at 30 μg/g for adults over 75 years (Kosnett*et al.,* 1994).

Storage of lead in different compartments of bone is also an age-dependent process. During in- fancy and childhood, lead is deposited in trabecular bone because it is the most active site of remodeling; whereas, in adults lead is deposited in both trabecular and cortical bone (Aufderheide*et al.,* 1992). Although by adulthood most of the lead burden is stored in cortical bone and teeth, what is stored in trabecular bone is partially labile and is released back into the bloodstream and soft tissues by diffusion as well as resorption (Fleming *et al.,* 1997). Bone lead can contribute to elevated blood lead levels long after the exposure no longer exists (Klein *et al.,* 1998). Situations that increase bone turnover, such as pregnancy, lactation, postmenopausal osteoporosis, hyperthyroidism, and cisplatin chemotherapy, have been shown to increase blood lead levels as a result of the mobilization of bone stores. Bone lead is also readily transferred to the fetal skeleton during pregnancy (Gulson*et al.*,2003).

2.3 Toxic Effects and Mechanism of Lead Toxicity

Lead affects at least three major organ systems such as: (1) the central and peripheral nervous systems; (2) the heme biosynthetic pathway; and (3) the renal system. Clinical manifestations differ somewhat between children and adults. In the child, the most serious symptoms are found in the central nervous system with subtle effects (e.g., decreased intelligence quotient and cognitive effects) occurring at lower levels and severe effects (e.g., seizures, encephalopathy) occurring at higher levels. Chelation therapy has reduced the mortality rate and morbidity substantially at higher levels. However, chelation therapy at lower levels (< 45 μg/dL), has not been shown to be as effective as removal of the lead source from the child's environment. Children are much more sensitive than adults to the neurocognitive and behavioral effects of lead, probably primarily for two reasons: (1) children absorb 40 to 50% of dietary lead whereas adults absorb about 10%; and (2) the nervous system develops rapidly in the young child. The blood lead threshold for neurocognitive and behavioral effects is probably lower in children than in adults (Lanphear*et al.,* 2005).

In the child, lead appears to have an effect on renal function even at levels below 10 μg/dL (Longhman*et al.,*1998). This especially true if the lead exposure occurs over a sustained period of time. Subtle abnormalities in renal tubular function, associated with aminoaciduria, glycosuria, and increased excretion of low-molecular weight proteins can occur (Longhman*et al.,* 1998). Lead has been clearly demonstrated to produce tubular nephrotoxicity and chronic interstitial nephritis in humans and rodents after chronic

exposure. In addition, lead in the kidney interferes with activation of Vitamin D 1, 2-dihydroxy cholecalciferol, a dependent process (Longhman*et al.,* 1998).

Lead interferes in the formation of active Vitamin D, which has an important role in its influence on calcium metabolism. Calcium is under tight homeostatic control in all cells. The active form of Vitamin D is produced, primarily, from the activation of Vitamin D by sunlight on the skin. The circulating hormone binds to Vitamin D Receptors (VDRs) in the nucleus of cells in the gastrointestinal tract, kidney and bone. This binding activates a cascade of events to increase calcium absorption (Longhman*et al.,* 1998). Due to their similar biochemical nature, lead can be absorbed by this mechanism especially in children who have decreased calcium intake. In addition, calbindin-D, the binding protein that aids in calcium transport, binds to lead with high affinity and may increase transport of lead in low calcium states (Onalaja*et al.,* 2000). It is known that lead interferes with the utilization of iron for the formation of heme. This probably occurs in every cell, although it is best studied in the blood-forming organs. In chronic, moderately severe lead poisoning, anemia is commonly found (Anderson *et al.,* 1996). A decrease in hemoglobin is reported to occur in iron-sufficient children when blood lead concentration exceeds 60 μg/dL. The anemia is a normocytic, normochromic, well-compensated hemolytic anemia. However, in children with iron deficiency, the decrease in hemoglobin may occur at lower blood lead levels and present as a microcytic anemia.

Anemia in lead poisoning results from impairment of hemoglobin production and changes in the red blood cell membrane. Lead’s interference in heme biosynthesis is

characterized by several unique enzyme blockades causing increased urinary delta-aminolevulinic acid (ALA), urinary coproporphyrin, and erythrocyte zinc protoporphyrin. While anemia may not be seen until blood lead concentrations are markedly elevated, the effect on hemoglobin synthesis occurs at lower levels (Anderson *et al.*, 1996). ALA dehyratase is inhibited at levels of 15 μg/dL in children. At levels of 30 μg/dL, elevation in erthyrocyteprotoporphyrin may be seen. Finally, at levels of 40, reduced hemoglobin synthesis may be found. The basophilic stippling of red cells is due to the presence of aggregated ribosomes, which may also include mitochondrial fragments. Conditions, such as lead poisoning, can result in altered ribosomes to have a higher propensity to aggregate. With staining, this appears as increased basophilic granulation (Bull., 2006).

The central nervous system can be affected by lead in children. Over the past several decades, epidemiologic studies have demonstrated that chronic, low-level lead poisoning may lead to CNS injury in young children (Weitzman *et al.*, 2005). Earlier studies suggested that altered electrophysiologic responses and adverse effects on intelligence quotient occurred at blood lead concentrations of 30 μg/dL or higher(Weitzman *et al.*, 2005). However, more recent data suggests environmental lead exposure in children at blood lead concentrations < 7.5 μg/dL is associated with cognitive deficits (Lanphear*et al.*, 2005). Studies have suggested that a permanent pattern of cognitive dysfunction may result from lead poisoning in the first several years of life (White *et al.*, 1993). It should be noted that the variability in blood lead testing allows for statistical significance to be seen at levels, only, above 5 μg/dL. Acute lead poisoning may produce encephalopathy in

children. Ataxia, altered state of consciousness, and seizures have been reported in children with blood lead concentrations over 80 μg/dL (White*et al.*,1993).

Oxidative stress has been the most important mechanism in lead toxicity. Lead catalyze oxidative reactions and generate reactive oxygen species. These reactive oxygen species (ROS) inhibit the production of sulfhydryl antioxidants, inhibit enzyme reactions impairing heme production, cause inflammation in vascular endothelial cells, damage nucleic acids and inhibit DNA repair, and initiate lipid peroxidation in cellular membranes (Patrick 2006).

2.3.1 Oxidative Stress

Oxidative stress represents an imbalance between the production of free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Flora, 2011), has been reported as a major mechanism of lead induced toxicity. Under the influence of lead, onset of oxidative stress occurs on account of two different pathways operating simultaneously; first comes the generation of ROS, like hydroperoxides (HO), singlet oxygen and hydrogen peroxide (HO), and second, the antioxidant reserves become depleted (Flora *et al.*,2002). The antioxidant defenses of the body come into play to nullify the generated ROS.

The most important antioxidant found in cells is glutathione (GSH). It is a tripeptide having sulfhydryl groups and is found in mammalian tissues in millimolar concentrations. It is an important antioxidant for quenching free radicals (Mates, 2000). Glutathione

exists in both reduced (GSH) and oxidized form (GSSG). The reduced state of glutathione donates reducing equivalents (H+ e) from its thiol groups present in cysteine residues to ROS and makes them stable. After donating the electron, it readily combines with another molecule of glutathione and forms glutathione disulfide (GSSG) in the presence of the enzyme glutathione peroxidase (GP). GSH can be regenerated from GSSG by the enzyme glutathione reductase (GR). Under normal conditions, 90% of the total glutathione content exists in reduced form (GSH) and around 10% is in the oxidized form (GSSG). Under conditions of oxidative stress, the concentration of GSSG is much higher than that of GSH.

Lead shows electron sharing capability that results in the formation of covalent attachments. These attachments are formed between the lead moiety and the sulfhydryl groups present in antioxidant enzymes, which are the most susceptible targets for lead and which eventually get inactivated. Lead inactivates glutathione by binding to sulfhydryl groups present in it. This results in synthesis of GSH from cysteine via the γ-glutamyl cycle, which is usually not effective in replenishing the supply of GSH (Hultberg*et al.,* 2004). Similarly, lead inactivates enzymes like δ-amino levulinic acid dehydratase (ALAD), glutathione reductase(GR), glutathioneperoxidase (GP) and glutathione-S-transferase, which further depresses the glutathione levels (Ahamed and Siddiqui, 2007).

A few other notable antioxidant enzymes that are rendered inactive by lead include super oxide dismutase (SOD) and catalase (CAT). Decrease in SOD concentration reduces the

disposal of superoxide radical, whereas reduction in CAT impairs scavenging of superoxide radical. Apart from targeting the sulfhydryl groups, lead can also replace the zinc ions that serve as important co-factors for these antioxidant enzymes and inactivates them (Flora *et al.,* 2007).

Lipid peroxidation is another biomarker of oxidative stress and is one of the most investigated consequences of ROS on lipid membranes. The generated free radicals capture electrons from the lipids present inside the cell membranes and damages the cell. Apart from lipid peroxidation, lead also causes hemoglobin oxidation, which directly causes RBC hemolysis. This occurs due to inhibition of ALAD, which results in an increased concentration of substrate ALA in both blood and urine. These elevated ALA levels generate hydrogen peroxide and superoxide radical and also interact with oxyhemoglobin, resulting in the generation of hydroxyl radicals (Patrick, 2006). Progression of all the above mentioned mechanisms makes the cell extremely vulnerable to oxidative stress and may lead to cell death.

2.3.2 Reactive Oxygen Species and Oxidative Stress

Cells acquire energy derived from oxidative phosphorylation linked to oxygen utilization. Approximately 1-3% of electrons passing through the respiratory chain in the mitochondria will react with oxygen to generate superoxide anion instead of water (Valko*et al.,* 2007). Superoxide anion can be converted by other enzymes to other reactive species. All of these species are called reactive oxygen species (ROS), and

include superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), Singlet oxygen ($^1O_2$) and hydroxyl radical (OH) and anion (OH^-).

Actually, low concentrations of ROS can participate in regulatory mechanisms, acting as messengers to regulate cell growth, differentiation, and the immune system (Nordberg et al., 2001). For example, ROS can stimulate extracellular signal-regulated kinase (ERK) for cell growth (Seifried*et al.,* 2007). It has been shown that hydrogen peroxide and nitric oxide act as signaling molecules and these active species can be used to kill the invading microbes. ROS can affect the signal transduction pathways in cells leading to cell proliferation or cell death including apoptosis and necrosis. These effects mediated by ROS are dependent on the dosage and the duration of ROS and are cell-specific (Seifried*et al.,* 2007). ROS can also be generated by exogenous sources such as environmental toxicants, ionizing radiation, and ultraviolet radiation (Valko*et al.,* 2007). Among the species of ROS, hydroxyl radical is the most highly reactive, whereas H_2O_2 is less active (Nordberg *et al.,* 2001).

2.3.2.1 Superoxide Anion

Superoxide anion can be formed from oxygen during enzymatic catalysis, e.g., xanthine oxidase, lipoxygenase, and cyclooxygenase (Thannickal*et al.,* 2000). Furthermore, superoxide anion can also be generated by NADPH-oxidase in phagocytic immune cells to act as a messenger of signal transduction pathways (Nordberg *et al.,* 2001). Due to its negative charge, superoxide anion is not able to freely cross membranes and therefore, cannot escape the organelle where it is produced (Thannickal*et al.,* 2000). Superoxide

anion is considered to be a primary ROS and it can interact with other molecules to produce secondary ROS (Valko*et al.*, 2007). For example, in the presence of metals, the reaction of superoxide ion and hydrogen peroxide will produce hydroxyl radicals. Superoxide anion can also spontaneously dismutate to hydrogen peroxide and oxygen in aqueous solution, especially at lower pH or in the presence of superoxide dismutase (SOD) (Thannickal*et al.*, 2000).

2.3.2.2 Hydrogen peroxide

Hydrogen peroxide is one of the less reactive ROS and unlike superoxide anion, hydrogen peroxide can penetrate biological membranes and is used to act as a signaling molecule (Nordberg*et al.*, 2001). Most of the intracellualar hydrogen peroxide is contributed by dismutation of superoxide anion, but hydrogen peroxide can be formed by the 2-electron reduction of oxygen mediated by other enzymes (Massey, 1994). Although hydrogen peroxide is less reactive than other ROS, it can interact with other molecules to generate more highly reactive ROS. In the presence of transition metals, hydroxyl radicals can be generated from hydrogen peroxide via the Fenton reaction, and these radicals are highly reactive (Nordberg *et al.*, 2001). Superoxide anion can react with metal ions to recycle the transition metal ions. Hydrogen peroxide can also react with chloride to form hypochlorous acid and can be removed by catalases, glutathione peroxidases, and peroxiredoxins.

$$H_2O_2 + Fe^{2+} \longrightarrow OH + OH^- + Fe^{3+}$$ (Fenton reaction)

$$Fe^{3+} + O_2^- \longrightarrow Fe^{2+} + O_2$$

$H_2O_2 + O_2^- \longrightarrow OH + OH^- + O_2$ (Haber-Weiss reaction)

$H^+ + Cl^- + H_2O_2 \xrightarrow{} HOCl + H_2O$

2.3.2.3 Hydroxyl Radical

The highly reactive hydroxyl radical has a half-life of approximately 10^{-9}s in aqueous solutions. It is the most dangerous ROS because it can react with almost all chemicals including cellular macromolecules such as DNA, lipid, and proteins. Hydroxyl radicals can be generated via the Fenton reaction in vitro. However, the Fenton reaction is not favorable in vivo because of the limited availability of free transition metal ions (Valko*et al.*, 2006). Hydroxyl radicals can be generated through various mechanisms. In addition to the Fenton reaction, hydroxyl radicals can be generated from decomposition of water by ionizing radiation, and hydroxyl radicals are also generated from reactions involving alkylhydroperoxides (Valko*et al.*, 2006).

2.3.2.4 Nitric Oxide

Nitric oxide (NO) is formed from the reaction of L-arginine and oxygen catalyzed by NO synthetase using NADPH.

$$\text{L-arginine} + O_2 + \text{NADPH} \xrightarrow{\text{NO Synthase}} \text{L-citrulline} + .NO + NADP^+$$

NO has a short half-life of a few seconds in aqueous solution (Valko*et al.*, 2006). Since NO is readily able to cross biological membranes, it acts as a messenger mediating

neutrotransmission, dilation of blood vessels, inhibition of platelet aggregation, angiogenesis, neurotransmission, and functions of the immune system (Govers*et al.,* 2004). Nitrosative stress is caused by excess NO that is able to interact with superoxide anion to form peroxynitrite anion (ONOO-), which is highly reactive causing DNA fragmentation and lipid peroxidation. Nitrosative stress affects immune defense; peroxynitrite anion readily crosses the membrane to effect cytotoxic actions on invading microbes (Marnett*et al.,* 2000). Moreover, peroxynitrite is able to interact with carbon dioxide to form the reactive intermediate ($ONOOCO^{2-}$), which will further oxidize proteins (Carr *et al.,* 2000).

2.3.3 Ionic Mechanism of Lead Toxicity

Ionic mechanism of action of lead mainly arises due to its ability to substitute other bivalent cations like Ca , Mg , Fe and monovalent cations like Na, though bivalent cations are more readily substituted, affecting various fundamental biological processes of the body (Lidsky and Schneider, 2003). Significant effects have been found on various fundamental cellular processes like intra and intercellular signaling, cell adhesion, protein folding and maturation, apoptosis, ionic transportation, enzyme regulation and release of neurotransmitters (Garza *et al.,* 2006). The ionic mechanism contributes principally to neurological deficits, as lead, after replacing calcium ions, becomes competent to cross the blood-brain barrier (BBB) at an appreciable rate. After crossing the BBB, lead accumulates in astroglial cells (containing lead binding proteins). Toxic effects of lead are more pronounced in the developing nervous system comprising immature astroglial cells that lack lead binding proteins. Lead easily damages the

immature astroglial cells and obstructs the formation of myelin sheath, both factors involved in the development of BBB.Lead, even in picomolar concentration, can replace calcium, thereby affecting key neurotransmitters like protein kinase C, which regulates long term neural excitation and memory storage (Flora *et al.,* 2012). It also affects the sodium ion concentration, which is responsible for numerous vital biological activities like generation of action potentials in the excitatory tissues for the purpose of cell to cell communication, uptake of neurotransmitters (choline, dopamine and GABA) and regulation of uptake and retention of calcium by synaptosomes. This interaction between lead and sodium seriously impairs the normal functioning of the aforementioned sodium dependent processes (Bressler*et al.,* 1999).

2.4 Natural Antioxidant

An antioxidant is a substance which, when present at a low concentration as compared to that of the oxidizable substrate, can prevent the oxidation of that substrate. Generally, an antioxidant can prevent lead toxicity in three ways (Garcia and Gonzalez, 2008):

1. By inactivating the generated ROS at molecular level, thereby terminating the radical chain reaction (chain breaking).
2. By chelating the lead ion and preventing further formation of ROS.
3. By chelating lead and maintaining it in a redox state, which leads to its incompetency to reduce molecular oxygen.

Natural antioxidants can be categorized into enzymatic and nonenzymatic. Enzymatic antioxidants like superoxide dimutase (SOD), catalase (CAT), glutathione (GSH) are

produced endogenously in the cells, whereas nonenzymatic antioxidants like carotenoids, flavonoids, vitamins, and minerals are constituents of many fruits, vegetables, nuts, grains and some meats (Flora,2009). The amount of antioxidants present under normal physiological conditions are adequate to quench the free radicals that are generated at a normal physiological rate. Any further increment in the concentration of free radicals (due to environmental or natural causes) can cause an imbalance between the free radicals and antioxidants, leading to oxidative stress (Blokhina*et al.,* 2003). This is where the role of exogenous antioxidants becomes important. They are taken through the diet or in the form of supplements to maintain the homeostasis between free radicals and antioxidants and thus prevent various deleterious effects, like heavy metal toxicity, inflammation, cancer, aging, cardiovascular and brain disorders (Willcox*et al.,* 2004; Pietta, 2000).

2.5 Role of Antioxidants in Lead Induced Oxidative Stress

Lead stimulate oxidative stress, a state that involves the generation of free radicals beyond the permissible limits, depleting at the same time the antioxidant reserves and thus hampering the ability of the biological system to reverse the resulting effects. Free radicals generation starts a chain reaction that results in lipid peroxidation, disruption of cell membrane, protein oxidation and oxidation of nucleic acids like DNA and RNA leading to cancer (Gurer and Ercal, 2000). The following natural antioxidant have been investigated and put forth for the treatment of lead induced oxidative stress.

2.5.1 Superoxide Dismutase

Superoxide Dismutase as a major antioxidant enzyme, play a crucial role in scavenging $O\bullet^{2-}$. The superoxide dismutase family is specialized in eliminating superoxide anion radicals derived from extracellular stimulants, including ionizing radiation and oxidative insults, together with those primarily produced within the mitochondrial matrix as byproducts of oxygen metabolism through the electron transport chain (Miao *et al.,* 2009). Three distinct isoforms of SOD have been identified and characterized in mammals: copper-zinc superoxide dismutase (Cu/ZnSOD); encoded by the sod1 gene), manganese superoxide dismutase (MnSOD); encoded by the SOD 2 gene, and extracellular superoxide dismutase (ECSOD) encoded by the SOD 3gene.These forms of SOD exhibit similar functions, but characteristics of their protein structure, chromosome localization, metal cofactor requirements, gene distribution, and cellular compartmentalization are distinctly different from one another (Miao *et al.,*2009).

2.5.2 Glutathione Peroxidases

Glutathione peroxidase is the general name for a family of multiple isozymes that catalyze the reduction of H_2O_2 or organic hydroperoxides to water or corresponding alcohols using reduced glutathione (GSH) as an electron donor;

$$H_2O_2 + 2GSH \rightarrow GS\text{-}SG + 2H_2O$$

In mammalian tissues, there are four major selenium-dependent glutathione peroxidases (GPX) and phospholipid hydroperoxide glutathione peroxidase, which incorporates cysteine instead of selenocysteine in the conserved catalytic motif (Margis*et al.*, 2008).

GSH the main antioxidant in CNS, is the most abundant small molecule, nonproteinthiol in cells present in millimolar concentration in the brain (Dringen*et al.*, 2003). It consists of a tripeptide of glutamate, cysteine and glycine characterized by a reactive thiol group and γ-glutamyl bond. Reduced GSH can nonenzymatically act directly with free radicals, notably superoxide radicals, hydroxyl radicals, nitric oxide, and carbon radicals for their removal. GSH peroxidase and GSH reductase can act enzymatically to remove H_2O_2 and maintain GSH in a reduced state (Dringen*et al.*, 2003).

2.5.3 Catalase

Catalase is a ferriheme-containing enzymethat is responsible for the conversion of hydrogen peroxide (but not other peroxides) to water (Droge, 2002). It is localized in peroxisomes and may also be found in cytoplasm and mitochondria. It has a minor role at low levels of hydrogen peroxide generation but becomes more important at higher levels of hydrogen peroxide production.Catalase is a porphyrin-containing enzyme of high molecular weight which destroys hydrogen peroxide in high concentration by catalysing its two- electron dismutation into oxygen and water (Schonbaum and Chance, 1976):

$$2H_20_2 \longrightarrow 2H_20 + 0_2$$

Alternatively, it can act as a peroxidase at low hydrogen peroxide concentration, using reducing co-substrates such as alcohols, including ascorbate and phenols:

$$RHXH + H_2O_2 \longrightarrow RX + 2H_20$$

2.5.4 Vitamins

The role of vitamins particularly B, C and E, has been found to be extremely significant and competitive in fighting toxicological manifestations of lead poisoning. These vitamins may chelate lead from the tissues along with restoring the pro/antioxidant balance. The role of various prominent vitamins are:

2.5.4.1 *Ascorbic acid (vitamin C)*

Ascorbic acid is probably the most widely studied vitamin when it comes to the prevention of lead induced oxidative stress. Its property of quenching ROS along with metal chelation makes it a potential detoxifying agent for lead (Tariq, 2007,Das and Saha, 2010). A recent study done by Chang *et al.*,(2011) showed the defensive effect of ascorbic acid on oxidative stress, developed in the hippocampus of lead exposed suckling rats. They reported that introduction of ascorbic acid during pregnancy and lactation caused to some extent amelioration of oxidative stress in the developing hippocampus.

Shan *et al.,* (2009) examined the defensive effects of ascorbic acid and thiamine against the toxic effects of lead on testes of mice. Exposure to lead exhibited a significant decrease in epididymal sperm count and motility, along with the induction of apoptosis through activation of caspase-3, Fas/Fas-L and Bcl-2. Co-administration of ascorbic acid and thiamine reverted the oxidative stress in a concentration dependent manner, as well as DNA damage and apoptosis induced by lead in rat liver cells (Wang*et al.*,2007). Supplementation of ascorbic acid in combination with silymarin was able to reduce acute hepatotoxic lead toxicity (Shalan*et al.,* 2005).

2.5.4.2 *Vitamin E (α-tocopherol)*

Vitamin E is a fat soluble vitamin with numerous biological functions (Flora, 2002). It possesses powerful anti-oxidative properties, operative in the membrane to prevent lipid peroxidation by obstructing the free radical chain reaction. Sajitha*et al.,* (2010) had reported that Vitamin E administered to rats counteracted the deleterious effect of lead by scavenging free radicals and thus preventing oxidative stress. Lead induced ALAD inhibition in the erythrocytes was found to be reversed by the treatment with vitamin E (Rendon-Ramirez *et al.,* 2007).

Effect of vitamin E in combination with other antioxidants has been found to be more pronounced than its individual administration. Flora *et al.,*(2003) had reported that co-administration of Vitamin E with monoisoamyl derivative (MiADMSA), which is a thiolchelator, exerts an elevated recovery from lead burden in rats. Interestingly, α-tocopherol is capable of reducing ferric iron to ferrous iron thereby to acting as a pro-oxidant. Moreover, the ability of α-tocopherol to act as a pro-oxidant (reducing agent) or antioxidant depends on whether all of the α-tocopherol becomes consumed in the conversion from ferric to ferrous iron or whether, following this interaction, residual α-tocopherol is available to scavenge the resultant ROS (Yamamoto and Nike, 1988).

2.5.5 Garlic (*Allium Sativum*)

2.5.5.1 Origin and history

Garlic(*Allium sativum*) is a member of the Liliaceae family, (*Allium sativum*) is a cultivated food highly regarded throughout the world. Originally from Central Asia,

garlic is one of the earliest of cultivated plants (Londhe*et al.,* 2011). The name " *Allium sativum*" is derived from the Celtic word "all", meaning burning or stinging, and the Latin "sativum"meaning planted or cultivated. The English word, garlic, is derived from the Anglo-Saxon "gar-leac" or spear plant, referring to its flowering stalk (Bhandari, 2012). Garlic (*Allium sativum)* besides being used as food, has been used as medicinal plant for over 4000 years for a variety of ailments including headache, bites, intestinal worms and tumors (Block *et al.,* 1985).

It is one of the earliest documented examples of plants employed for treatment of disease and maintenance of health. It was in use at the beginning of recorded history and was found in Egyptian pyramids and ancient Greek temples (Bhandari, 2012). Applications of garlic have been documented in ancient medical texts from Egypt, Greece, Rome, China and India. Garlic was administered to provide strength and increase work capacity for labourers in many cultures. Almost 25 centuriesago, Hippocrates, the Father of Medicine, stated "let food be thy medicineand let medicinebe thy food" (Bhandari, 2012). Garlic (*Allium sativum*) has a broad spectrum of activities: antibacterial, anticarcinogenic, hypolipidemic, hypoglycemic, antifungal, antiatherosclerotic, and antioxidant (Sharma *et al.,* 2010



Figure 2.1: Garlic (AliumSativum)

2.5.5.2 *Chemical composition of garlic*

Garlic contains at least 33 sulphur compounds, several enzymes, 17 amino acids, and minerals such as selenium (Bhandari *et al.,* 2012). Of all the Allium species, garlic contains a higher concentration ofsulphur compounds. Its pungent odour and many of its medicinal effects are due to the sulphur compounds. Approximately 1% alliin (S-allylcysteinesulfoxide) is present in dried, powdered garlic. Allicin (diallylthiosulfinateordiallyl disulfide), which is the most biologically active compound in garlic, does not exist until it is crushed or cut (Icieke*t al.,* 2009). Sulphur compounds: alliin, allicin, ajoene, diallyltrisulfide, sallylcysteine, vinyldithiines, allylpropyl disulfide, S-allylmercaptocystein and others Enzymes: allinase, peroxidases, myrosinase and others Amino acids and their glycosides: arginine and others Selenium, germanium, tellurium and other trace minerals. Additionally, garlic also contains a high concentration of selenium, which is responsible, in part, for garlic's antioxidant and cancer-preventive effects ((Bhandari *et al.,* 2012)).

2.6 Nervous System

The human nervous system is the most complex system in the human body which is formed by a network of more than billion nerve cells, assisted by many glial cells. Nerve tissue is distributed throughout the body as an integrated communication network. The nervous system is divided into central nervous system and peripheral nervous system.

The central nervous system is made up of brain and spinal cord. It is formed by neurons and supporting cells called neuroglia. Peripheral nervous system (PNS) is formed by

neurons and their processes present in all regions of the body. It consists of cranial nerves, arising from brain and spinal nerves, arising from the spinal cord. It is also divided into two subdivisions: Somatic nervous system and Autonomic nervous system.

The brain is made up three major division: 1. the prosencephalon known as forebrain which consist of telencephalon and diencephalon. Telencephalon include cerebralhemispheres, basal ganglia, hippocampus and amygdaloid nucleus. 2. Mesencephalon also known as midbrain, 3. Rhombencephalon consisting of metencephalon which forms by pons and cerebellum and myelencephalon formed by medulla oblongata.

2.6.1 Cerebellum

2.6.1.1 *Morphology*

The cerebellum, or "small brain," develops from the embryologic rhombic lip, a zone of cells between the alar and roof plates at the level of the pontine flexure. Although it develops from a "sensory" region (the rhombic lip), the cerebellum is concerned primarily with motor function (Afifi*et al.,* 2005). The cerebellum is located in the posterior fossa of the skull, separated from the occipital lobes by a dural fold, the tentorium cerebelli. It overlies the dorsal surfaces of the pons and medulla oblongata and contributes to the formation of the roof of the fourth ventricle.

The cerebellum consists of a midline vermis and two laterally placed hemispheres. The parts of the hemispheres adjacent to the vermis are known as the paravermal or

intermediate zones. The cerebellum is connected to the midbrain, pons, and medulla oblongata by three pairs of peduncles which contain afferent and efferent fibres (Clarke *et al.,* 1996).

2.6.1.2*Cellular layers of the cerebellum*

Thecerebellar cortex has three (3) layers: an outer molecular layer, a central layer of large purkinje cells, and an inner granule layer (Luiz*et al.,* 2003). Five cell types are distributed in the different cortical layers. Basket and stellate cells are in the molecular layer, Purkinje cells are in the Purkinje cell layer, and granule and Golgi cells are in the granule cell layer(Luiz*et al.,* 2003). Of these five cell types, the Purkinje cell constitutes the principal neuron of the cerebellum, since it is the only cerebellar neuron that send its axons outside the cerebellum. All the other cells are intrinsic neurons and establish connections within the cerebellum (Afifi*et al.,* 2005). The purkinje cells have conspicuous cell bodies and their dendrites are highly developed. These dendrites occupy most of the molecular layer as shown in fig 2.2.

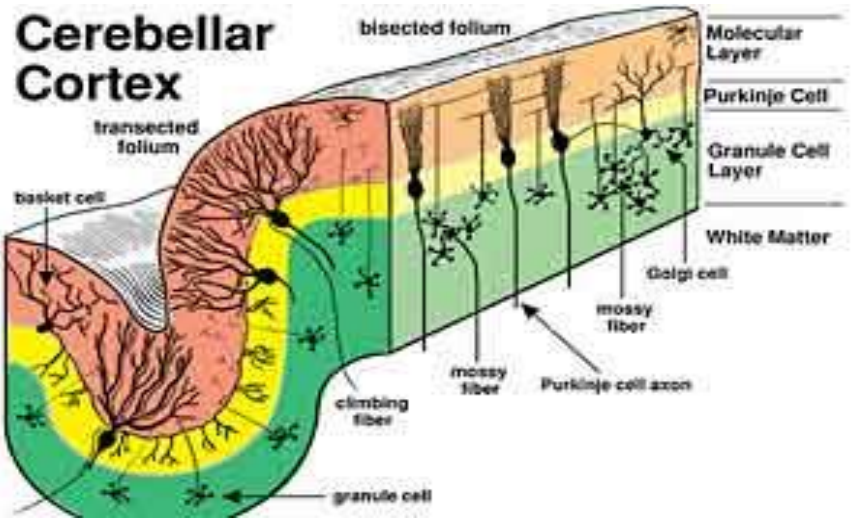


Figure 2.2: Cellular layers of the cerebellum showing the outer molecular layer (brown), the purikinje cell layer (yellow) and the granule cell layer (green) (Linaset al.,2004).

2.6.1.3 *Function of the cerebellum*

The cerebellum have the following functions:

- ❖ Coordination of Movement; the cerebellum controls the timing and pattern of muscle activation during movement.
- ❖ Maintenance of equilibrium in conjunction with the vestibular system
- ❖ Regulation of muscle tone and modulation of spinal cord and brain stem mechanism involved in postural control.

2.6.2 Hippocampus

2.6.2.1 *Morphology and formation of the hippocampus*

Anatomically, the hippocampus is an elaboration of the edge of the cerebral cortex (Gray, 2000, Chris, 2008). In rodents, the hippocampus, is a horseshoe-shaped 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 the sibiculum collectively make up the hippocampal formation, which is said to be part of the limbic system as shown in Figure 2.2. The hippocampus and the dentate gyrus belong to the allocortex and has simplified laminar pattern compared with the neocortex.

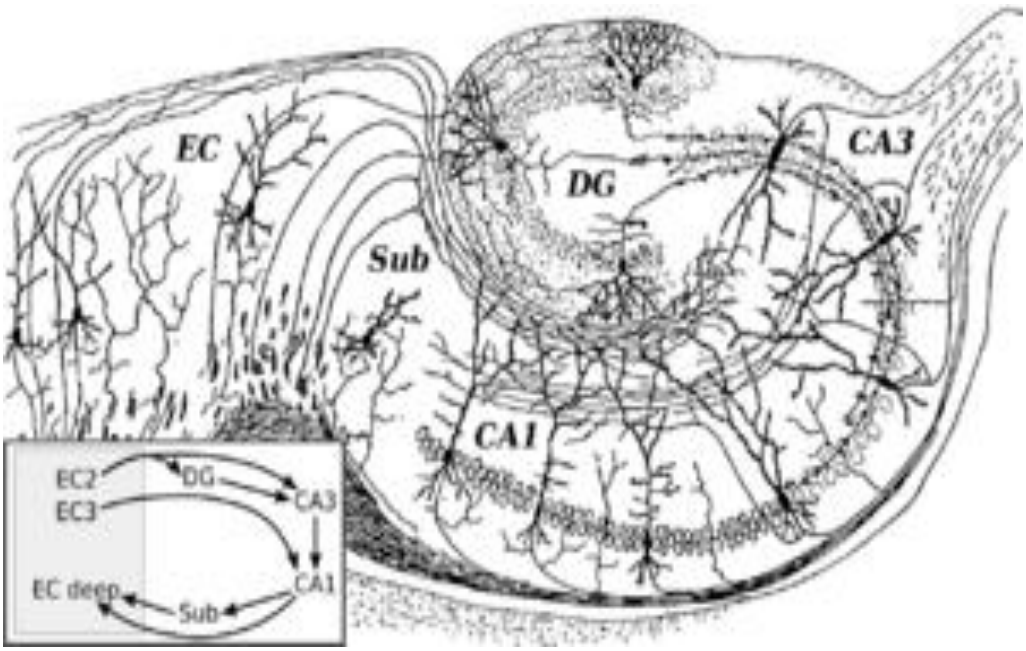


Figure 2.3 Cells and layers of the hippocampus (Annabelle, 2009).

KEY

EC: Entorhinal cortex

Sub: Subiculum

CA1: CornuAmmonis 1

CA3: CornuAmmonis 3

DG: Dentate gyrus

2.6.2.2 *The cells and layers of hippocampus*

Although Ramon y Cajal described seven laminae in the hippocampus, it is customary to combine the different laminae into three major layers; the molecular layer, the pyramidal cell layer, and the stratum oriens (polymorphic layer) (Afifi*et al.*, 2005).The pyramidal cell layer is divided into a zone in which the pyramidal cells are compact and a zone in which the pyramidal cells are less compact. The boundary between the compact and less compact zones of the pyramidal layer separates the two divisions of the hippocampus into the superior division and the inferior division. The hippocampus has been subdivided further into fields designated as cornuAmmonis 1, 2, 3, and 4 (CA_1 through CA_4). CA_1, the largest hippocampal field in humans, is located in the superior division at the interface between the hippocampus and the subiculum (Chris, 2008). CA_2 and CA_3 are in the inferior division within the hippocampus. CA_4 constitutes the transition zone between the hippocampus and the dentate gyrus.

There are basically two types of neurons in the hippocampus: the principal neurons (pyramidal cell) and the intrinsic neurons (polymorphic cell, basket cell) (Afifi*et al.*, 2005). The pyramidal neurons in the pyramidal cell layer are the principal neurons of the hippocampus. They are the only neurons with axons which contribute to the outflow tract from the hippocampus. Pyramidal neurons vary in size and density in different regions of the hippocampus(Afifi*et al.*, 2005). They are smaller and more densely packed in the superior region than in the inferior region. Basal dendrites of pyramidal neurons are oriented towards the ventricular surface; apical dendrites are oriented towards the molecular layer. Both types of dendrites arborize extensively and are rich in dendritic

spines. Axons of pyramidal cells are directed towards the ventricular surface, where they gather to form the alveus and fimbria and finally join the fornix as the outflow tract from the hippocampus(Luiz*et al.,* 2003). Intrinsic neurons have axons which remain within the hippocampus. Because of the irregularity of their perikarya and dendrites, they are referred to as polymorphic neurons. They are situated in the stratum oriens. Their irregularly oriented dendrites arborize locally, while their axons ramify between pyramidal neurons and arborize around the perikarya of pyramidal neurons in a basket formation (hence the term basket cells).

2.6.2.3 *The dentate gyrus*

The dentate gyrus is composed of a similar series of strata namely; a molecular layer, a granular cell layer, and a polymorphic layer. The molecular layer is continuous with that of the hippocampus. The granular layer is made up of small, densely packed granular cells whose axons form the mossy fiber system which links the dentate gyrus and the hippocampus. The cells in the polymorphic layer are varied and include pyramidal and basket cells. Unlike the hippocampus, the output of the dentate gyrus does not leave the hippocampal formation (Afifi*et al.,* 2005).

2.6.2.4 *Subiculum*

Like the hippocampus and the dentate gyrus, the subiculum is composed of three layers: a molecular layer, a pyramidal layer, and a polymorphic layer. The polymorphic layer originates in the adjoining entorhinal cortex. Axons of pyramidal neurons in the

subiculum, like those in the hippocampus, contribute to the output of the hippocampal formation.

2.6.2.5*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. It 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.

CHAPTER THREE

3.0 Materials and Methods

3.1 Experiment rats

Forty five(45) Wistar rats (both sexes) were used in this study and were purchased from the Animal House Centre, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria Nigeria. The ratswere maintained at the Animal House, Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria Nigeria, under controlled conditions.

Experimental Groups:

TheWistar rats wererandomly divided into nine groups (N=5) as follows:

1- Control group (CG): each animal received with distilled water.

2- Low dose lead group (LLG): each animal receivedwith 10% ofLD_{50} of lead acetate (60mg/kg body weight) dissolved in distilled water once a day.

3- High dose lead group (HLG): each animal received with 20% of $LD_{50}$of lead acetate (120mg/kg body weight) dissolved in distilled water once a day.

4- Low dose Garlic extract + (LGE + LLG): each animal received aqueous garlic extract (300mg/kg body weight) and 10% of $LD_{50}$of leadacetate (60mg/kg body weight) dissolved in distilled water once a dayby oral gavages.

5- Low dose Garlic extract + High dose lead group (LGE + HLG): each animal received aqueous garlic extract (300mg/kg body weight) and 20% of $LD_{50}$of lead acetate (120mg/kg body weight) dissolved in distilled water once a dayby oral gavages.

6- High dose Garlic extract + Low dose lead group (HGE + LLG): each animal received aqueous garlic extract (500mg/kg body weight) and 10% of $LD_{50}$of lead acetate (60mg/kg body weight) dissolved in distilled water once a dayby oral gavages.

7- High dose Garlic extract + High dose lead group (HGE +HLG): each animal received aqueous garlic extract (500mg/kg body weight) and 20% of $LD_{50}$of lead acetate (120mg/kg body weight) dissolved in distilled water once a dayby oral gavage.

8- Standard drug (Succimer) + low dose lead group (SD + LLG): each animal received Meso 2,3dimercaptosuccinic acid (30mg/kg body weight) and 10% of $LD_{50}$of lead acetate (60mg/kg body weight) dissolved in distilled water once a day.

9- Standard drug (Succimer) + High dose lead group (SD + HLG): each animal received Meso 2,3dimercaptosuccinic acid (30mg/kg body weight) and 20% of $LD_{50}$of lead acetate (120mg/kg body weight) dissolved in distilled water once a day.

All the treatments were given orally once a day for twenty one (21) days.

Table 3.1: showing groups of Wistar rats and their treatment

Groups	*No of rats*	*Treatment (Dosage/ body weight)*	*Duration*
1	5	Distilled water (Control)	1^{st} – 21^{st} day
2	5	Low dose lead acetate (60mg/kg body weight)	1^{st} – 21^{st} day
3	5	High dose lead acetate (120mg/kg body weight)	1^{st} – 21^{st} day
4	5	low dose garlic extract (300mg/kg body weight) + low dose lead acetate (60mg/kg body weight)	1^{st} – 21^{st} day
5	5	Low dose garlic extract (300mg/kg body weight) + high dose lead acetate (120mg/kg body weight)	1^{st} – 21^{st} day
6	5	High dose garlic extract (500mg/kg body) + low dose lead acetate (60mg/kg body weight)	1^{st} – 21^{st} day
7	5	High dose garlic extract (500mg/kg body weight) + high dose lead acetate (120mg/kg body weight)	1^{st} – 21^{st} day
8	5	Meso 2,3 dimercaptosuccinic acid (30mg/kg body weight) + low dose lead acetate (60mg/kg body weight)	1^{st} – 21^{st} day
9	5	Meso 2,3 dimercaptosuccinic acid (30mg/kg body weight)+ high dose lead acetate	1^{st} – 21^{st} day

3.2 Lead Acetate and Its Preparation

High dose: 20% (120mg/kg body weight) of the LD_{50} (600mg/kg body weight) of the lead acetate was dissolved in 1ml of distilled water for each of the adult Wistar rat according to their body weight.

Low dose: 10% (60mg/kg bw) of the LD_{50} (600mg/kg body weight) of the lead acetate was dissolved in 1ml of distilled water for each of the adult wistar rat according to their body weight according to the method of Magaji*et al.,* (2014).

3.3 Source of Garlic and Preparation of the Aqueous Extract:

Fresh garlic bulbs was obtained from AbubakarRimi market, Kano and authenticated at the Department of Biological Science (Voucher Number: 423), Ahmadu Bello University Zaria, Nigeria. The garlic bulbs were then separated, peeled and washed with distilled water. After drying in a shed, the clean garlic bulbs were crushed with an electric grinder and then dissolved in distilled water. The extract was then decanted carefully using muslin cloth (Senapati*et al.,* 2000).

3.4 Source of Succimer (Meso 2,3dimercaptosuccinic acid)

Succimer (DMSA) was purchased from ABCAM Plc with quote order reference number (#1887607) and product key (ab142294).

3.5 Neurobehavioural Study

3.5.1 Assessment of spatial memory

Spatial memory and learning test (Morris Water Maze).

Morris water maze is a behavioural neuroscience test to study spatial memory and learning according to the method of Morris,(1981). It is a very accurate study of learning, memory, and spatial working and can also assess damage to cortical regions of the brain (D′Hooge*et al.,* 2001).

The basic procedure for the Morris water maze test is that the rat will be placed in a small pool of water which contains an escape platform hidden a few millimetres away and below the water surface as shown in Plate I. Many factors can influence the performance, including sex, the environment in which they were raised, exposure to drug etc (Wongwitdecha*et al.,* 1996).



Plate I: Morris water maze test

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 will be able to locate the platform in a shorter time. As the rat was learning to locate the hidden platform, any improvement in the time to find the platform was recorded (Liu *et al*, 2011). After the learning, the rats were administered with lead and aqueous extract once a day over a period of three (3) weeks and the above procedures were repeated during and after the administration of aqueous garlic extract. The memory of the rats were evaluated by the time the rats spent in locating the platform before, during and after administration of lead and the aqueous garlic extract as shown in Plate I.

3.5.2 Beam walking Test

Fine motor coordination and balance was assessed by beam walking test. The apparatus used; consisted of 1 meter beam with flat surface resting 50cm above the table top on two poles. A black box was placed at the end of the beam as the finishing point as shown in Plate II. Performance on the beam was quantified by measuring the time it takes for the Wistar rat to traverse the beam and the number of paw slips that occur in the process. The test was done in four (4) days; three (3) of training and two (1) of testing respectively.



Plate II: Beam Walking Test

3.6 Animal Sacrifice and Tissues Processing

3.6.1 Sacrifice

At the end of the experiment, the ratswere deeply anaesthetized with chloroformin the sacrificing chamber. An incision was made through the skin and muscles of the skull. The skull was opened through the midsagittal suture and the brain tissue was removed carefully, washed in normal saline and fixed in normalized fixative containing 10% formaldehyde in 0.01M PBS at room temperature. After the fixation, the tissues were taken to the histology laboratory of Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria, Nigeria for tissue processing.

3.7 Biochemical Analysis

Blood samples were collected and centrifuged at 1000rmp for 10mins and the serum was collected and used for the estimation of oxidative stress markers as follows;

3.7.1 Measurement of lipid peroxidation level (MDA)

MDA is commonly used as an index of lipid peroxidation. MDA content was assayed using method described by Ohkawa*et al.*,(1979) modified by Atawodi*et al.*,(2011). And the specific activity was expressed as nmol/mg protein.

3.7.2 Measurement of Antioxidant Enzymes Activities

The SOD activity was estimated according to Fridovich*et al.,* (1989); CAT according to Abei's method (1974) and GSH activity assayed by Ellman's method (2004). The activities of SOD, CAT and GSH were defined as u/ml, u/mg protein and µg/ml respectively.

3.8 Tissues Processing

The fixed tissues were processed routinely and stained using Haematoxylin and Eosin (Hand E) and cresyl violet special staining methods.

3.8.1 Routine Paraffin Sectioning

The fixed tissues were removed from bouin's fluidand dehydrated using ascending grades of alcohol series. This method involves dehydration of tissue in two (2) changes of 70% alcohol and two (2) changes of 90% alcohol, three (3) changes of 95% alcohol and three (3) changes of absolute alcohol, each of the stage lasted for 30 minutes. The dehydrated tissues were cleared in two (2) changes of xylene for two (2) hours; the clearing helped to remove opacity from the dehydrated tissues thereby making them transparent. The cleared tissues were infiltrated by immersion into molten paraffin wax to allow solidification. The embedded tissues were blocked in rectangular block and then the blocks were cut in 5µm serial sections using rotary microtome. The tissue sections were allowed to float in water at 30^{o}C to help the spreading of the paraffin ribbons. The clean slides were used to pick the tissue sections from the warm water bath. The slides were then left to dry and later stained using haemotoxylin and eosin (Hand E) and cresyl violet staining methods.

3.8.2Staining

3.8.2.1*Haematoxylin and eosin (H and E) staining method*

The method of H and E staining technique involved hydrating the tissue sections in descending grades of alcohol from 100%, 95%, 90% and finally 70%. Each of these steps

lasted for three (3) minutes and the tissues were then washed in running tap water. The tissues were stained with haematoxylin for twenty five (25) minutes, washed in water and differentiated in acid alcohol. The tissues were then counter stained with eosin and blued in scott water. The tissues were hydrated in ascending grades of alcohol and cleared with xylene for three (3) changes of five (5) minutes each. The tissues were then mounted with cover slips using a mounting media and later viewed under a light microscope and photomicrographs taken using digital amscope.

3.8.2.2*Cresyl violet staining*

Cresyl violet staining method was used to stain Nissl substances in the cytoplasm of neurons in the formalin fixed tissue. This stain was commonly used to identify the neuronal structure in the brain and spinal cord tissues.

The fixed tissues were de-waxed with xylene for two (2) changes in three minutes and then rehydrate in absolute alcohol for two (2) changes in three minutes. The rehydrated tissues were then stained in 0.1% cresyl violet for 4-15 minutes. The tissues were then quickly rinsed in tap water to remove the excess stain, Washed in 70% ethanol and dehydrated using absolute ethanol for two (2) in three (3) minutes. Finally the tissues were cleared with xylene for (2) changes and mount using DPX.

3.9Data Analysis

All the results were analysed using Statistical Package for Social Scientist (SPSS version 18) and the results were expressed as mean ±SEM. The statistical significance between

the means were analysed using one way analysis of variance (ANOVA) and P- value ≤ 0.05 was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of aqueous Garlic extract on lead induced changes on Body Weight of the Rats

The results on the effect of lead administration on body weight changes are shown in Table 4.1. The results showed a significant difference in weight betweenthe Control and the treated Groups at (P□ 0.05). There was a gradual increase in the mean body weight of the ratsin the Control group (Group one) as shown in Table 4.1. However, there was a gradual decrease in body weight of the ratsin the lead treated groups namely Groups 2, 3,4, and 5 as compared with the ratsin the Control group.

In Groups 2 and 3, there was a gradual decrease in the final body weight and when compared with the ratsin the Control group was statistically significant (P□ 0.01), after three weeks of lead administration. On the other hand, the ratsin the lead and aqueous garlic extract or Succimertreated groups, there were gradual increases in the body weight which when compared with the ratsin the Control group(Group one) were not significant after second and third weeks of administration but was statistically significant after the first week of administration(P□ 0.05) as shown in Table 4.1.

Table 4.1 Effect of aqueous Garlic extract on lead induced changes on body weight of Wistar rats

Group	N	0 Week Mean ±SEM(g)	1ST Week Mean ±SEM(g)	2nd Week Mean ±SEM(g)	3rd Week Mean ±SEM(g)
1 Control	5	096.00±0.71	103.00±2.52	119.00±5.57	121.33±1.86
2 60mg/kg Pb^{2+}	3	142.20±5.45**	137.20 ±5.33 **	137.20±5.33**	136.20±5.33**
3 120mg/kg Pb^{2+}	3	144.60±4.42 **	137.60±3.80**	137.60±3.80**	134.60±3.80**
4 60mg/kg Pb^{2+} + Extract	3	138.60±4.45 **	138.60±5.90**	135.60±5.90**	137.00±5.08**
5 120mg/kgPb^{2+}+ Extract	4	119.00±6.54 **	125.00±3.00*	125.00±3.00**	125.00±2.65
6 60mg/kg Pb^{2+} + Extract	4	096.80±0.49	133.40±1.17**	138.80±7.13	143.20±6.95
7 120mg/kgPb^{2+}+ Extract	3	120.00±6.18 **	137.40±3.42**	137.40±3.42*	141.80±3.48
8 120mg/kgPb^{2+}+ DMSA	3	096.80±0.49	128.00±1.30**	128.00±1.30	138.40±1.17
9 120mg/kgPb^{2+}+DMSA	3	099.25±6.10	119.40±1.81	119.40±1.81	125.20±1.66

All the values are expressed as Mean ± SEM, the value in the same column with the same superscript are significantly different at *P = □ 0.05or **P = □ 0.01.
g□ grams
N□ number of animal per group

4.2 Effect of aqueous Garlic extract on lead induced changes on Neurobehavioral Studies

4.2.1 Effect of aqueous Garlic extract on lead induced changes on Spatial Learning and Memory Using Morris Water Maze Test

The results of the effect oflead acetate exposure on spatial learning and memory using Morris water maze Test are shown in Table 4.2. The result showed that after four days of Morris water maze training for all groups (Control and treated Groups), the ratslearnt how to locate the hidden platformindicated by the decreasing mean time taken by the ratsto complete Morris water maze which showed no significant difference between groups (P 0.05). However, after the administration, the result showed that there was a reduction in the mean time taken by the ratsin Group one (Control) to complete Morris water maze task as shown in Table 4.2. The result also showed that the ratsin lead acetate treated Groups; two (2), three (3), four (4) and five (5) respectively, had increases in the meantime taken to complete Morris water maze task which when compared with the ratsin Group one (Control) was found to be statistically significant (P 0.01).

However, the ratsin the lead and aqueous garlic extract or Succimer treated Groups had a marked reduction in the meantime taken to complete Morris water maze task which when compared with the ratsin Group one (Control) the difference was not significant(P 0.05).

Table 4.2 Effect of aqueous Garlic extract on lead induced changes on Morris Water Maze Test

Group	N	0 Week Mean ±SEM(s)	1ST Week Mean ±SEM(s)	2nd Week Mean ±SEM(s)	3rd Week Mean ±SEM(s)
1 Control	5	05.20±0.84	04.35±0.28	01.89±0.23	02.17±0.28
2 60mg/kg Pb^{2+}	3	05.80±1.58	12.67±0.72**	09.49±1.09**	16.06±0.52**
3 120mg/kg Pb^{2+}	3	06.11±0.88	11.16±0.88**	11.79±0.68**	17.23±1.02**
4 60mg/kg Pb^{2+} + Extract	3	04.06±1.18	03.22±1.22	05.31±0.62**	09.73±0.57**
5 120mg/kgPb^{2+}+ Extract	4	03.63±0.71	02.49±0.27	03.70±0.45	16.89±0.82**
6 60mg/kg Pb^{2+} + Extract	4	07.49±2.84	01.52±0.18	01.89±0.42	01.69±0.16
7 120mg/kgPb^{2+}+ Extract	3	07.06±3.25	04.81±0.38	03.59±0.22	03.75±0.42
8 120mg/kgPb^{2+}+ DMSA	3	11.36±1.98	04.61±0.98	03.39±0.49	02.48±0.36
9 120mg/kgPb^{2+}+DMSA	3	12.49±4.82	03.92±0.54	03.43±0.23	03.36±0.42

All the values are expressed as Mean ± SEM, the value in the same column with the same superscript are significantly different at P □ 0.01
s□ second
N□ number of animal per group

4.2.2 Effect of aqueous Garlic extract on lead induced changes on Motor Coordination Using Beam Walking Test

The results of beam walking test are shown in Table 4.3. The results showed that the time taken by the ratsto traverse the beam was analyzed. The results showed a significant difference across Groups 2, 3, 4, 5, 6, 7, 8 and 9 (P□ 0.05), compared with the Control Group. In the Control Group (Group one), the result showed that there was no increase in the meantime taken by the ratsto traverse the beam while there was significant increase in the meantime taken by the ratsin lead treated Group 2, 3, and 4 to traverse the beam which when compared with the Control Group was found to be statistically significant (P□ 0.05).

In week 3, the results, showed a significant increase in the time (s) taken to cross the beam by the ratsin the experimental Groups 2, 3, 4, 5, 6, 7, 8, and 9 when compared to the ratsin Group 1 (Control). The result showed a significant decrease in the time (s) taken by the ratsin Groups 6, 7, 8, and 9 to cross the beam when compared to the ratsin Groups 2, 3, 4 and 5 (P□ 0.05).

Table 4.3Effect of aqueous Garlic extract on lead induced changes onmotor coordination using beam walking assay of Wistar rat

Group	N	0 Week Mean ±SEM(s)	1ST Week Mean ±SEM(s)	2nd Week Mean ±SEM(s)	3rd Week Mean ±SEM(s)
1 Control	5	41.95±1.31	41.66±0.85	42.16±1.26	42.94±3.61
2 60mg/kg Pb^{2+}	3	60.08±4.01**	82.34±3.97**	57.51±1.38**	73.02±2.63**
3 120mg/kg Pb^{2+}	3	52.63±1.06**	83.32±1.34**	54.88±1.29**	75.66±3.13**
4 60mg/kg Pb^{2+} + Extract	3	55.24±0.89**	69.59±5.32**	55.85±0.85**	68.81±5.55**
5 120mg/kgPb^{2+}+ Extract	4	53.40±0.87**	79.91±4.34**	55.50±1.15**	78.27±5.69*
6 60mg/kg Pb^{2+} + Extract	4	56.29±1.09**	54.20±1.41*	52.87±0.37*	57.20±1.38
7 120mg/kgPb^{2+}+ Extract	3	55.46±1.42**	59.77±3.96**	52.22±2.42*	63.39±4.44
8 120mg/kgPb^{2+}+ DMSA	3	54.33±0.96**	54.65±1.01*	46.52±2.51	56.65±0.94
9 120mg/kgPb^{2+}+DMSA	3	54.33±0.70**	54.68±1.59*	50.37±3.70	56.24±1.15

All the values are expressed as Mean ± SEM, the value in the same column with the same superscript are significantly different at *P = □ 0.05 or **P = □ 0.01.
s□ second
N□ number of animal per group

4.3 Effect of aqueous Garlic extract on lead induced changes on Oxidative Stress Biomarker

The results of the effect of lead acetate on the antioxidant enzyme activities and lipid peroxidation are shown in the Table 4.4. The result of superoxide dismutase (SOD) activity, showed that there was a significant decrease in SOD activities in Groups 2, 3, and 4 when compared to SOD activities in the Control Group (Group 1)(P□ 0.05), while there was a non-significant increase in SOD activities in Groups 5, 6, 7, 8, and 9 when compared to the Control Group.

The results of GSH analysis, showed that there was a significant decrease in GSH activities in Group 2, 3, and 5 when compared to the Control Group (P□ 0.05), while there was a non-significant increase in GSH activities in Groups 4, 6, 7, 8, and 9 when compared to the Control Group. The result of Catalase (CAT), showed that there was a significant decrease in CAT activity in Groups 2, 3, and 4 when compared to CAT activities in the Control Group (Group 1) (P□ 0.05), while there was a non-significant increase in CAT activities in Groups 5, 6, 7, 8, and 9 when compared to the Control Group.

The result of lipid peroxidation (LPO) level, showed that there was significant increase in lipid peroxidation (LPO) level in Group 2, 3, and 4 when compared to lipid peroxidation (LPO) level in the Control Group (P□ 0.05), while there was a non-significant increase in lipid peroxidation (LPO) level in Groups 5, 6, 7, 8, and 9 when compared to the Control Group

Table 4.4 Effect of aqueous Garlic extract on lead induced changes onoxidative stress markers

Group	N	SOD Mean ±SEM	GSH Mean ±SEM	CAT Mean ±SEM	MDA Mean ±SEM
1 Control	5	155.10±2.35	22.26±1.46	6.63±0.39	6.30±0.17
2 60mg/kg Pb^{2+}	3	136.35±1.89*	11.29±1.04*	2.75±0.24**	7.95±0.48*
3 120mg/kg Pb^{2+}	3	137.22±2.43*	12.33±0.46*	2.63±0.37**	8.11±0.47*
4 60mg/kg Pb^{2+} + Extract	3	136.08±3.19*	21.67±1.66	4.56±0.53*	7.86±0.54*
5 120mg/kgPb^{2+}+ Extract	4	156.87±1.20	10.71±0.91**	6.14±0.20	6.68±0.29
6 60mg/kg Pb^{2+} + Extract	4	140.90±1.99	26.66±2.63	5.91±0.52	6.72±0.11
7 120mg/kgPb^{2+}+ Extract	3	158.17±6.53	25.83±1.07	6.70±0.42	6.58±0.43
8 120mg/kgPb^{2+}+ DMSA	3	159.19±4.08	27.22±4.74	6.69±0.37	6.76±0.16
9 120mg/kgPb^{2+}+DMSA	3	161.90±4.90	25.56±1.46	7.18±0.61	6.80±0.33

All the values are expressed as Mean ± SEM, the value in the same column with the same superscript are significantly different at *P = □ 0.05or **P = □ 0.01

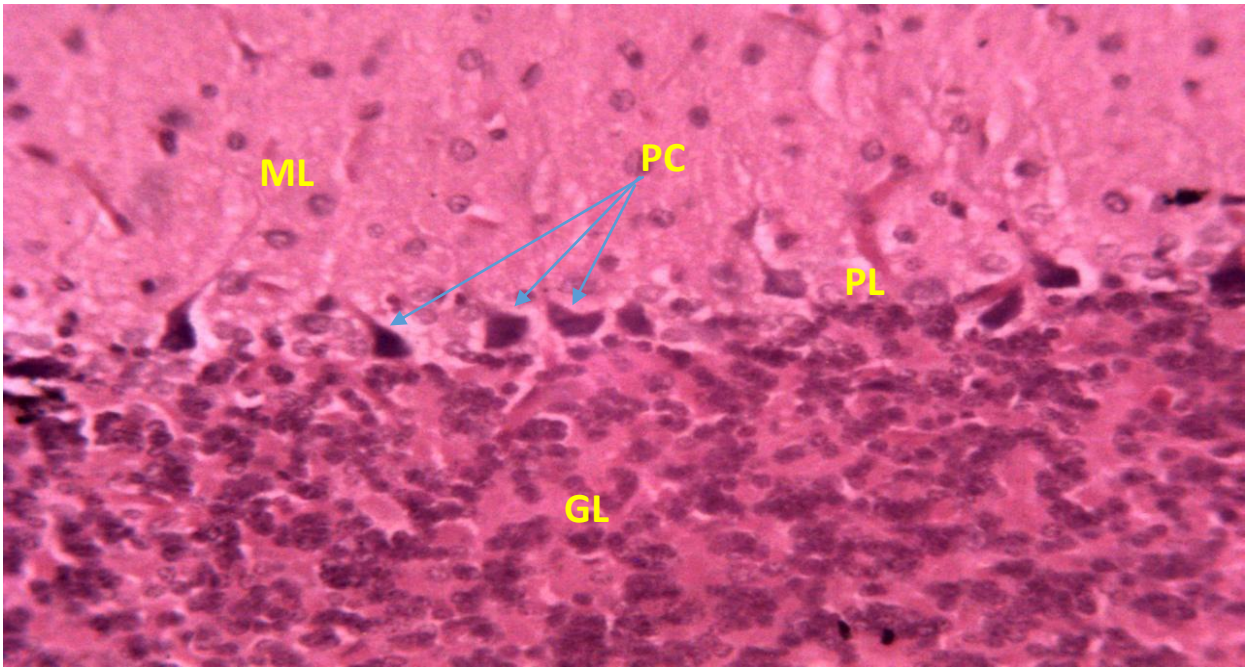
4.4 Histological Studies:

The results from histological observations showed histological changes in the tissues studied. The cerebellum of the ratsin the control group (Group 1), showed normal cytoarchitecture with clearly defined three layers namely; an outer Molecular layer, inner Granular layer and intermediate Purkinje layer with normal purkinje cells as shown in Plates 1 and 2, while the cerebellum of the ratsexposed to low and high doses of a Lead acetate (Group 2 and 3) only,showed marked neurodegeneration of layers of the hippocampus and cerebellum with much of the Purkinje layer beenaffected as shown in Plates 3, 4, 5 and 6. The cerebellum of the ratsexposed to low doses of the lead acetate and aqueous garlic extract; lead acetate and 30mg/kg body weight of Succimershowed similar cytoarchitecture with the Control Group with normal molecular layer, granular layer and middle purkinje layer as shown in Plates 7,8,11,12,15 and 16. However, the cerebellum of the ratsexposed to high doses of the lead acetate and aqueous garlic extract; lead acetate and 30mg/kg body weight of Succimer as shown in Plates 9, 10, 13, 14, 17 and 18 revealed mild neurodegenerationof the cerebellum layer as compared with the ratsin the Control group.

The histological studies showed normal histoarchitectureof the hippocampus in the Control group with normal pyramidal cell layer with pyramidal cells as shown in Plates 19 and 20 while the ratstreated with lead acetate only (Groups 2 and 3) demonstrated gross disruption of the hippocampus and loss of its basic histological form as shown in Plates21, 22, 23 and24 respectively.The majority of the Pyramidal cells were shrunken, irregular, abnormal in outline and ill-identified cytoplasmic processes were also

present.The pyramidal cells also appeared relatively smaller than in the Control Group (Group 1) and pyramidal cells showedpyknotic nuclei.

Plates 25, 26, 27 and 28 showed the hippocampus of the ratstreated with lead acetate and extract at low dose (Groups 4 and 5) which showed relatively disorganized pyramidal cell layer as compared with the ratsin the Control group. The pyramidal cells appear normal with deeply stain nuclei. However, Plates 29, 30, 31 and 32 showed the hippocampus of the ratstreated with lead acetate and aqueous garlic extract at high doses (Groups 6 and 7) and also Plates 33, 34, 35 and 36 showed the hippocampus of the ratstreated with lead acetate and 30mg/kg body weight ofSuccimer (Groups 8 and 9) with sparse of Pyramidal cells in the Pyramidal cell layer which was approximately similar to that of the Control group, and the nuclei showed somewhat deeply stained appearance.



Pate III: A section of the cerebellum of the Control group (G1), showing normal orientation of the molecular layer (ML), granular layer (GL), and purkinje layer (PL) with normal Purkinje cells (PC) (H and E Stain x250).

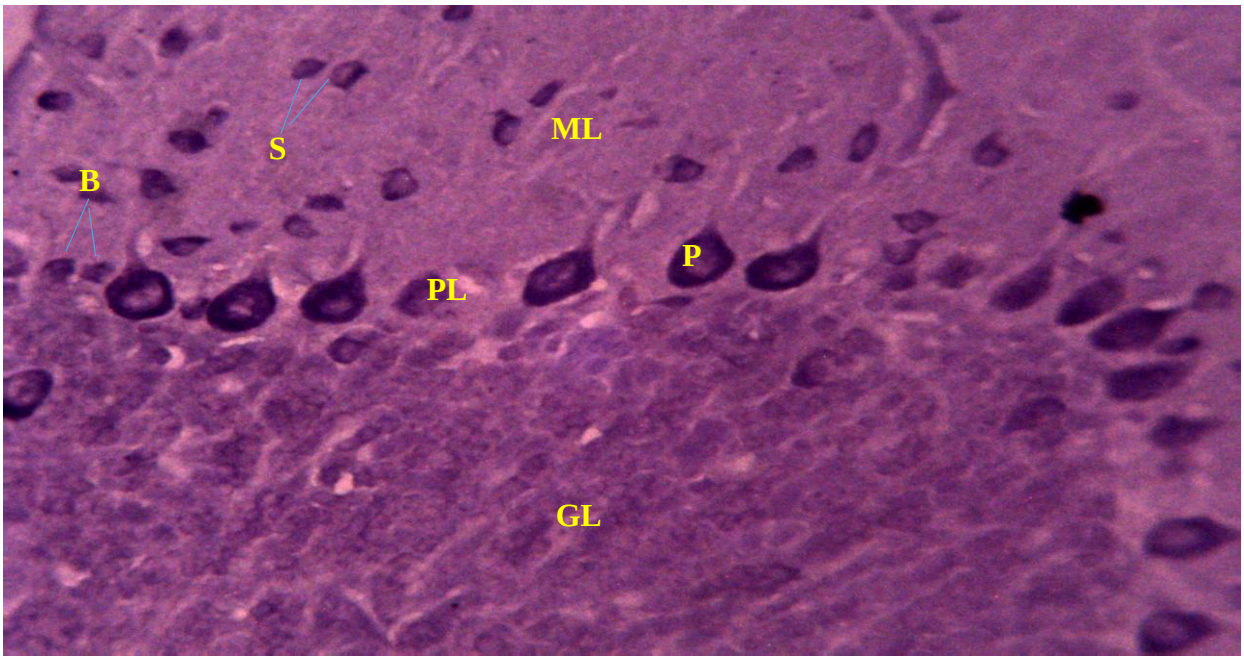
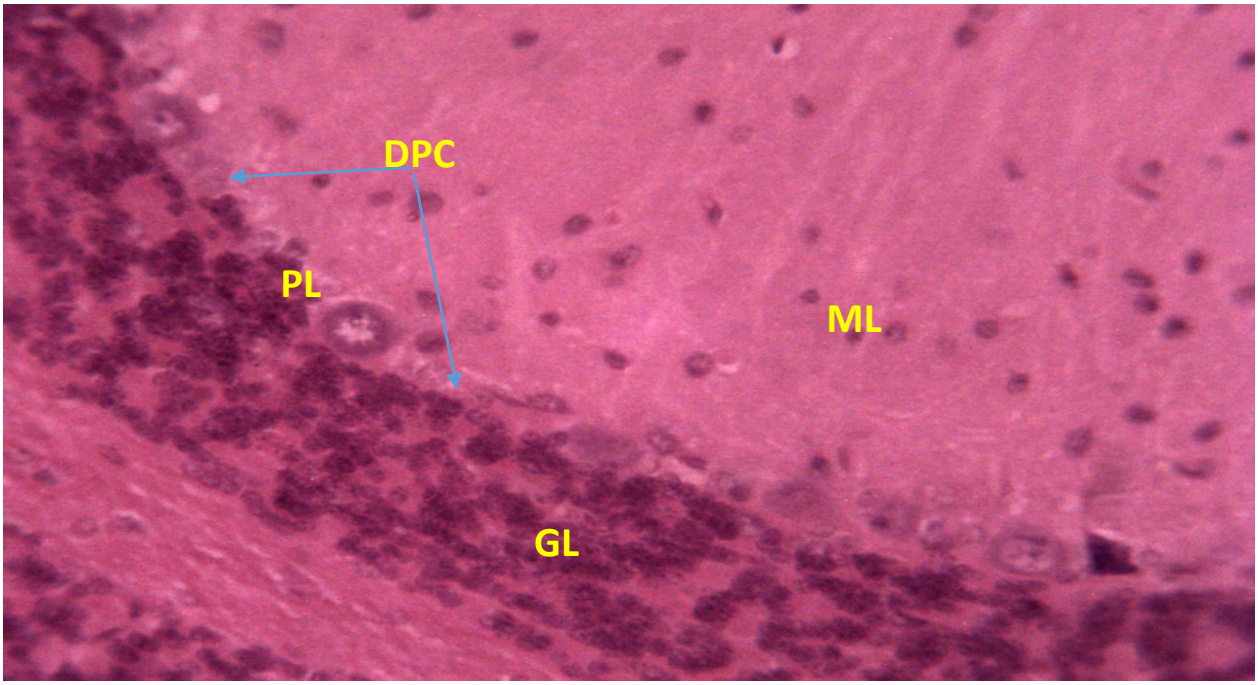


Plate IV: A section of the cerebellum of a Control group (G1), showing normal granular layer (GL), molecular layer (ML) containing the outer satellite cells (S) and the inner basket cells (B) located near the Purkinje cells (P) and Purkinje cell layer (PL) with large flask shaped, uniformly arranged purkinjecells with a vesicular nuclei (Cresyl violet x250).



Pate V: A section of the cerebellum of the low lead treated group (G2), showing normal molecular layer (ML), Purkinje cell layer (PL) with some degenerated Purkinje cells (DPC) and less dense granular layer (GL) compared with the Control Group (H and E Stain X250).

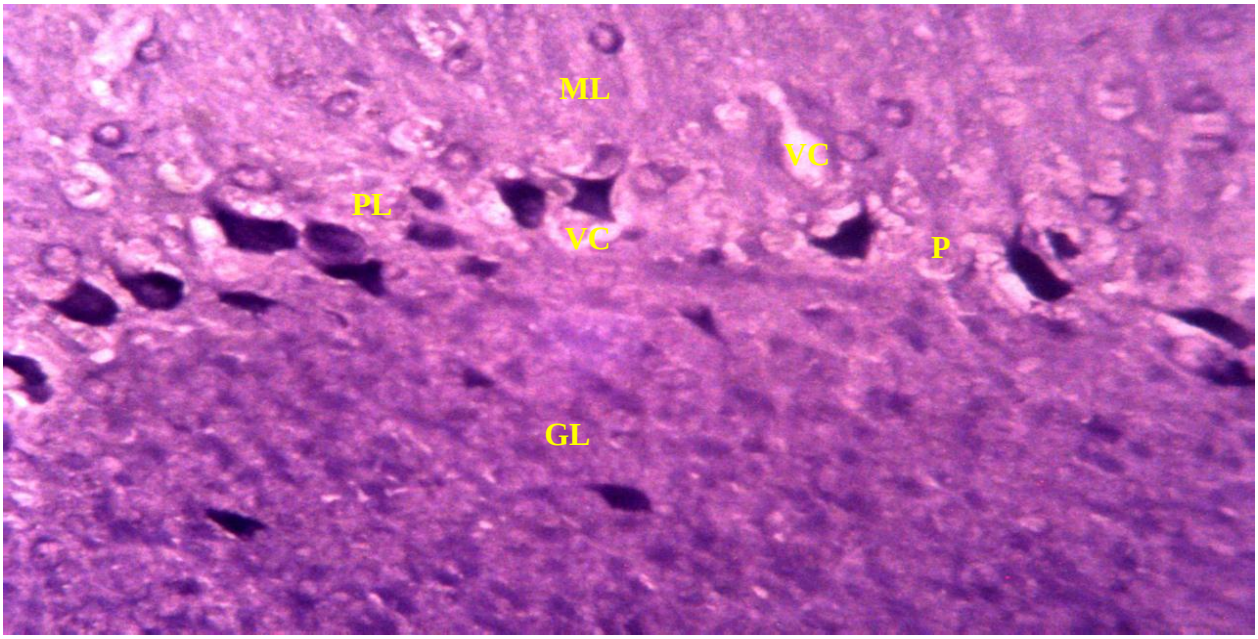
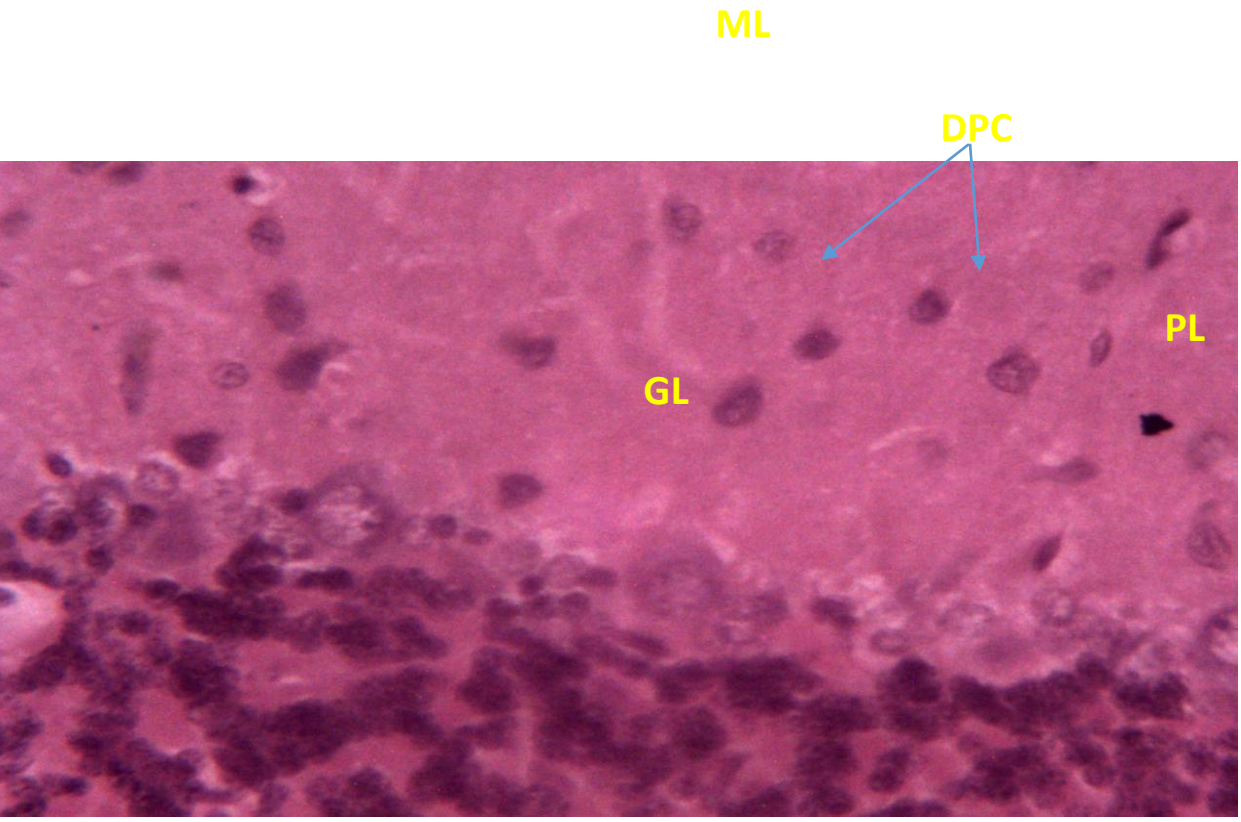


Plate VI: A section of the cerebellum of a low lead treated group (G2) showing granular layer (GL), vacuolations (VC) in the molecular layer (ML) and Purkinje layer (PL). There wasdegeneration and distortion of Purkinje cells (P) with increase of the intensity of staining for Nissl granules (Cresyl violet x250).



Pate VII: A section of the cerebellum of the high lead treated group (G3), showing molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with degenerated Purkinje cells (DPC) (H and E Stain X250).

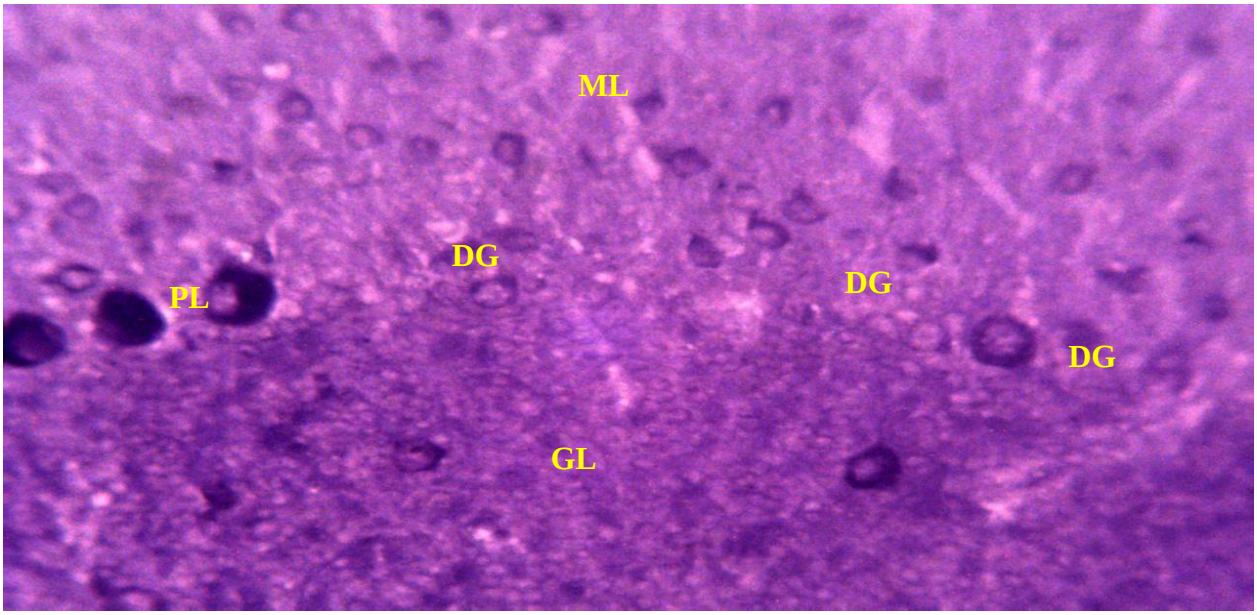


Plate VIII: A section of the cerebellum of a high lead treated Group (G3), showing molecular layer (ML), granular layer (GL) and purkinje layer (PL). There was degeneration (DG) of the Purkinje cells of the Purkinje layer with increase in the intensity of staining for theNissl granules (Cresyl violet x250).

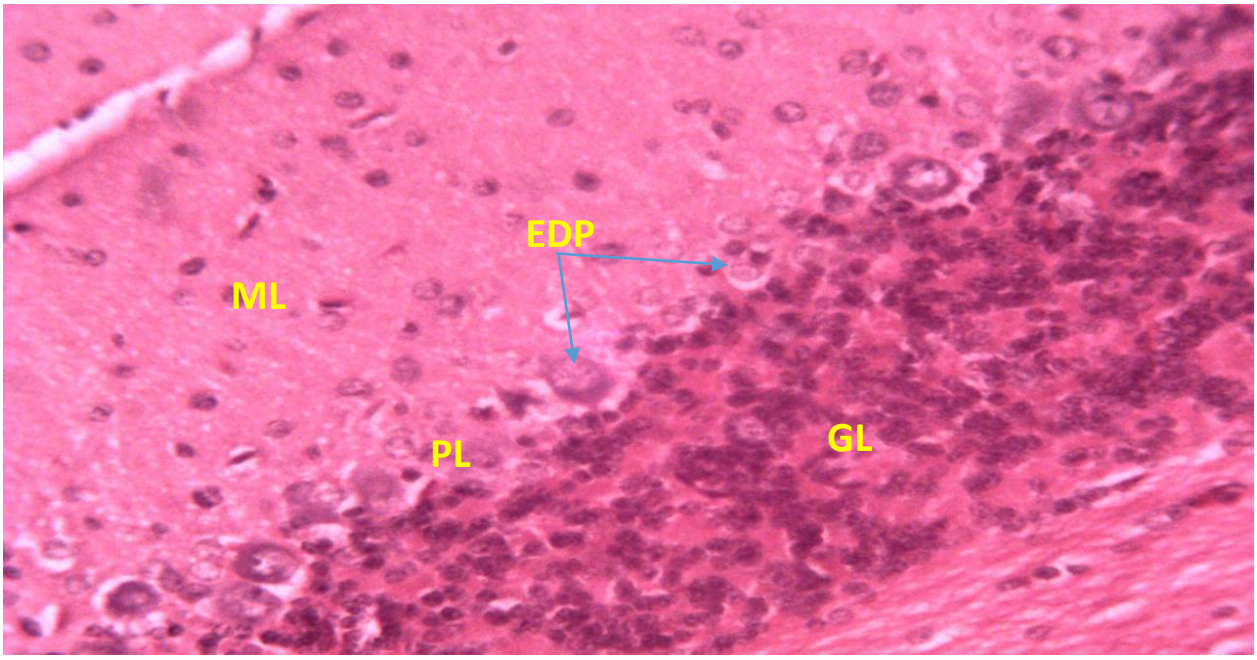


Plate IX: A section of the cerebellum of the low lead treated and low garlic extract dose group (G4), showing normal molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with enlarged and degenerating Purkinje cells (EDP) (H and E Stain X250).

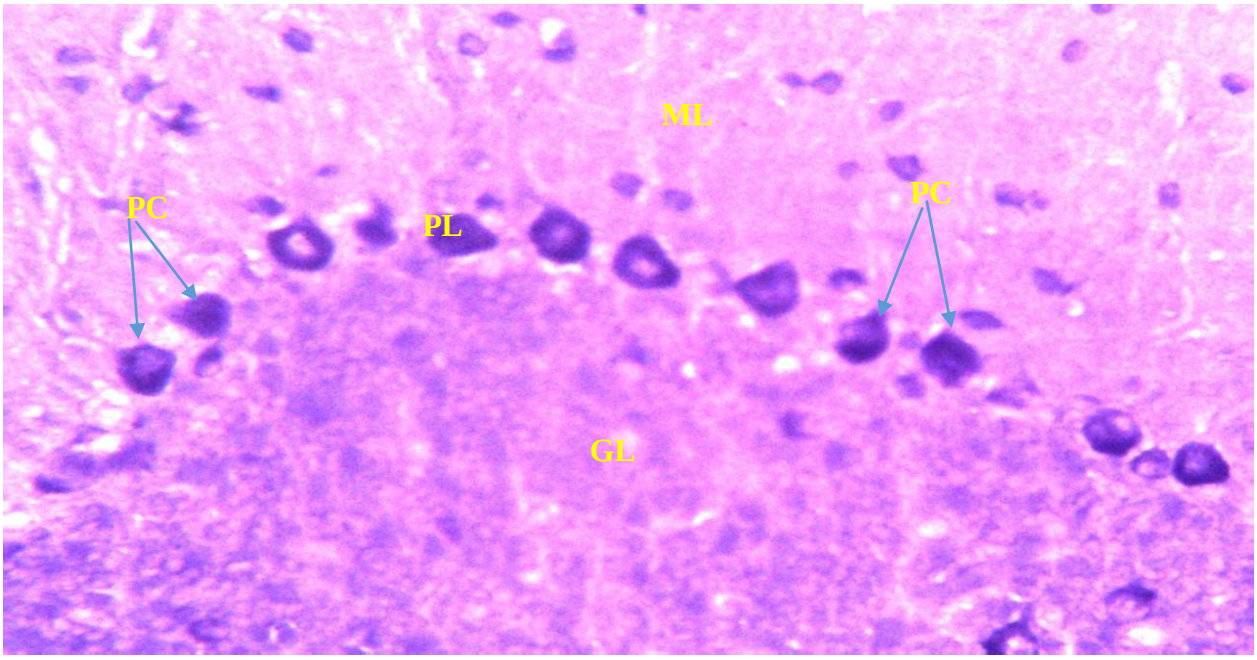


Plate X: A section of the cerebellum of a low lead + garlic extract treated group (G4), showing Molecular layer (ML), granular layer (GL) and Purkinje layer (PL). There was less degeneration and distortion of a Purkinje cells (P) with increase of the intensity of staining for Nissl granules (cresyl violet x250).

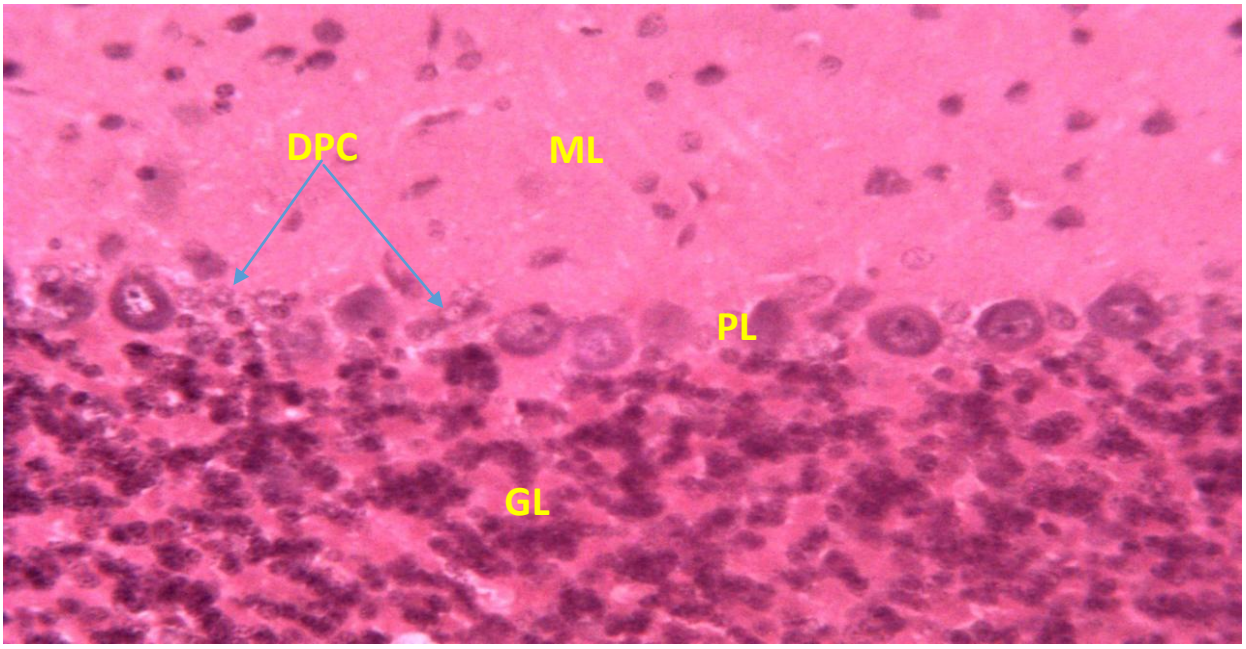


Plate XI: A section of the cerebellum of the high lead and low garlic extract dose treated (G5), showing normal molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with degenerating Purkinje cells (DPC) (H and E Stain X250).

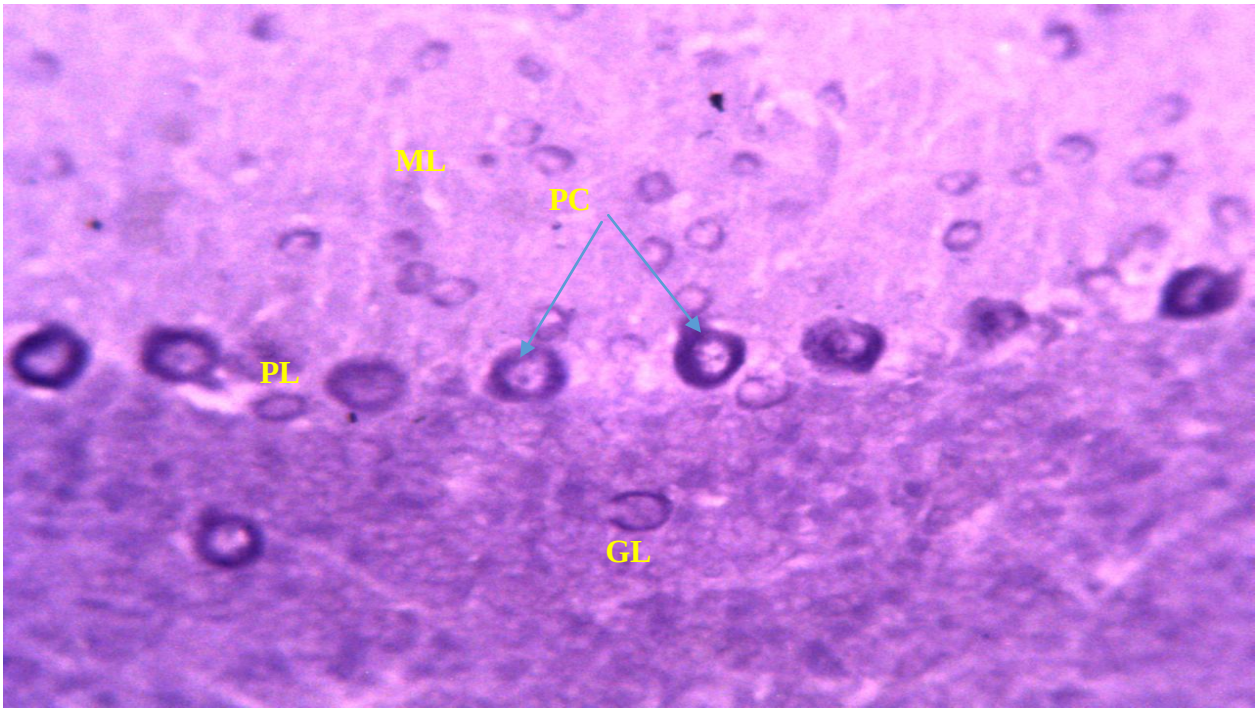


Plate XII: A section of the cerebellum of a high lead + low garlic extract treated group (G5), showing molecular layer (ML), granular layer (GL) and Purkinje layer (PL). The Purkinje cells (PC) are more or less normal compared with the Control group (G1)(Cresyl violet x250).

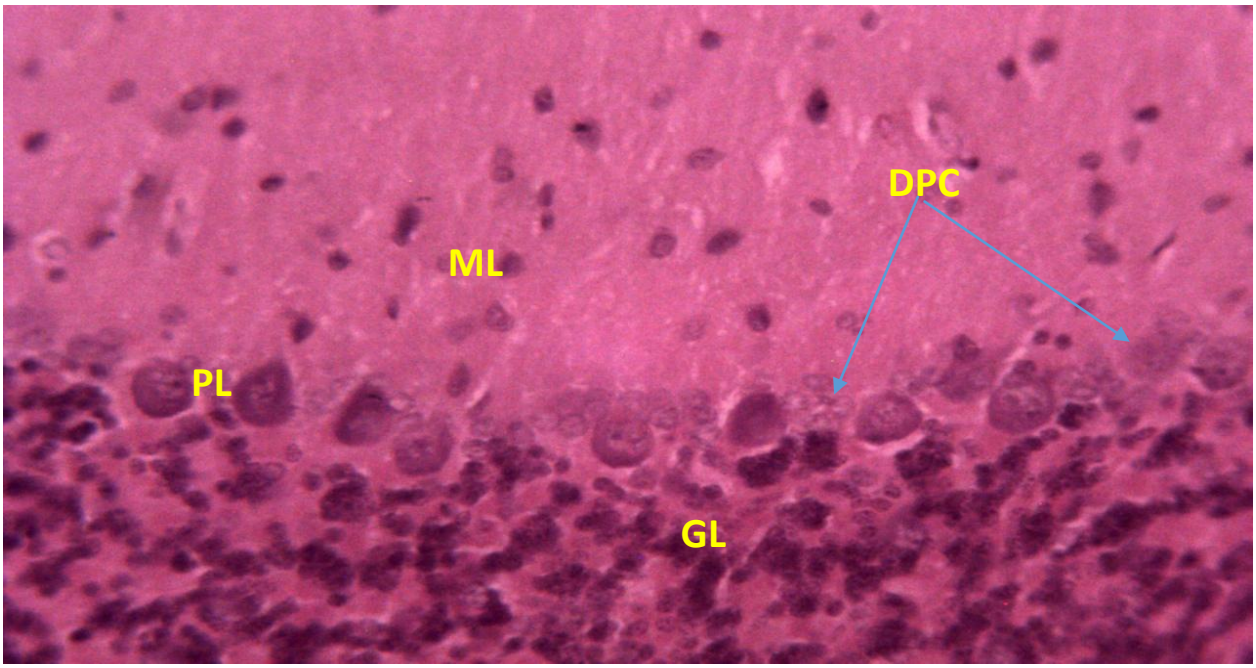


Plate XIII: A section of the cerebellum of the low lead and high garlic extract dose treated group (G6), showing normal molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with few degenerating Purkinje cells (DPC) compared with the Control group (G1) (H and E Stain X250).

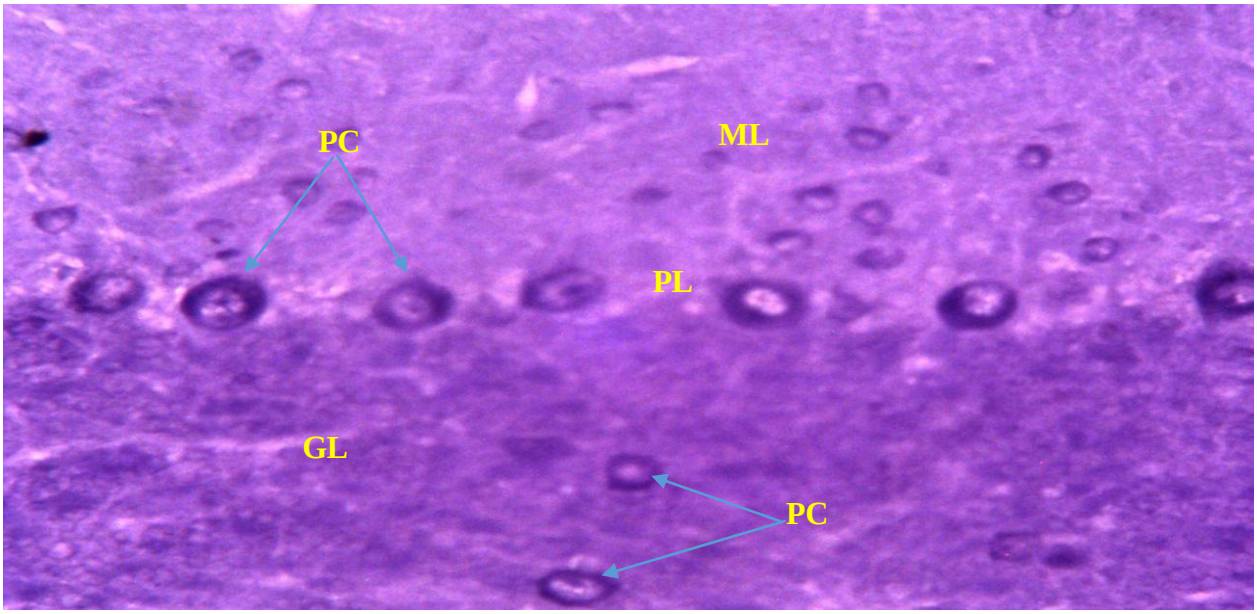


Plate XIV: A section of the cerebellum of a low lead + high garlic extract treated group (G6), showing the molecular layer (ML), granular layer (GL) and Purkinje layer (PL). The Purkinje cells (PC) are more or less normal and some Purkinje cells were observed in the granular layer (cresyl violet x250).

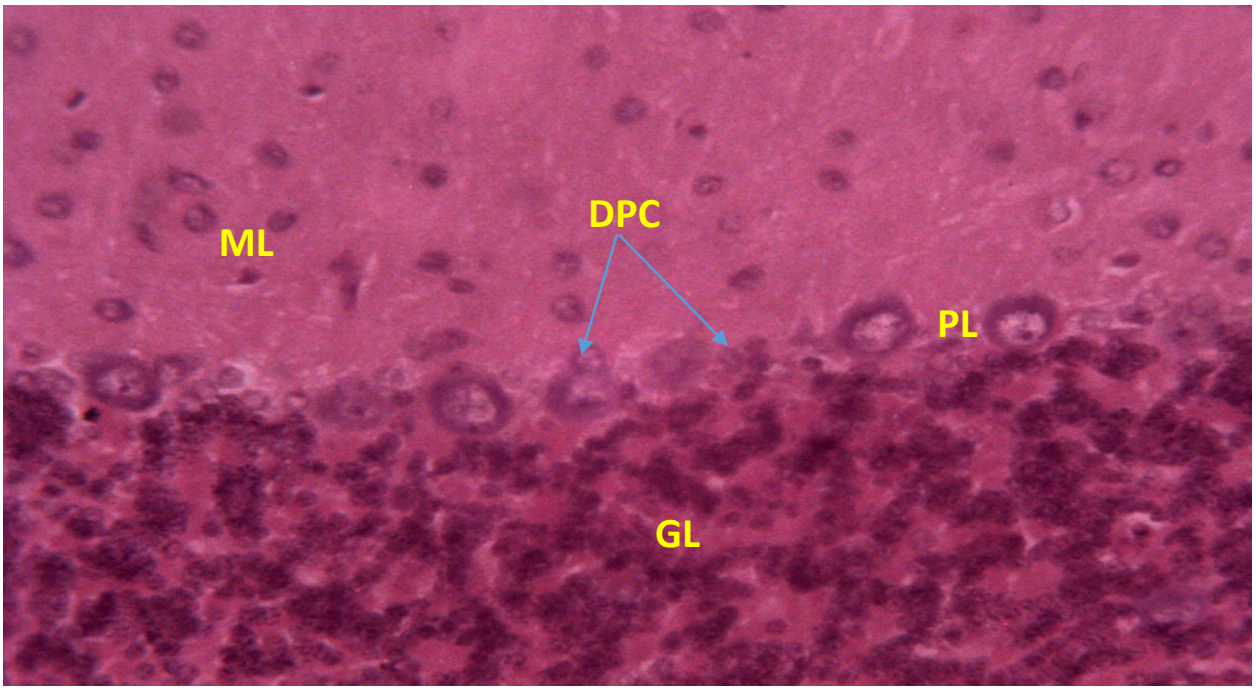


Plate XV: A section of the cerebellum of the high lead and high garlic extract dose group (G7), showing preserved normal molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with few degenerating Purkinje cells (DPC) (H and E Stain X250).

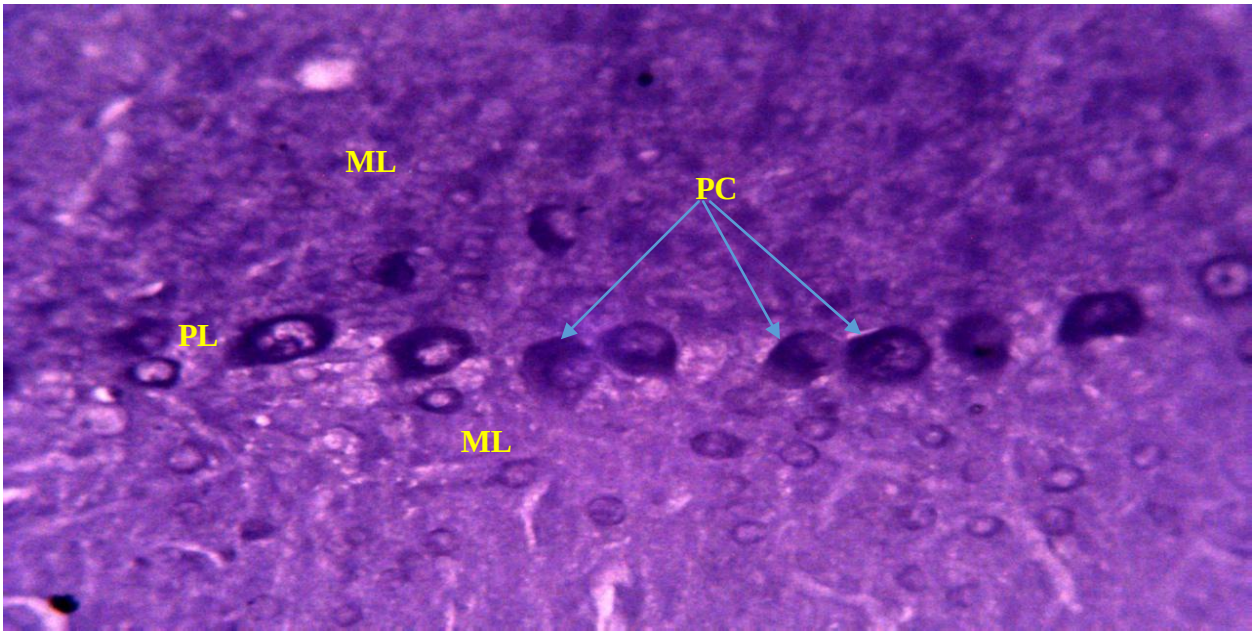


Plate XVI: A section of the cerebellum of a high lead + high garlic extract treated group (G7), showing molecular layer (ML), granular layer (GL), Purkinje cell layer (PL) with large flask shaped, uniformly arranged Purkinje cells (PC) with a vesicular nuclei as compared with the Control (Cresyl violet x250).

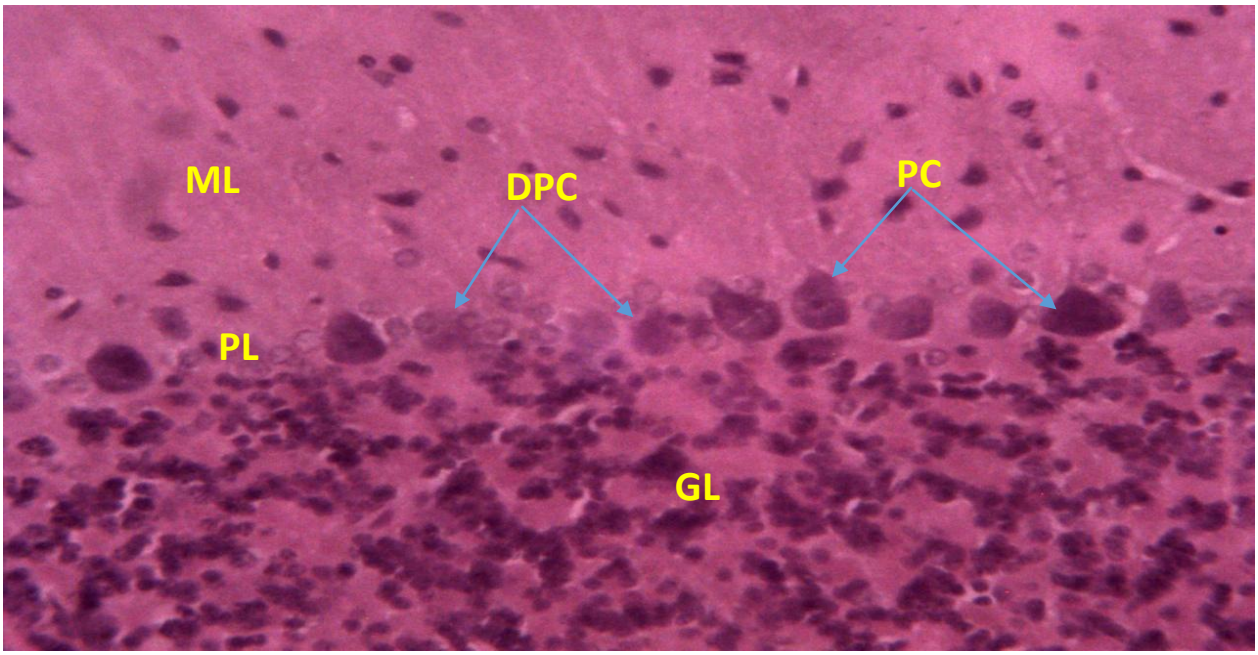


Plate XVII: A section of the cerebellum of the low lead and 30mg/kg b w DMSA treated group (G8), showing preserved normal molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with some normal Purkinje cells (PC) and few degenerating Purkinje cells (DPC) (H and E Stain X250).

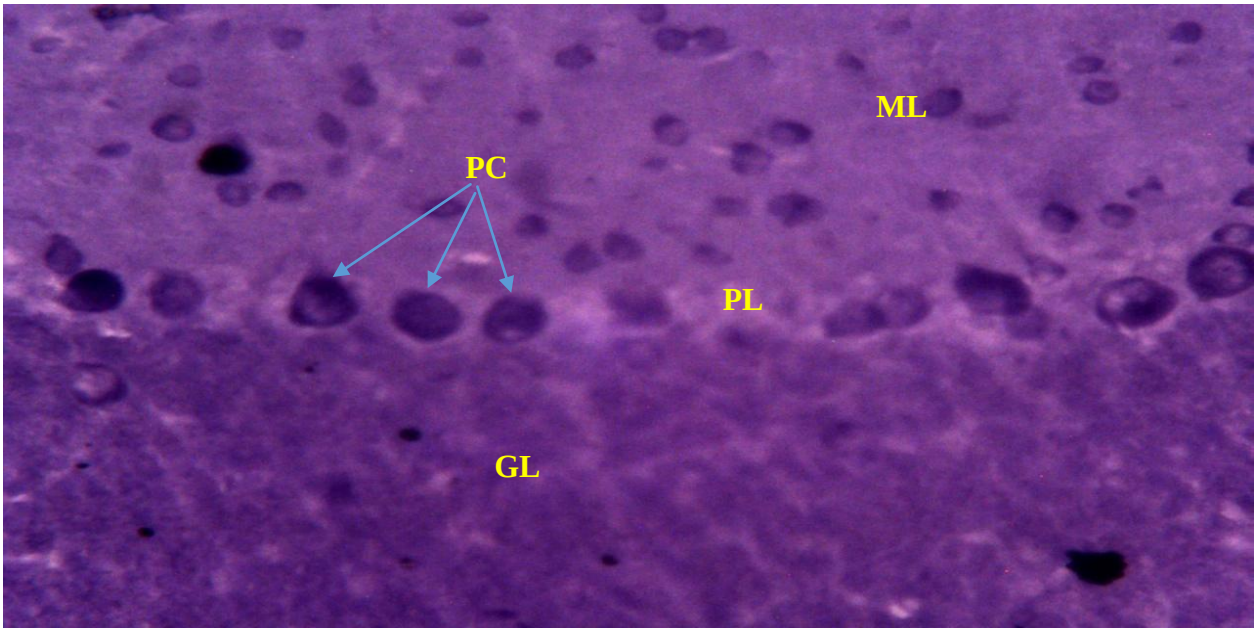


Plate XVIII: A section of the cerebellum of a low lead + 30mg/kg b w DMSA treated Group (G8), showing molecular layer (ML), granular layer (GL), Purkinje cell layer (PL) with large flask shaped, uniformly arranged Purkinje cells (PC) with clumping of nuclei as compared with the Control group (Cresyl violet x250).

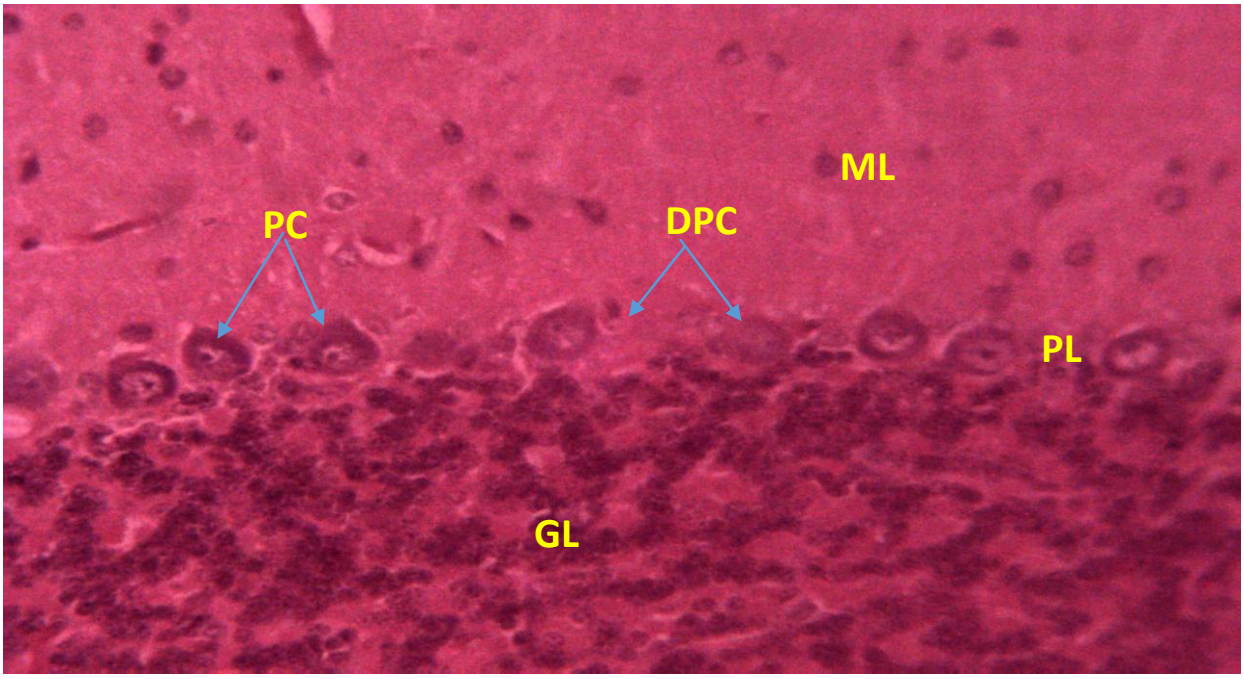


Plate XIX A section of the cerebellum of the high lead and 30mg/kg b w DMSA treated group (G9), showing preserved normal molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with normal Purkinje cells (PC) and few degenerated Purkinje cells (DPC) (H and E Stain X250).

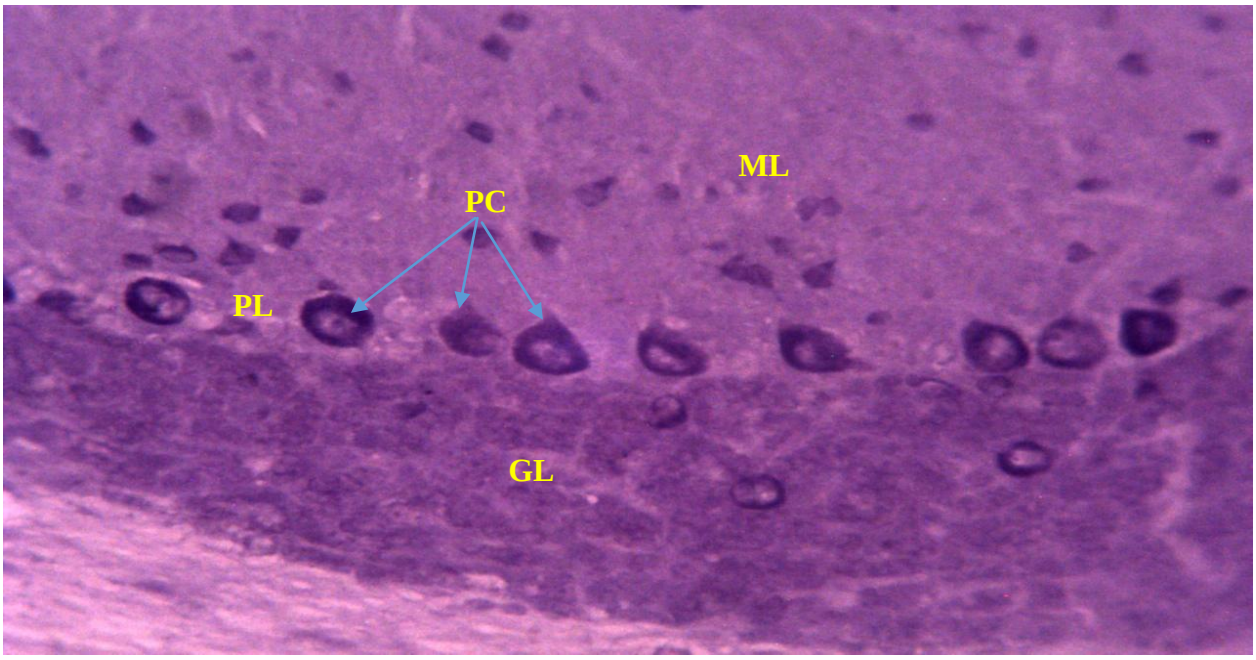


Plate XX: A section of the cerebellum of a high lead + 30mg/kg b w DMSA treated Group (G9), showing molecular layer (ML), granular layer (GL), Purkinje cell layer (PL) with large flask shaped, uniformly arranged Purkinje cells (PC) with a vesicular nuclei as compared with the Control group (cresyl violet x250).

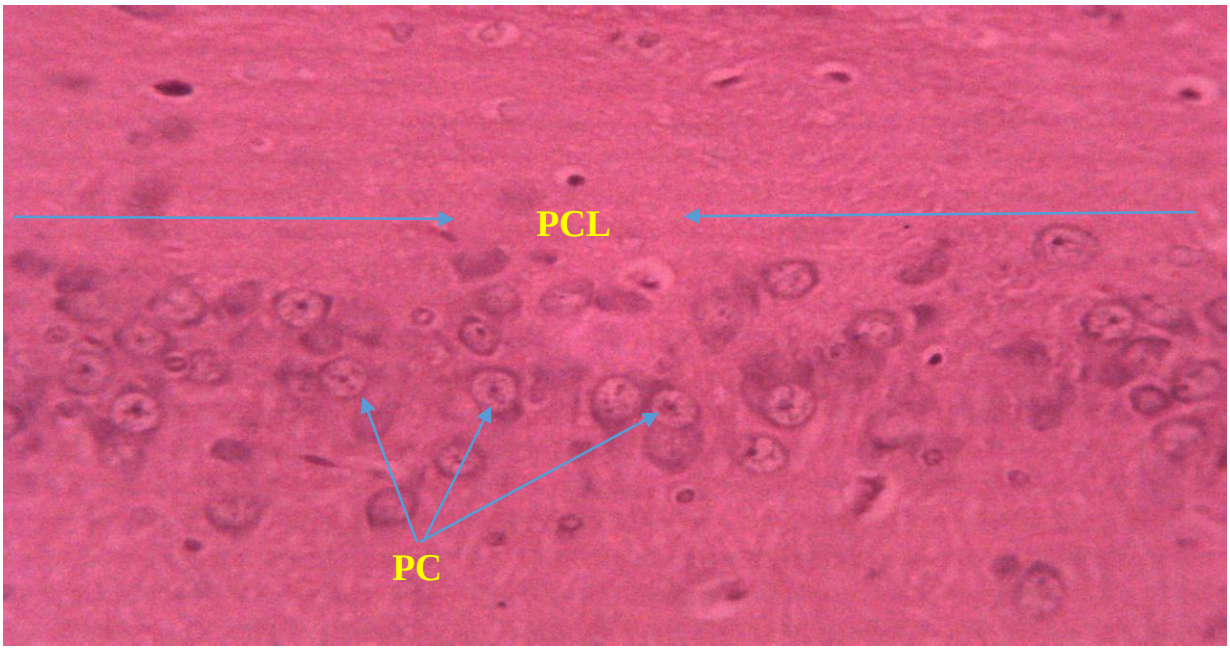


Plate XXI: section of the hippocampus of the Control Group (G1), showing normal pyramidal cell layer (PCL) with normal pyramidal cells (PC) (Hand E X250).

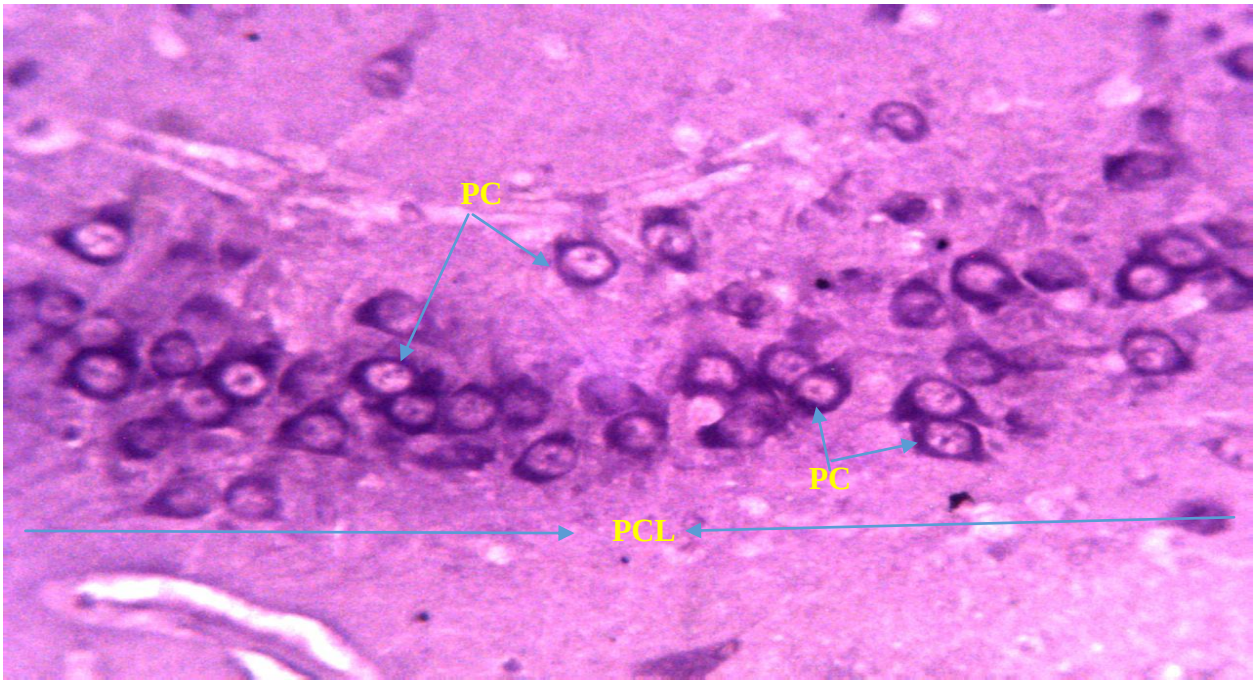


Plate XXII: A section of the hippocampus of the Control Group (G1), showing normal pyramidal cell layer (PCL), and pyramidal cells with vesicular nuclei (PC) (cresyl violet x250).

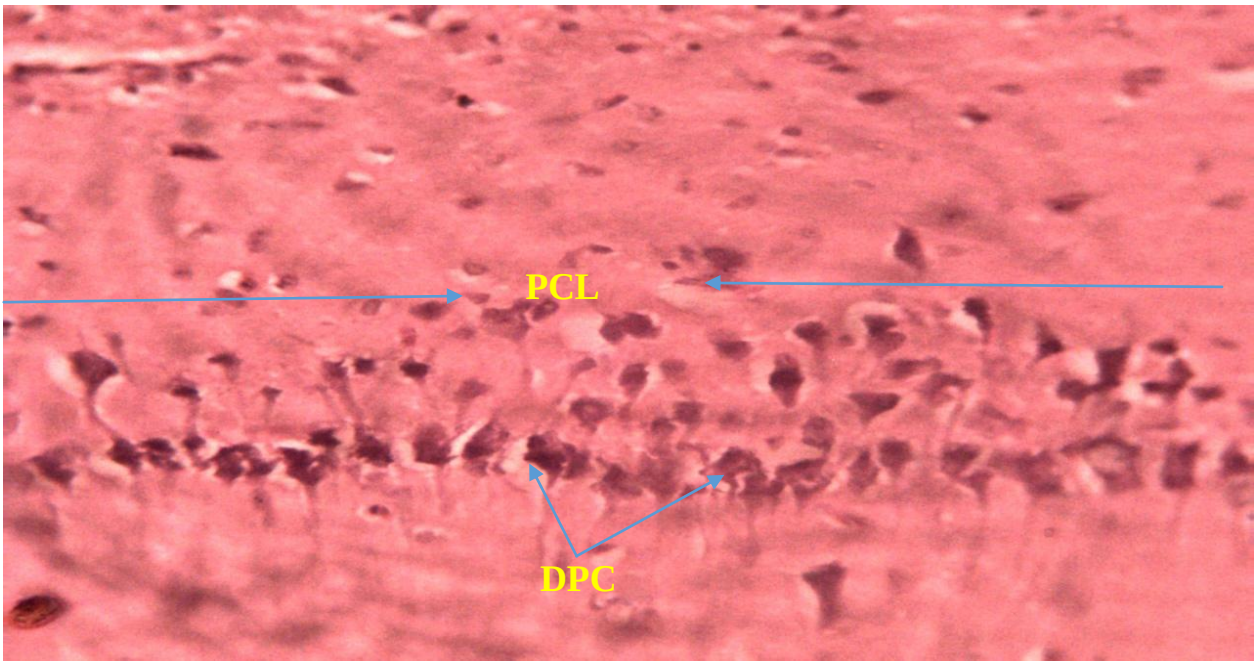


Plate XXIII: A section of the hippocampus of the low lead treated Group (G2), showing pyramidal cell layer (PCL) with degenerating pyramidal cells (DPC). The cell appear smaller than that of the Control Group cell (H and E X250).

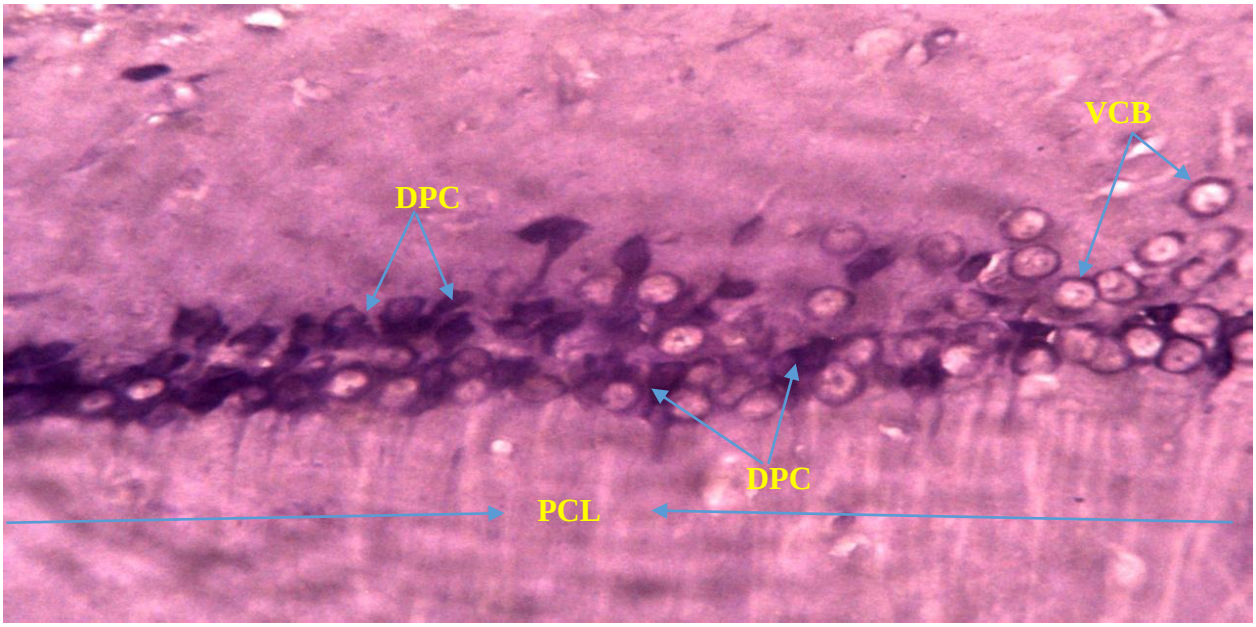


Plate XXIV: A section of the hippocampus of the low lead treated Group (G2), only showing pyramidal cell layer (PCL) with degenerated pyramidal cells (DPC) and vacuolated cell body (VCB) (cresyl violet x250).

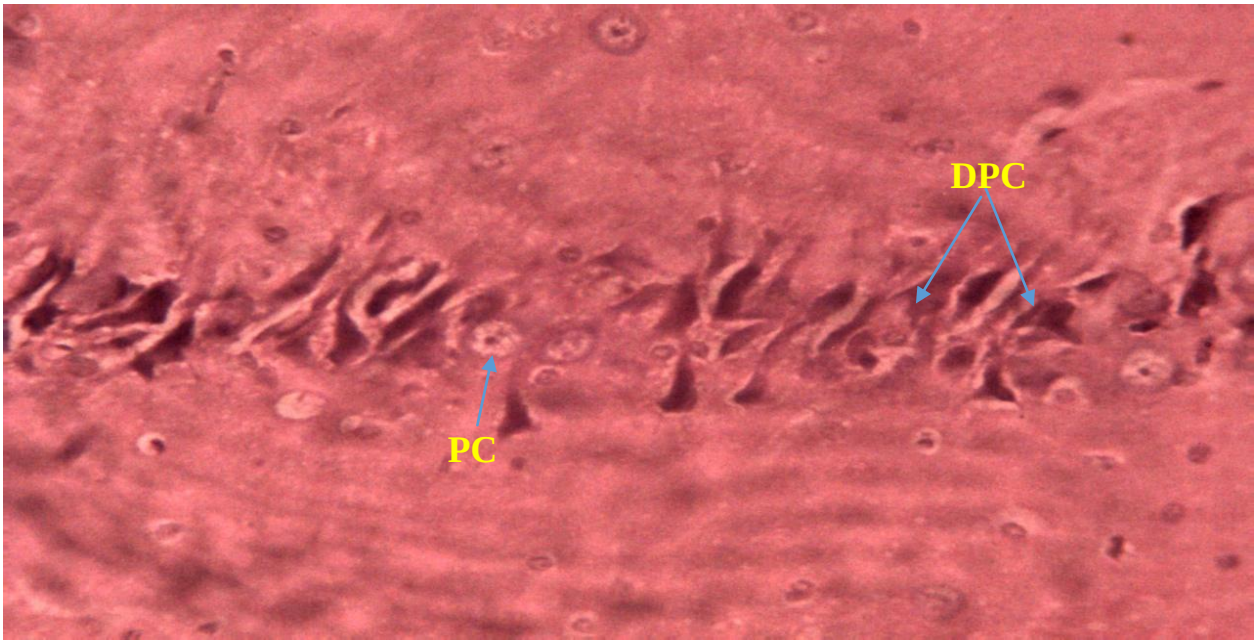


Plate XXV: A section of the hippocampus of the high lead treated Group (G3),showing pyramidal cell layer with degenerating pyramidal cells (DPC)and few of normal pyramidal cells (PC) (Hand E X250).

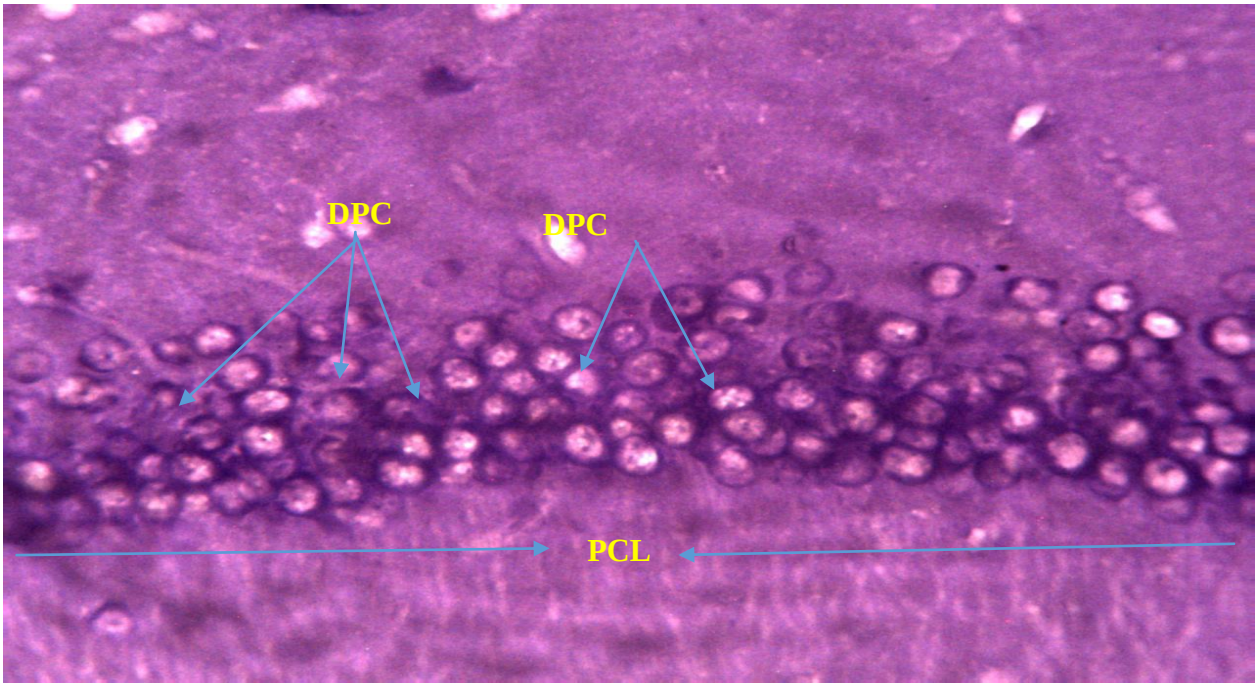


Plate XXVI: A section of the hippocampus of the high lead treated Group only (G3), showing pyramidal cell layer (PCL) with vacuolated pyramidal cells (VPC) and some degenerated pyramidal cells (DPC) (Cresyl violet x250).

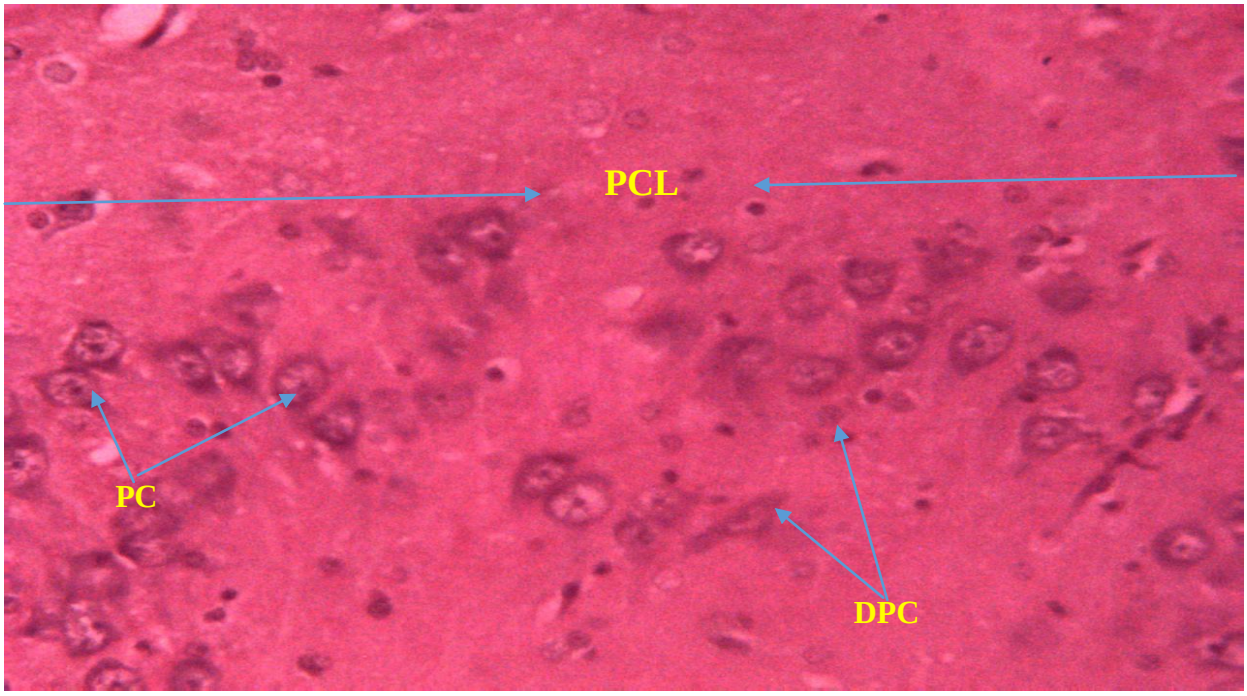


Plate XXVII: A section of the hippocampus of the low lead and low garlic extract treated Group (G4), showing pyramidal cell layer (PCL) with normal pyramidal cells (PC) and some degenerated pyramidal cells (DPC) (Hand E X250).

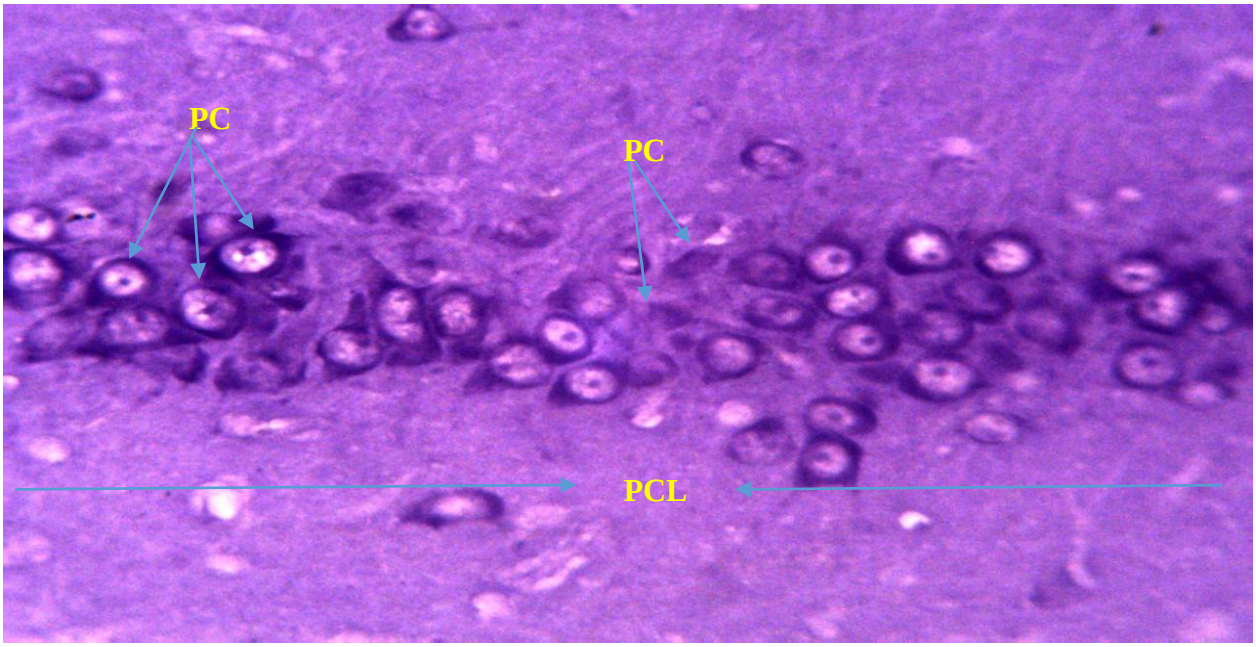


Plate XXVIII: A section of the hippocampus of the low lead and low garlic extract treated Group (G4), showing pyramidal cell layer (PCL) with normal pyramidal cells (PC) and some few degenerated pyramidal cells (DPC). They are more or less normal compared with Control Group (Cresyl violet x250).

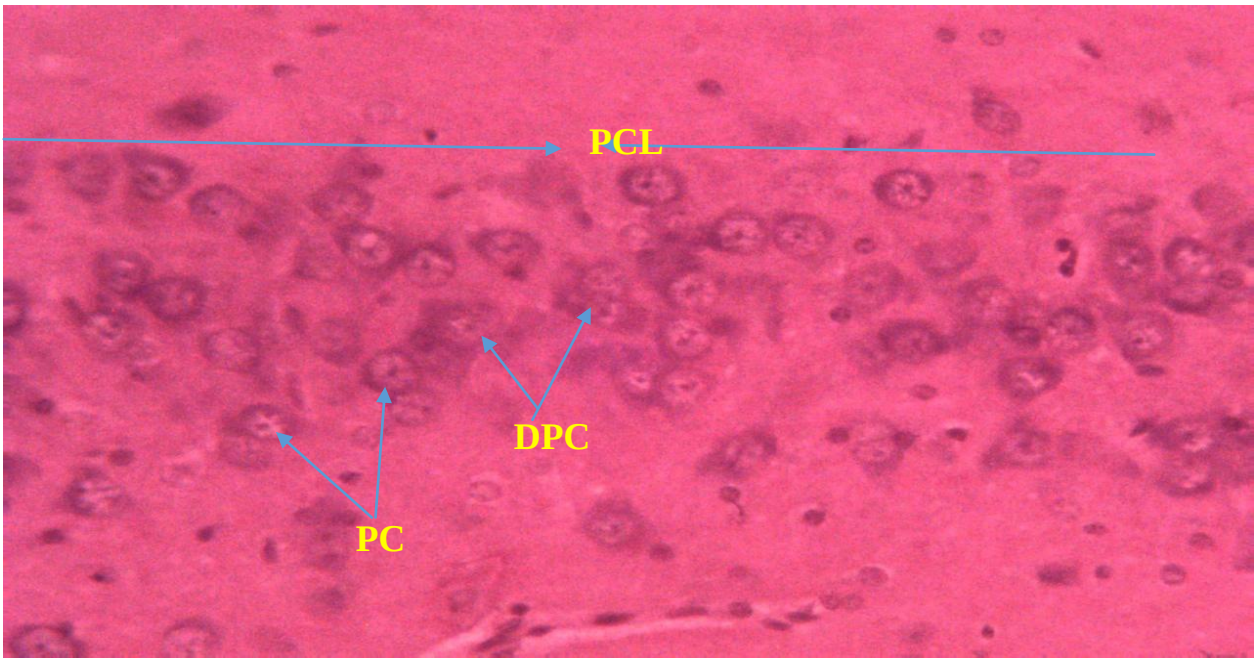


Plate XXIX: section of the hippocampus of the high lead and low garlic extract treated Group (G5), showing pyramidal cell layer (PCL) with normal pyramidal cells (PC) and some degenerated pyramidal cells (DPC) (Hand E X250).

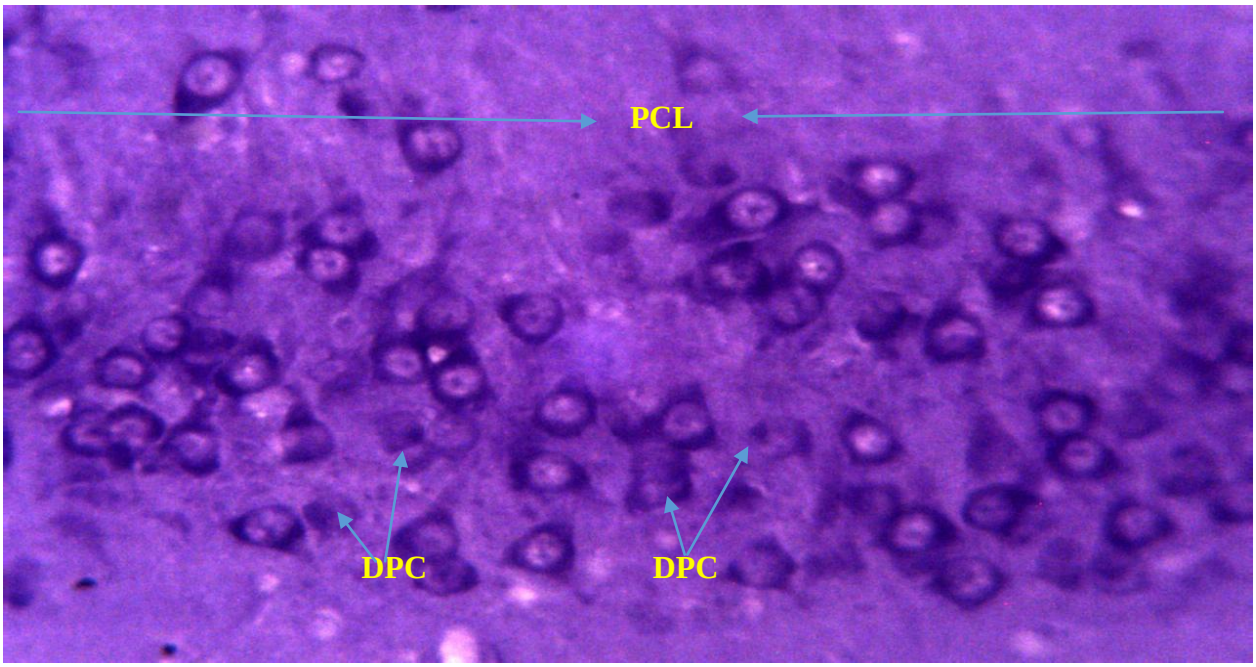


Plate XXX: A section of the hippocampus of the high lead and low garlic extract treated Group (G5), showing disorientation of the pyramidal cell layer (PCL) with some degenerated pyramidal cells (DPC) (Cresyl violet x250).

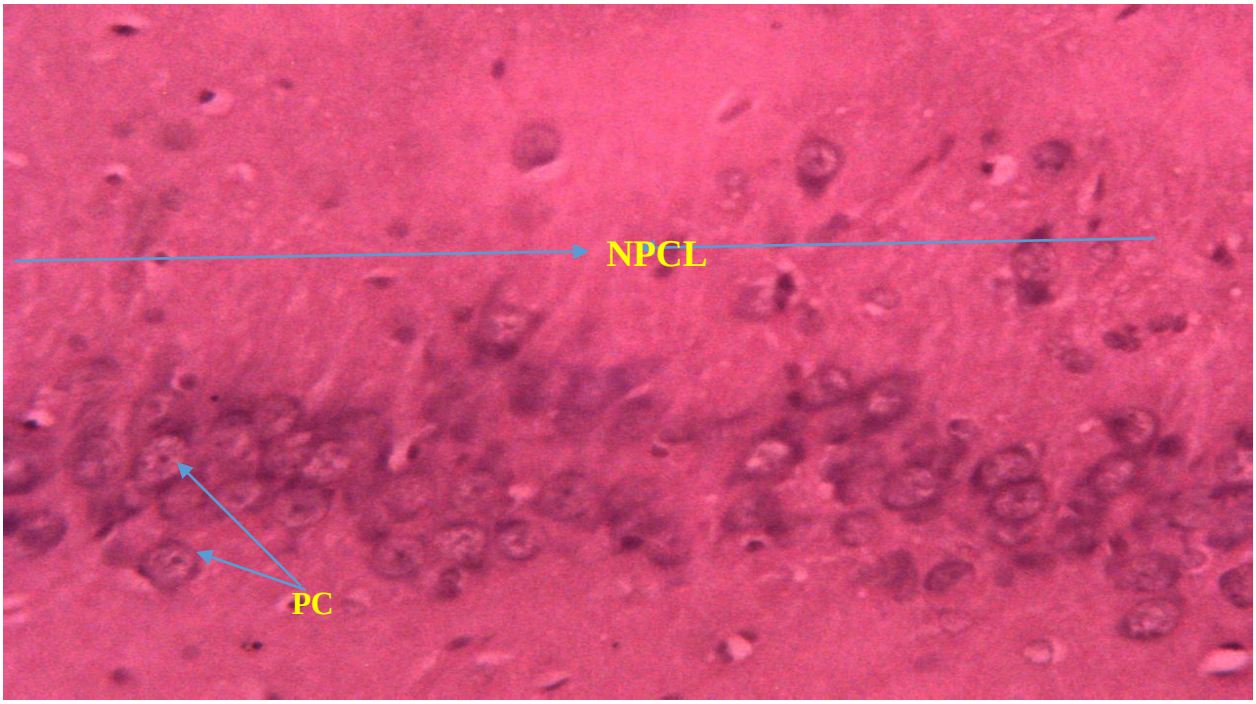


Plate XXXI: A section of the hippocampus of the low lead and high garlic extract treated Group (G6), showing normal pyramidal cell layer (NPCL) with relatively normal pyramidal cells (PC) (Hand E X250).

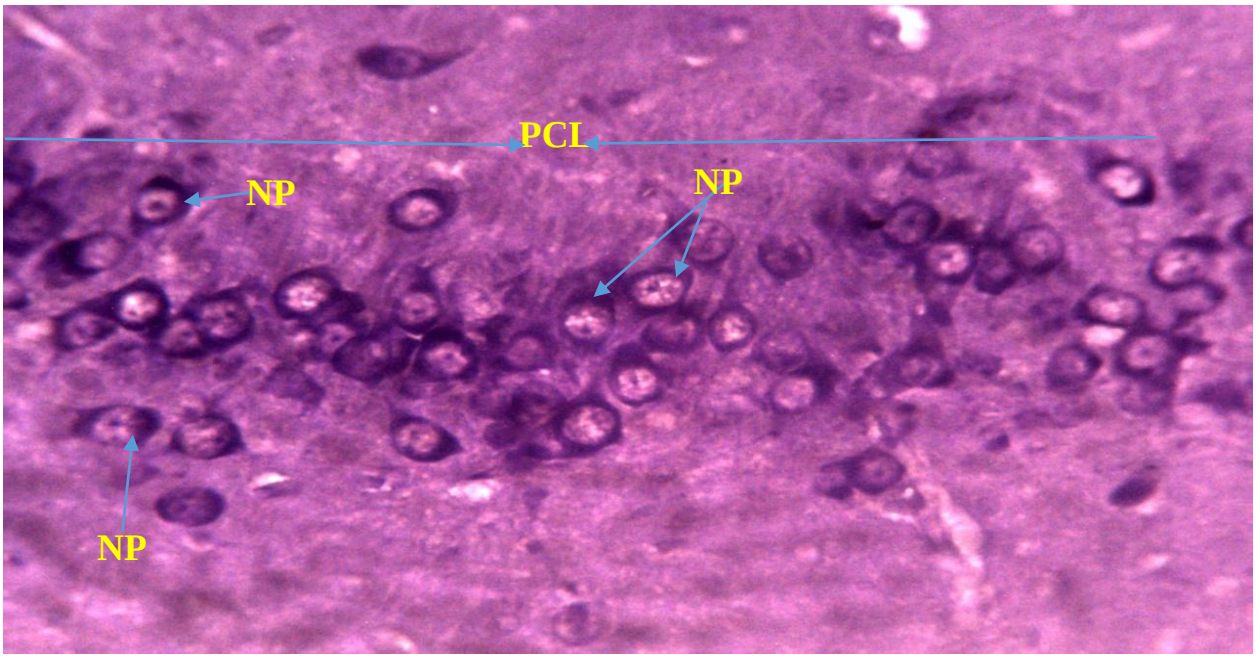


Plate XXXII: A section of the hippocampus of the low lead and high garlic extract treated Group (G6), showing normal pyramidal cell layer (PCL), pyramidal cells with vesicular nuclei (NP) as compared with the Control group (Cresyl violet x250).

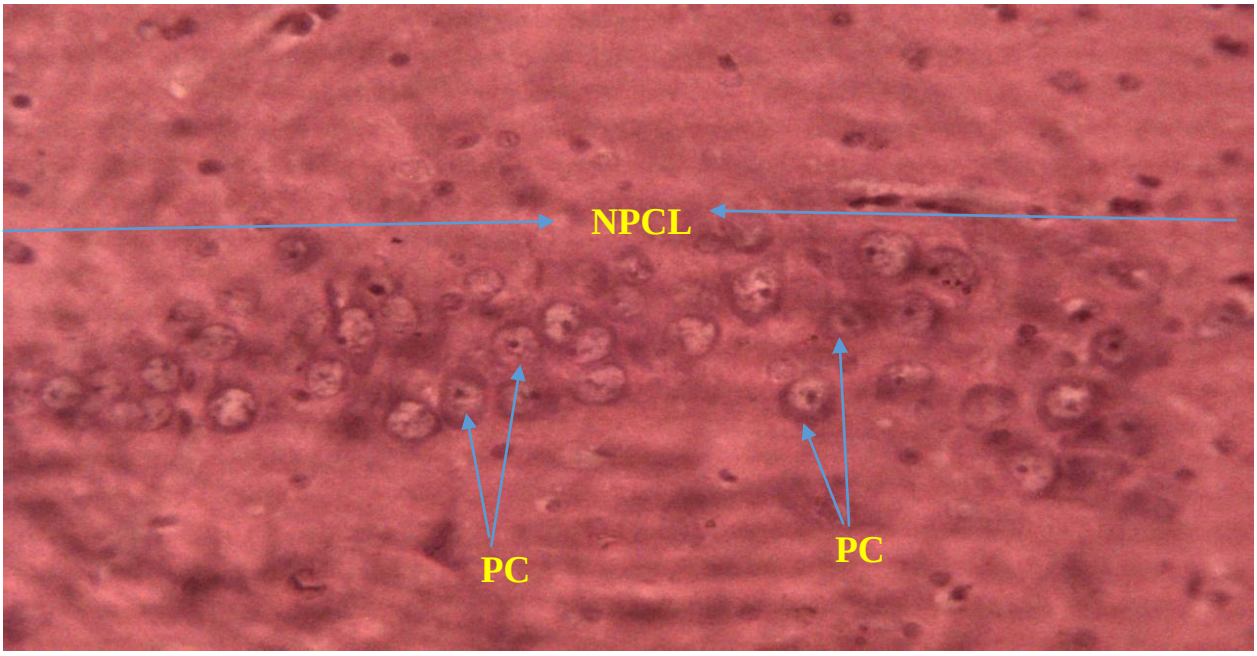


Plate XXXIII: A section of the hippocampus of the high lead and high garlic extract treated Group (G7), showing normal pyramidal cell layer (NPCL) with normal pyramidal cells (PC) (Hand E X250).

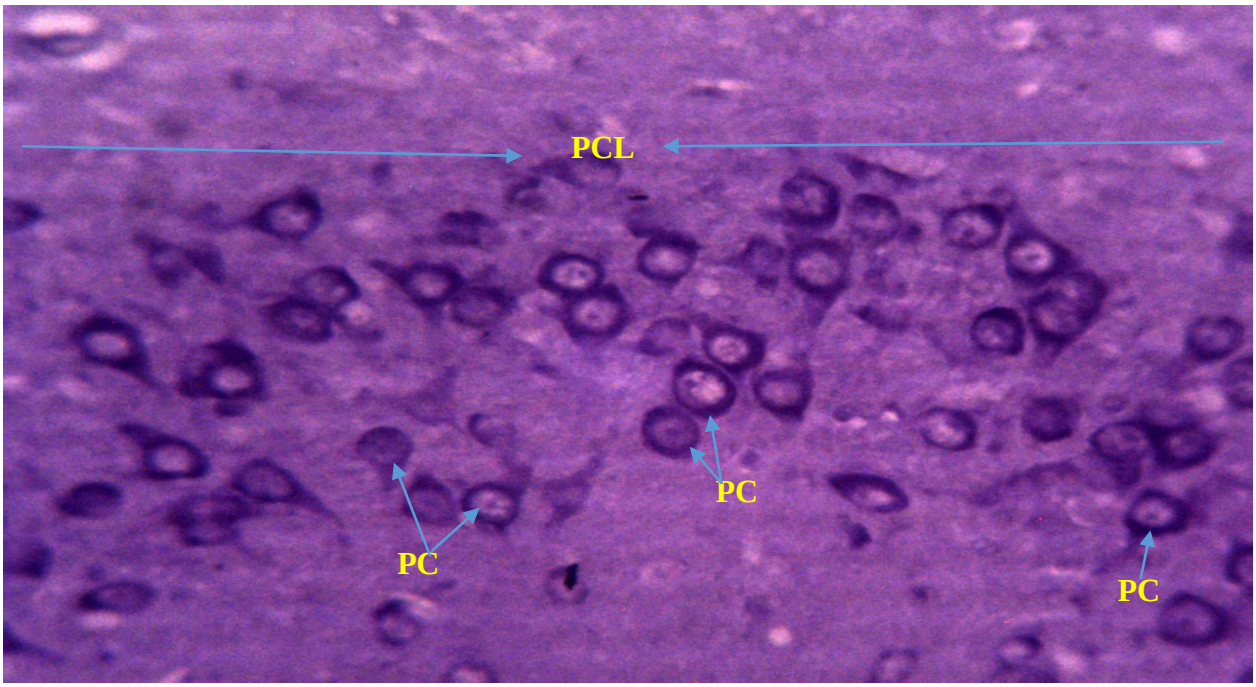


Plate XXXIV: A section of the hippocampus of the high lead and high garlic extract treated Group (G7), showing disorientation of the pyramidal cell layer (PCL) with normal pyramidal cells (PC) (Cresyl violet x250).

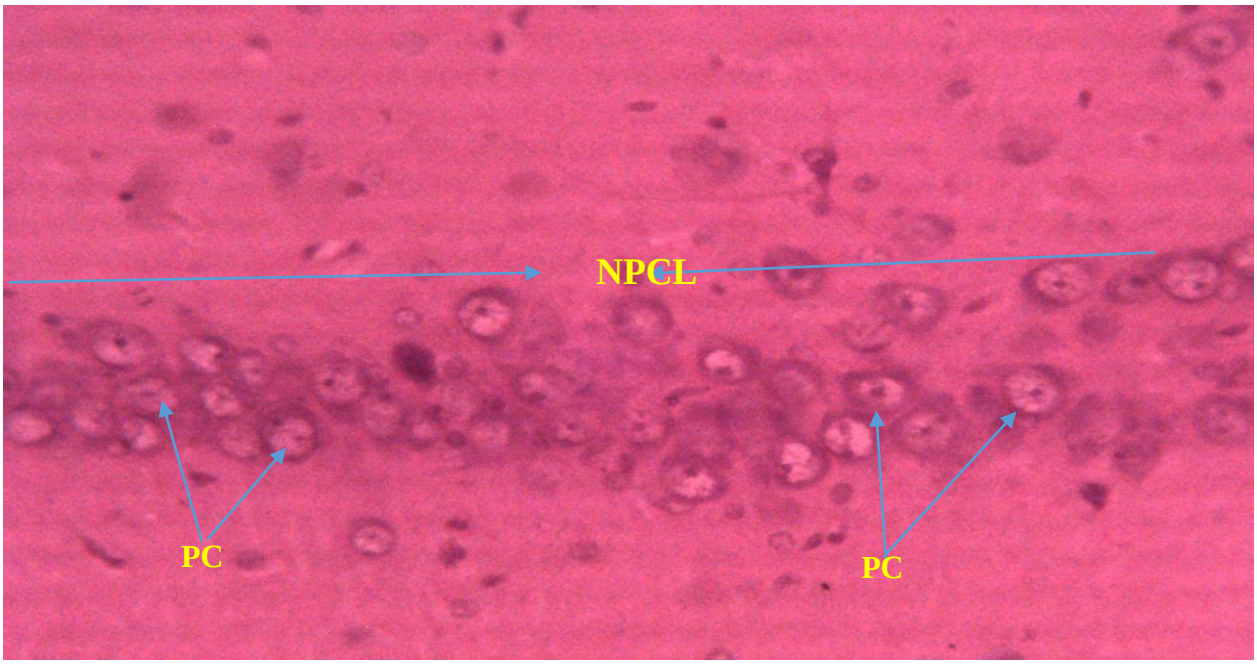


Plate XXXV: section of the hippocampus of a low lead + 30mg/kg b w DMSA treated Group (G8), showing normal pyramidal cell layer (NPCL) with normal pyramidal cells (PC) (Hand E X250).

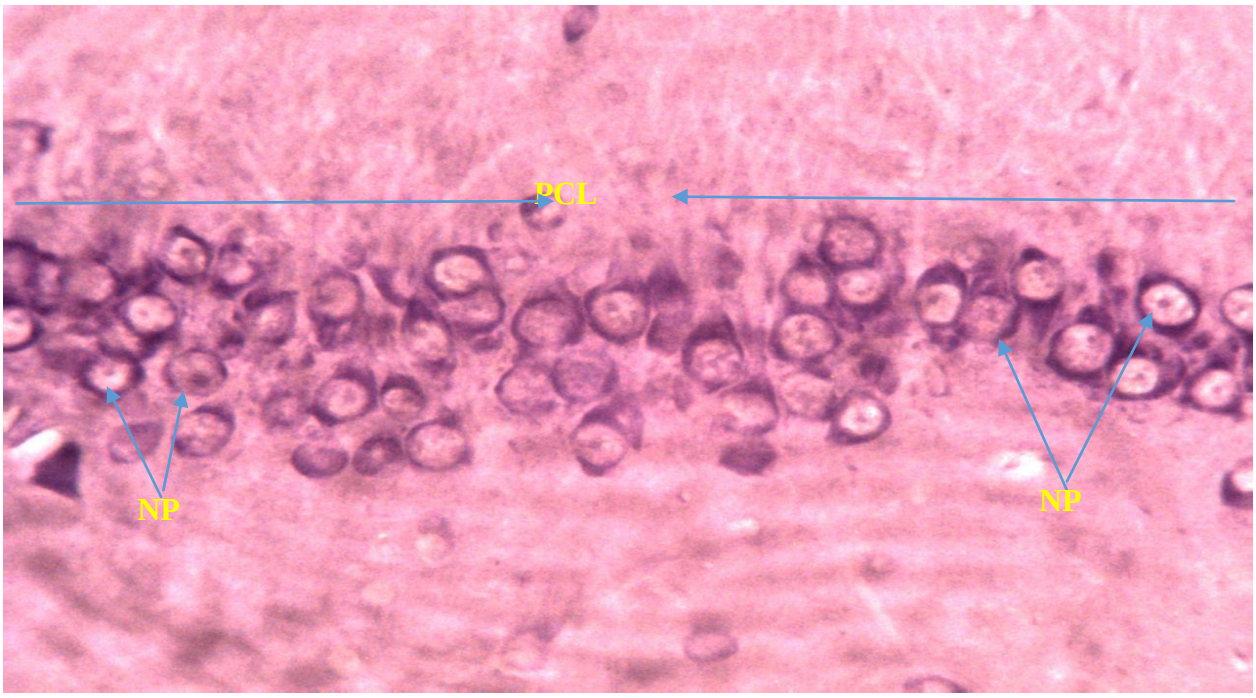


Plate XXXVI: A section of the hippocampus of the low lead and 30mg/kg b w DMSA treated Group (G8) showing normal pyramidal cell layer, pyramidal cells with vesicular nuclei (NP) as compared with the Control Group (Cresyl violet x250).

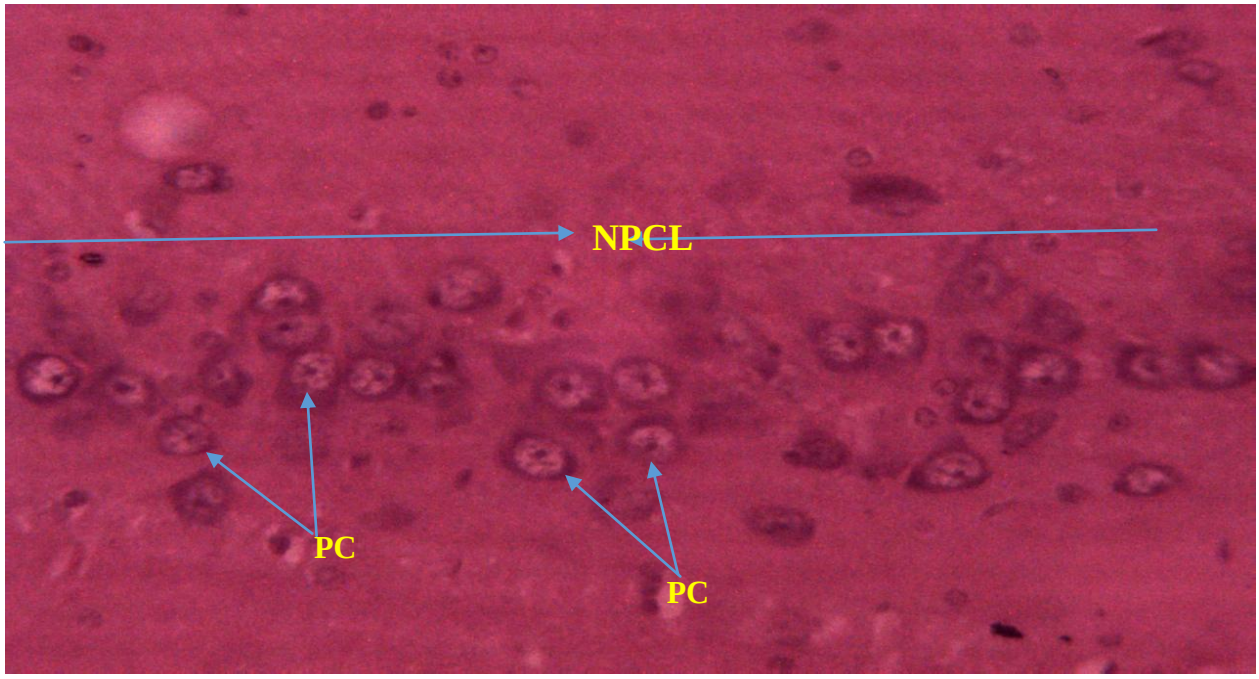


Plate XXXVII: A section of the hippocampus of a low lead + 30mg/kg b w DMSA treated Group (G9), showing normal pyramidal cell layer (NPCL) with normal pyramidal cells (PC) (Hand E X250).

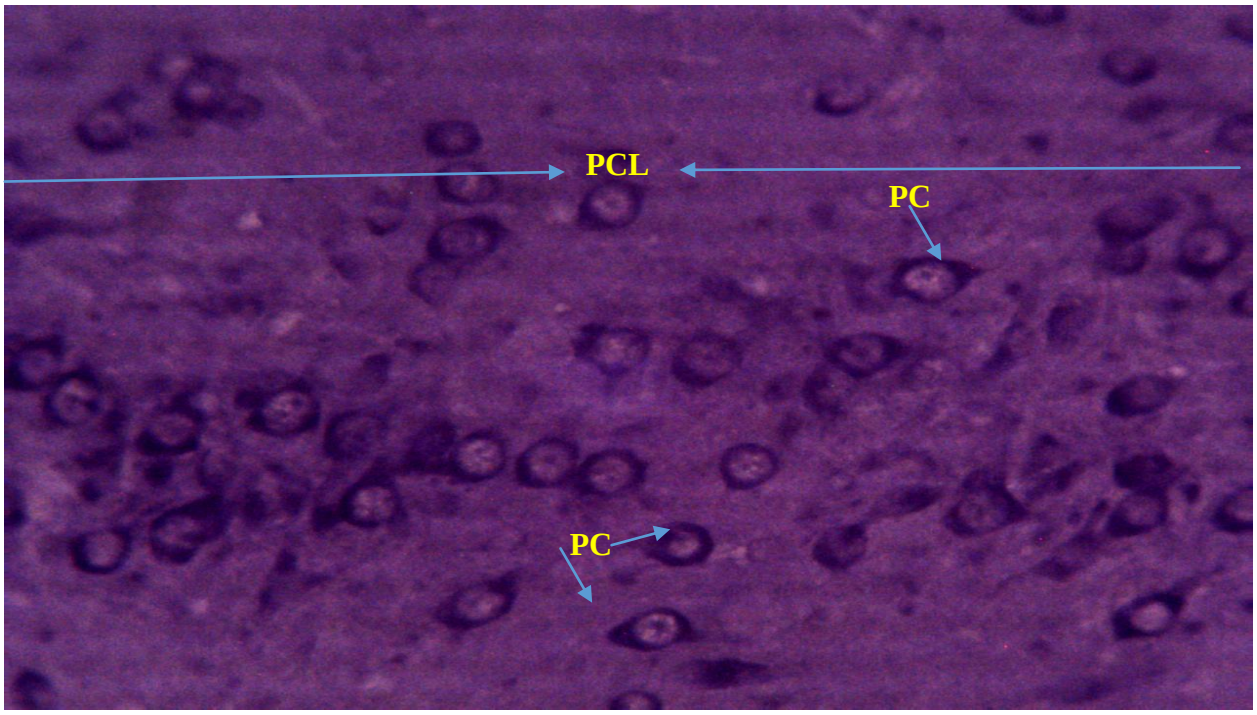


Plate XXXVIII: A section of the hippocampus of the high lead and 30mg/kg b w DMSA treated Group (G9) showing disorientation of the pyramidal cell layer (PCL) with normal pyramidal cells (PC) (Cresyl violet x250).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of aqueous Garlic extract on lead induced changes on Body Weight

It has been reported that ratsthat are continuously exposed to heavy metals such as mercury (Hg), cadmium (Cd), arsenic (As) and lead (Pb) usually results in reduction in the body weight(Nwokocha*et al.,* 2011). The present study was carried out to study the effect of lead induced changes on the hippocampus and cerebellum of adult Wistar rats. Our study showed that there were reduction in mean body weight of the ratsin the lead treated groups onlywhen compared with the ratsin the Control group.

This reduction in the mean body weight with continuous exposure to lead might be explained on the basis of anorexia which is induced by lead exposure (Klaassen, 2001). Another possible explanation for the reduction of the mean body weight may be the decreased muscle mass and cachexia due to the oxidative stress induced by lead. This is because there was evidence that heavy metal toxicity was associated with oxidative stress (Yeh*et al.,* 2009) which according to many researchers was associated with muscle wasting and cachexia leading to low body weight (Buck *et al.,* 1998).

The result of the effect of lead on body weight of the adult Wistar rats agreed with many other studies of the effect of lead on body weight of adult Wistar rat. One of such studies was the work of Amjad*et al.,*(2013) in which lead was given, to study its effect on the body and kidney weights of Wistar Albino Rats which wasameliorated by Ginkgo biloba Extract. The result of the present study also showed that ratstreated with lead acetate and

garlic extract or Succimer(DMSA) showed slight increase in the body weight which when compared with the ratsin the Control Group was not significant. The slight increase in the mean body weight in treated groups, which were given both lead acetate as well as aqueous garlic extract, may be attributed to the antioxidant property of Garlic.

5.2 Effect of aqueous Garlic extract on lead induced changes on Neurobehavioral Changes

On the sixth day of training no significant differences in meantime taken by the ratsto complete the Morris water maze taskwere observed which indicated that on day six all groups learned how to find the hidden platform. During administration, the result of the neurobehavioral studies in the present study showed that there was decrease in the meantime taken by the ratsin Group one (Control) completing the Morris water maze task while there were significant increase in mean time taken to complete the Morris water maze task in the lead treated Group only (Groups 2and 3).

This could be due to degeneration of hippocampalpyramidal cells, valuolations and loss of basic histological form of the pyramidal cells that appears smaller than normal. These destruction of pyramidal cells implies that activity from the brain region that projects into the pyramidal layer of the hippocampus will also be lost such as memory and learning ability (Wolf, 2009; Quirino, 2012).The result obtained from the present study agreed with many other studies on the effect of lead on neurobehavioral changes. One of such work was byBazrgar*et al.*,(2015), on the effect of postnatal lead exposure on the Spatial Learning and Memory in Male rats.

The main finding of the present study was that the administration of aqueous garlic extract or Succimer (DMSA) caused better performance in Morris water maze task compared to both the Control and the lead treated Group only. The ratstreated with lead and aqueous garlic extract or Succimer had decrease mean time taken to complete Morris water maze test. This could be due their ability to preservehippocampal pyramidal cells against lead induced toxicity.

On the other hand, the result of the present study on motor coordination showed a significant increase in the meantime taken by the ratsin lead treated groups to traverse the beam which when compared with the Control group was found to be significant.This could be attributedto thehistomorphological changes in the cerebellum which consequently interfered with the motor ability of ratsin the experimental group. While the results inleadand aqueous garlic extracttreated Groupsshowed that there were slightly decrease in the meantime taken by the ratsto traverse the beam which indicated the effectiveness of aqueous garlic extract on induced changes on the cerebellum.

5.3 Effect of aqueous Garlic extract on lead induced changes onOxidative Stress Biomarker

It has been shown that heavy metals induces oxidative stress and this has been the focus of toxicological research for the last decade to evaluate the possible mechanism of the toxicity(Valavanidis*et al.,* 2006).Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and the cell's ability to reduce ROS, detoxify reactive intermediates and repair damage that may occur in cellular molecules. This imbalance

may occur as a result of increased ROS production, a decrease in defense mechanisms or both (Joseph *et al.,* 2012). Even though the exact mechanism of lead toxicity is not very well known, however, many researches had reported that it can cause generation of ROS and inhibits the antioxidant enzyme activities in tissues (Franco *et al.*, 2009).

In the present study, the activities of the antioxidant enzymes; CAT, SOD and GSH has been found to decrease significantly in the lead treated ratscompared to the ratsin the Control Group and this could be attributed to increase in reactive oxygen species (ROS) generation. The reduced activity observed was in agreement with many other studies of the effect of lead on antioxidant system. One of such work was by Hatice*et al.*,(2015), on the effect of lead and mercury on antioxidant systems in rat brain and reported that the mercury chloride and lead nitrate exhibited a significant inhibition of superoxide dismutase, catalase, glutation peroxidase and glutathione-S-transferase activities and increasing of malondialdehyde levels. However, the enzymes activity (CAT, SOD and GSH) in the lead and aqueous garlic extract or Succimer (DMSA) increased significantly when compared with lead treated group only which is an indicator that aqueous garlic extract has beneficial effects against oxidative stress.

Malondialdehyde (MDA) is one of the oxidative damage products of lipid peroxidation. Its presence in tissues indicate oxidative stress (Joseph *et al.,* 2012). The present findings showed that there was significant increase in the MDA level in lead treated Groupsonly when compared with the ratsin the Control Group while there was no significant increase

in MDA level in the lead and aqueous garlic extract or Succimer (DMSA) treated group when compared with the Control Group ratsthis could due to oxidation of the lipid.

5.4 Histological studies

Several studies have revealed that lead (Pb^{2+}) exposure produces neurological damage and behavioral disruptions in humans and in experimental rats. It has been reported that lead (Pb^{2+}) exposure to brain results in behavioral alternation, learning and memory deficits (Soodi et al. 2008). Histological examinations of cerebellar and hippocampal sections in the present study of the Control, aqueous garlic extract and the standard drug (DMSA) Groups showed normal cytoarchitecture of the cerebellum and hippocampus while the histological sections of the cerebellum and hippocampus of the ratstreated with lead acetate only showed neurodegeneration and distortion of the cellular layer of cerebellum and hippocampus.

The histological sections of the Control group, aqueous garlic extract and the standard drug (DMSA) showed normal cytoarchitecture of the cerebellum with relatively little histological changes while the cerebellar sections of the ratsin the lead treated group showed evident of histological changes. These changes include distortion of the cerebellar layer especially the pyramidal cell layer, loss of cellular architecture of the Purkinje cells, deformity of nuclei,loss of Nissl substance from Purkinje cells, and loss of nuclear cytoplasmic differentiation and these could ultimately interfere with the motor activities of the cerebellum and other motor function like loss of fine movement, loss of grasping, maintenance of equilibrium and loss of regulation of muscle tone which are

modulated by the spinal cord and brain stem mechanisms involved in postural control.Morphological changes observed in cerebellar tissues in the present study are supported by previous neuropathological findings which showed changes in the cerebellumfollowing lead exposure. One of such works was the work of Hesham*et al.*,(2015) that studied if allicin combined with Vitamin B-complex have superior potentials than α-tocopherol alone in ameliorating lead acetate induced Purkinje cell alterations in rats.

In hippocampalsections of the Control Group rats, there were no sign of histological changes with normal pyramidal layer, however, histological section of the ratsin lead acetate treated group only (Groups 2 and 3), showed a number of histological changes ranging from degeneration and reduction of pyramidal cells, vacuolated cytoplasm, absence of Nissl's granules, few cells showed vesicular nuclei, while others revealed pyknotic nuclei.This implies that the activity of the hippocampus in memory formation and learning will be impaired and the role of the hippocampus that involved storage and retrieval of information will also be lost. These findings are in accordance with the work of Owolabi*et al.*,(2014), who studied the Effects of MoringaOleifera against Lead-Induced Disruption of the Hippocampus in Animal Models and reported that lead produced deleterious effects on tissues of the hippocampal formation disrupting its general histoarchitecture and cellular integrity whileMoringaoleifera leaf extract ameliorated the severity of lead toxicity.

Meanwhile, the histological sections of the ratstreated with lead acetate and aqueous garlic extract or the standard drug (DMSA), showed preserved normal histoarchitectureof the hippocampus. Pyramidal cells were prominent with deeply vesicular nuclei, presenting normal and healthy morphology. This suggests that aqueous garlic extract have a promising effects against lead induced toxicity in Wistar rat models.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

It can be concluded from the present study that;

- Lead acetate has induced changes in the body weight of the adult Wistar rats.
- Rats exposed to lead acetate (Pb^{2+}) showed impairment in spatial learning and memory in Morris water maze and Beam walking tasks, but rats that are exposed to the same concentration of lead (Pb^{2+}) and aqueous garlic extract did not show impairment in Morris water maze and Beam walking tasks performance as shown by the reduction in the time taken to perform the tasks.
- Aqueous garlic extract has positive effect on the lead induced changes on GSH, CAT, SOD activities and MDA levels. The study showed thatMDA levels were increased and enzyme (GSH, CAT, and SOD) activities weredecreased in lead treated rats compared to the control group while there was preserved effect of lead acetate in the rats treated with lead acetate and aqueous garlic extract or Succimer(DMSA).
- Lead induced histological changes ranging from degeneration and cytoplasmic vacuolationofnerve cells of the cerebellum and hippocampus in the lead treated group only while there was relatively little histological changes in the lead and aqueous garlic extract or the standard drug (DMSA) groups.

6.2 RECOMMENDATIONS

1. Further studies are recommended to investigatethe molecular and cellular mechanisms responsible for increases in antioxidant enzyme activity (CAT, SOD, and GSH) and decreases MDA level.
2. It is recommended that further work should be carried out in order to quantify the amount of lead that could possibly be deposited in the hippocampus and cerebellum.

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