THE EFFECTS OF MERCURY ON THE HIPPOCAMPUS, CEREBELLAR AND CEREBRAL CORTICES OF ADULT WISTAR RATS

BY

ABUBAKAR SADEEQ ADAMU B.Sc (A.B.U) 2009

(M.Sc./MED/05416/2010-2011)

A RESEARCH THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERISTY, ZARIA.

IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF SCIENCE IN HUMAN ANATOMY

DEPARTMENT OF HUMAN ANATOMY, FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

DECEMBER, 2012.

DECLARATION

I hereby declare that the research work reported in this thesis entitled "**The effects of Mercury on the Hippocampus, Cerebellar and Cerebral Cortices of Adult Wistar rats**" was carried by me in the Department of Human Anatomy, Faculty of Medicine, A.B.U, Zaria, under the supervision of Prof. H.O Kwanashie and Dr. A.O. Ibegbu. The information derived from the literatures has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree in any institution.

_____ _____

Abubakar Sadeeq Adamu **Date**

CERTIFICATION

This thesis entitled "**The Effects of Mercury on the Hippocampus, Cerebellar and Cerebral Cortices of Adult Wistar Rats**" by Abubakar Sadeeq Adamu 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 Prof (Mrs.) **H.O. Kwanashie** and **Dr. A.O. Ibegbu**. It is therefore approved for its contribution to knowledge and literary presentation.

_____ _____

Prof (Mrs.) H.O. Kwanashie **Date**
Chairman Supervisory Committee,
Department of Pharmacology and Therapeutics,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University, Zaria.

_____ _____

Dr. A.O. Ibegbu **Date**
Member Supervisory Committee,
Department of Human Anatomy,
Faculty of Medicine,
Ahmadu Bello University, Zaria.

_____ _____

Dr. Barnabas Danborno Date
Head of Department of Human Anatomy,
Faculty of Medicine,
Ahmadu Bello University, Zaria

_____ _____

Prof. Joshua Adebayo Date
Dean school of post graduate studies
Ahmadu Bello University, Zaria

DEDICATION

This research work is dedicated to Allah (S.W.A) for giving me the strength and health to carry out successfully the research reported in this thesis and also to my late Mum, Haj. Zuwairiyya Aliyu Adamu and my younger sister Hauwa'u Adamu.

ACKNOWLEDGEMENTS

In the name of ALLAH, the most gracious and most high. My profound gratitude goes to the chairman of my supervisory committee Prof. H.O. Kwanashie for her continuous supervision and constructive criticism throughout the course of my research and to a father, a teacher, a mentor and a member supervisory committee Dr. A.O. Ibegbu for his tireless contributions and encouragement towards the success of this study and my academic pursuit in general.

My unreserved appreciation goes to my beloved parents for giving me the opportunity and training in my academic carrier. They have supported me right from childhood and to the best of their ability instilled a high moral standard in me that will forever grow and reflect in my disposition.

My special thanks goes to my brothers Mohd lawal, Anas, Abbas and also my sisters Khadijat, Aisha and Hauwa'u for their understanding and support throughout the period of my studies.

My able uncle Bashir Garba (Gamblos), I sincerely appreciate your tireless contributions and support, no words can be used to express my appreciation, may Allah bless you and your family and give us the courage to do to others as you have done to us. God bless you once again.

I will forever remain grateful to Mr. James A. Timbuak, who out of no time, found time to seat with me for statistical analysis, may the good Lord shower His blessings

on you. And also to a grandfather, a mentor, Dean Faculty of Sciences and Director Center for Biotechnology A.B.U. Zaria, Professor A.J. Nok. God bless.

Dr. U. E. Umana, Dr. W. O. Hamman, Mr. S.A. Musa, all of Human Anatomy Department A.B.U and Mal Shehu Shika of CERT A.B.U Zaria. I must appreciate all your great contributions and suggestions. Dr. (Mrs.) Alawa, I also appreciate your meaningful criticisms. My unreserved appreciation goes to my friend, a brother and a colleague Adamu Lawal Hassan (Prof) who is always there for me throughout the work, I really appreciate.

Michael, Abdulrazak and Sakeenah Sambo, Zaharaddeen Garba your efforts must be well acknowledged likewise S.S Babale and his wonderful family thank you for your prayers and encouragement. Ibrahim Shehu, Abdulkadir Jauro and Isah Yahya Jibril and to my wonderful ..deeyah (Sadeeyah M. Sadi Mato) I will never forget those wonderful memories with you. God bless.

The tempo of my appreciation will be lost if I forget to mention the staff of pharmacology Department A.B.U Zaria especially Mr. John, Mal Aliyu, Mal. Moh'd and Mal Mua'zu, I so much appreciate your understanding. To my class mate, such as Adamu Lawal Hassan (Prof), Hadiza ,Sabdat, Progress, Isah and Oga Gani, Musa Kona (kuntus) and lastly Mubashir. Lastly, I thank all staffs of Human Anatomy, Pharmacology and therapeutic and Pathology Department A.B.U, Zaria and to each and every one who contributed to the development of this research work but their names are not mentioned. Thank you all and God bless.

TABLE OF CONTENTS

Title Page ……...I

Decalration…….……...…II

Certification…….....……III

Dedicationon……..…….IV

Acknowledgements……...V

Table of contents……VII

List of figures …….....IX

List of Tables……...…X

List of Plates……...……XII

Abstract……..……..….XII-XIII

List of appendices……XIV

CHAPTER ONE

1.0 Introduction…….……..…1

1.1 Background……..1

1.2 Aim and objectives……..4

1.2.1 Aim of the study…...…….……..…..4

1.2.2 Objective of the study…….…….....5

1.3 Scope of the study…….5

1.3 Significance of the study ……..…….5

CHAPTER TWO

2.0 Literature review…….…….…….6

2.1 Exposure to mercuric Compound..……..……..6
2.2 Toxicokinetics of Mercuric Compound ..……..…7
2.3 Mercurialism and its effects……...…...9
2.4 Mercury Exposure and Toxicity……..11
2.5 Memory……..……16
2.5.1 Types of memory……..……..…16
2.5.2 Sensory memory..……..……..…16
2.5.3 Short term memory……..16
2.5.4 Long term memory……..…17
2.6 Central nervous system.……....18
2.6.1 Hippocampus…….…….……..…18
2.6.2 Morphology and formation of the Hippocampus…….……...…18
2.6.3 The cell and layers of the Hippocampus……....19
2.6.4 The dentate gyrus…….……...……22
2.6.5 Functions of Hippocampus…….……....22
2.7 Cerebellum……..23
2.7.1 Morphology of the cerebellum…….…….……...…23
2.7.2 Cellular layer of the cerebellum…….…….……24
2.7.3 Function of cerebellum……..…….…….……25
2.8 Cerebrum…….26
2.8.1 Morphology of the cerebrum…….…..26
2.8.2 Cellular layers of the cerebral cortex…….....27
2.8.3 Functions associated with cerebrum……....32

CHAPTER 3

3.0 Materials and Method……..…...33
3.1 Materials……..33
3.2 Experimental design…… …...……...33
3.3 Experimental procedure/protocol……...…….……..…..35
3.4 Neurobehavioral studies……..36

3.4.1 Spatial learning and memory with Morris water maze method……..36

3.4.2 Motor activity test with Montoya staircase method …… ……..37

3.4.3 Training time……..……38

3.5 Animals sacrifice…….39

3.6 Tissue processing technique……39

3.6.1 H and E staining Method……....40

3.6.2 Special stain Beilschowsky…….……40

3.7 Neutron Activation Analysis……...41

3.7.1 Preparation of tissue for organic trace elements analysis…….. 41

3.8 Statistical analysis……....43

CHAPTER FOUR

4.0 Results……...44

4.1 Physical observation ……..…….……44

4.2 Body weight of animals…….……..44

4.3 Physical observation of animals in Montoya staircase box…….46

4.4 Trace elements Analysis using NAA-1……...46

4.5 Motor Activity Test using Montoya staircase test……..……..…49

4.6 The effects of mercury chloride on spatial learning and memory……..…51

4.7 Histological changes……53

CHAPTER 5

5.0 Discussion ……..……80

5.1 Effects of mercury on body weight…….80

5.2 Trace elements analysis……..…….81

5.3 Histology ……...…….83

5.4 Neurobehavioral studies……..……85

CHAPTER SIX

6.0 Conclusion and Recommendations……...88

6.1 Conclusion……...88

6.2 Recommendation……89

References……...……90

Appendix……...……...….101

LIST OF FIGURES

Figure 2.1 Sources of mercury exposure.....…...7

Figure 2.2 Hippocampus and associated structures of the limbic system.....…...19

Figure 2.3 Cells and layers of the hippocampus……20

Figure 2.4 Cellular layers of the cerebellum.....….26

Figure 2.5 The structure of the brain showing the cerebrum.....…27

Figure 2.6 The cellular layers of the cerebral cortex…….....…….31

Figure 3.1 Morris water maze pool……...37

Figure 3.2 Montoya staircase test……..39

Figure 4.1 Concentrations of copper and zinc in part per million of animals treated with mercury chloride…….....48

LIST OF TABLES

Table 3.1 Grouping, dosages and frequency of administration of mercury chloride..36

Table 4.1 Effects of oral administration of mercury chloride on body weight of Animals…….……...…....45

Table 4.2.The mean time taken to explore Montoya staircase for testing motor activity…….…….....50

Table 4.3 Effects of mercury chloride ingestion on spatial learning and memory using Morris water maze test……52

Table 4.4.The Mean Cell Count of the Hippocampus, Cerebellar and Cerebral cortices in adults Wistar Rats…….....55

LIST OF PLATES

Plate 1: Photomicrograph section showing of the cerebral cortex control group receiving distilled water (H and E X250)……..……56

Plate 2: Photomicrograph section showing of the cerebral cortex receiving 12.45 mg/kg/hg (H and E X250)…….……...57

Plate 3: Photomicrograph section showing of the cerebral cortex receiving 24.90 mg/kg/hg (H and E X250)……58

Plate 4: Photomicrograph section showing of the cerebral cortex receiving 24.90 mg/kg/hg (H and E X250)…….……59

Plate 5: Photomicrograph section showing of the cerebellum control group receiving distilled water (H and E X250)…….….60

Plate 6: Photomicrograph section showing of the cerebellum receiving 12.45 mg/kg/hg (H and E X250)……..…….61

Plate 7: Photomicrograph section showing of the cerebellum receiving 24.90 mg/kg/hg (H and E X250)…….……..62

Plate 8: Photomicrograph section showing of the cerebellum receiving 48.90 mg/kg/hg (H and E X250)……..……63

Plate 9: Photomicrograph section showing of the hippocampus control group receiving distilled water (H and E X250)…….……64

Plate 10: Photomicrograph section showing of the hippocampus receiving 12.45 mg/kg/hg (H and E X250)…….……...65

Plate 11: Photomicrograph section showing of the hippocampus receiving 12.45 mg/kg/hg (H and E X250)…….……...66

Plate 12: Photomicrograph section showing of the hippocampus receiving 12.45 mg/kg/hg (H and E X 250)……67

PLATE 13: Photomicrograph section of the cerebellum of the control group receiving distilled water (Bielschowsky stain ×250)……68

PLATE 14: Photomicrograph section of the cerebellum receiving 12.45 mg/kg/Hg (Bielschowsky stain ×250)……..69

PLATE 15: Photomicrograph section of the cerebellum receiving 24.90mg/kg/Hg (Bielschowsky stain ×250)……..70

PLATE 16: Photomicrograph section of the cerebellum receiving 49.80 mg/kg/Hg (Bielschowsky stain ×250)…….71

PLATE 17: Photomicrograph section of the cerebral cortex of the control group receiving distilled water (Bielschowsky stain ×250)……..…….72

PLATE 18: Photomicrograph section of the cerebral cortex receiving 12.45 mg/kg/Hg showing (Bielschowsky stain ×250)……73

PLATE 19: Photomicrograph section of the cerebral cortex receiving 24.90 mg/kg/Hg (Bielschowsky stain ×250)……..……74

PLATE 20: Photomicrograph section of the cerebral cortex for group receiving 49.80 mg/kg/Hg (Bielschowsky stain ×250)……75

PLATE 21: Photomicrograph section of the cerebral cortex for the control group receiving distilled water (Bielschowsky stain ×250)……76

PLATE 22: Photomicrograph section of the cerebral cortex for group receiving 12.45 mg/kg/Hg (Bielschowsky stain ×250)…….77

PLATE 23: Photomicrograph section of the cerebral cortex for group receiving 24.45mg/kg/Hg (Bielschowsky stain ×250)……78

PLATE 24: Photomicrograph section of the cerebral cortex for group receiving 49.80 mg/kg/Hg (Bielschowsky stain ×250)……79

LIST OF APPENDICES

Appendix I Calculations ……...101

Appendix II The Mean and Standard error of Mean of Copper and Zinc in the brain tissue of adults Wistar rats after Neuron Activation Analysis……...…102

ABSTRACT

Mercury is a widespread environmental and industrial pollutant which is used in food preservation, cosmetics, pharmaceutical companies and laboratories. The aim of the present study was to investigate the possible effects of mercury (Hg) exposure on the histomorphology of the hippocampus, cerebellar and cerebral cortices, and also to evaluate the effects of mercury on spatial learning and memory, and motor activity with the aid of Morris water maze and Montoya staircase tests respectively. Twenty four adult Wistar rats of both sexes, with average weight of 210gms were randomly divided into four groups I, II, III, and IV with six animals per group. The animals in groups II, III, and IV were administered mercury chloride orally at the concentration of 12.45, 24.9 and 49.8 mg/kg body weight respectively while group I served as control and was administered with normal saline. The administration lasted for a period of 21 days (3 weeks). Animals were trained for both spartial learning and memory, and motor function using Morris water maze and Montoya stair case tests respectively. The brain tissues was fixed in Bouin's fluid and processed for histopathological studies using H and E and Bielschowsky stains. Brain tissues were analyzed for trace element analysis using Neutron Activation Analysis (NAA-1). The results from spartial learning and memory test was statistically significant after the first week of administration of mercury chloride in group IV animals, by the second week, there was an increase in time to find the platform, also by third week of exposure to low dose of mercury, there was a statistical significant increased in latency time taken to find the platform as an escape route by the rats. The result from Montoya

staircase test showed reduced exploratory motor activity in rats exposed to high concentration of $HgCl_2$ that was statistically significant in the third week. There was significant motor exploratory activity impairment due to mercury intoxication even at low doses. The trace element analysis with NAA-1 revealed a significant decrease in concentration of copper and zinc in the brain tissues of rats exposed to mercury The results also showed neurodegenerative changes in the hippocampus, cerebral and cerebellar cortices which range from cellular death and sparse distribution of cells and decreased in cell number which also contributed to the decreased in concentration of the copper and zinc concentrations in the hippocampus, cerebellar and cerebral cortices as well as loss in motor activity, and memory and learning deficit. The findings from this study showed that ingestion of mercury chloride has potential deleterious effects on brain as shown in the histopathology, decreased cell number, impairment of learning and memory, exploratory motor activity and reduction in essential trace element concentrations in the brain of Wistar rats.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Man in his environment is exposed to much potential hazards by heavy metals via bioaccumulation and biodegradation which is transferred in man via food chain due to anthropogenic activities (Wang *et al.,* 2007). Mercury, a heavy metal is a highly deleterious environmental pollutant that can lead to many health problems in the world (WHO, 1991). Mercury is known to the ancient Egyptians, Chinese and Indians as silver water (ATDRS, 1995). It was found in Egyptian tomb dating as far back as 1500BC. The name mercury was derived from the word *Hydragyrus* meaning water and silver or silver water. Mercury is a liquid and was named after the Roman god of mercury, known for its mobility and speed (Wang *et al.,* 2007; Berlin *et al.,* 2007).

Mercury exists in three forms (WHO, 1991 and 2003; ATDRS, 1995; Burger *et al*., 2011). These forms include: Elemental mercury also called metallic mercury, is element in its pure, un-combined form. Inorganic mercury compounds or mercury salts are more commonly found in nature, include Mercuric sulphide (HgS), Mercuric oxide (HgO) and Mercuric chloride ($HgCl_2$). Organic mercury is formed when mercury combines with carbon and other elements. Examples are dimethylmercury and phenyl mercuric chloride.

The following are some sources of mercury and its compounds**.** Industrial sources: mercury emitted from fossil fuels power and into the air by Mining co-operations, water bodies and land (Akagi, 1995; Bjomberg *et al.,* 2011). Mercury can be found in natural forms such as Mercury chloride that is found in higher densities in rocks and volcanic activities which can give half of the $HgCl_2$ present in nature (Bodek *et al.*, 1988; FAO, 1994; Park, 2000). Burning of fossil fuels such as petrol and gas, fumes, battery disposals, broken mercury thermometer and coal combustion are other high sources of emitting mercury and its compounds (ATDRS, 1995; Booth, 2005). Consumer products such as Photographic plates and toners contain high amount of mercury chloride (Goyer, 1986). Cosmetics examples include creams, perfumes, soaps and mascara.

There are many routes of exposure to mercuric compounds but the evidence of exposure is dependent on the levels of toxicity (WHO, 2005; Vimercati, 2001). These exposure routes include: Oral exposure can be via consumption of food products and grains preserved with mercuric compounds (WHO, 2005; EHD, 2002; Vupputuri *et al.,*2005). Inhalational exposure route can be from fumes, industrial actions of fossil fuel, odor and sewages in the form of mercury oxide (WHO, 1989; 1991 and 2005; Mohan *et al.,* 2011). Dermal exposure can be through the use of mercuric ointments, creams and some soaps which can result in disease conditions (ATDRS, 1989; Berlin, 2006; Nardofy *et al.,* 2000).

Mercurialism is known as mercury poisoning or toxicity in which the metallic mercury is absorbed via the skin and ingested or the vapor is taken via the lungs (Lim *et al.,* 2009). Children and women within the reproductive age are more susceptible to mercury poisoning (Sorensen *et al.,* 1999). In children mercury poisoning is known as acrodynia or pink disease. Mercury and its compounds have been shown to have effects on the respiratory, cardiovascular and reproductive systems, blood, hair, skin, and enzymes and many other organs and tissues (Murphy, 1976; ATDRS, 1992; Kosan *et al.,* 2001; WHO, 2003; Olieveri, 2000; Valera, 2008; Rao *et al*., 2009).

Mercury and its compounds' toxicity were evident in Nigata, Minamata, Iraq, Pakistan, Guatemala and Yatsishiro (Berlin, 2003; Mahafeey, 2004; Wang *et al.,* 2007; Sorensen *et al.,* 1999; EHD, 2002). Mercury toxicity in Nigeria was reported in some fishes from Lagos lagoon, Oboni land and Oyewo water of Niger Delta (Wegwu, 1999). In Northern Nigeria, there was toxicity via the use of traditional cosmetic called kohl in some states like Kastina, Sokoto, and some other Northern states (Onyeike *et al.,* 2002).

Mercury and its compounds have been shown to also have effects on the growth, weight, renal system, liver and psychological disturbances to mention but a few (Murphy, 1976; ATDRS, 1992; Kosan *et al.,* 2001; WHO, 2003; Oliveri, 2000; Valera *et al.,* 2008; Rao, 2001). Signs and symptoms of mercury poisoning include; Irritability, excitability, restlessness of the skin and eyes, headache, dizziness, difficulty in walking, frequent urination (Amin- zaki *et al.,* 1974; WHO, 2005;

ATDRS, 2007 and 2011). Mercury has no known nutritional or biomedical importance but has various applications and uses (W.H.O, 2003 and 2005). Mercuric compounds are used in embalmment and in preservation of anthropological species and specimens. It was used for the treatment of syphilis before the advent of antibiotics. In Chinese and Tibetan medicine, it was used in healing injuries and fractures. Mercuric chloride are used as fungicides to control root maggot of cabbage and onions while Pharmaceutical companies use mercury (thimerosal) in vaccines and in syrups, pills and blue masses. In cosmetics, mercuric compounds are widely used in the production of creams, ointments, perfumes, mascara and others. Miscellaneous uses include mercury switches, liquid mirrors, batteries, thermometers and many others. Mercury has no any nutritional value and still has various uses/application which exposes both human and animals to its toxic effects which could be either short or long term.

1.2 AIM AND OBJECTIVES OF THE OF THE STUDY

1.2.1 Aim of the Study

The aim of the study is to investigate the effects of Mercury chloride exposure on the brain tissues mainly the hippocampus, cerebellar and cerebral cortices using adult Wistar rats.

1.2.2 Objectives of the Studies

The objectives of the study are to

1. Investigate the histopathological changes in the hippocampus, cerebellar and cerebral cortices induced by mercury chloride exposure on the adults Wistar rats.
2. Evaluate the effects of mercury chloride exposure on spatial learning and memory using Morris Water maze method.
3. Assess the motor activity of adult Wistar rats using Montoya Staircase test.
4. Determine the effects of mercury chloride exposure on the concentration of trace elements present in the brain tissues of adult Wistar rats.

1.3 SCOPE OF THE STUDY

The study will be limited to general histomorphological and degenerative changes of hippocampus, cerebellum and cerebrum, studies on spartial learning and memory, motor function and neurochemical changes that may occur in the brain tissues as a result of exposure to mercury chloride.

1.4 SIGNIFICANCE OF THE STUDY

People are exposed to the potential risk of heavy metals via different routes which can lead to many health problems, as such this study is to create awareness on the effects of exposure to mercury and its compounds (mercuric chloride) directly or indirectly, particularly on the central nervous system.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 EXPOSURE TO MERCURIC COMPOUNDS

There are many routes of exposure to mercuric compounds but the evidence of exposure depends on the level of toxicity (Berlin, 2006; WHO, 2005). These routes include:

i. Oral exposure: This can be via consumption of food stuff preserved with mercuric compounds (FAO, 1994; FAD, 1998). Fishes and shells for example contain mercury in their muscle, example of these fishes are Tilapia, King mackerel, Shark and file fishes (Gunderson, 1988; Clarkson *et al.,* 1989 and 2003; Vupputuri *et al.,* 2000; Dewaily *et al.,* 2008).

ii. Inhalational exposure: Can be from fumes, industrial actions of fossil fuel power, odor and sewages in the form of mercury oxide and some cosmetics in the form perfumes (Berlin, 1986; Clarkson, 2003; WHO 1991 and 2003).

iii. Dermal exposure: Via the used of mercuric ointments, creams and some soaps which can result to disease condition. However, dermal or ocular exposure can occur when coming in contact with mercury and its compound (ATDRS, 1995; Altmann *et al.,* 2008; Yannai, 2002; WHO, 2003).

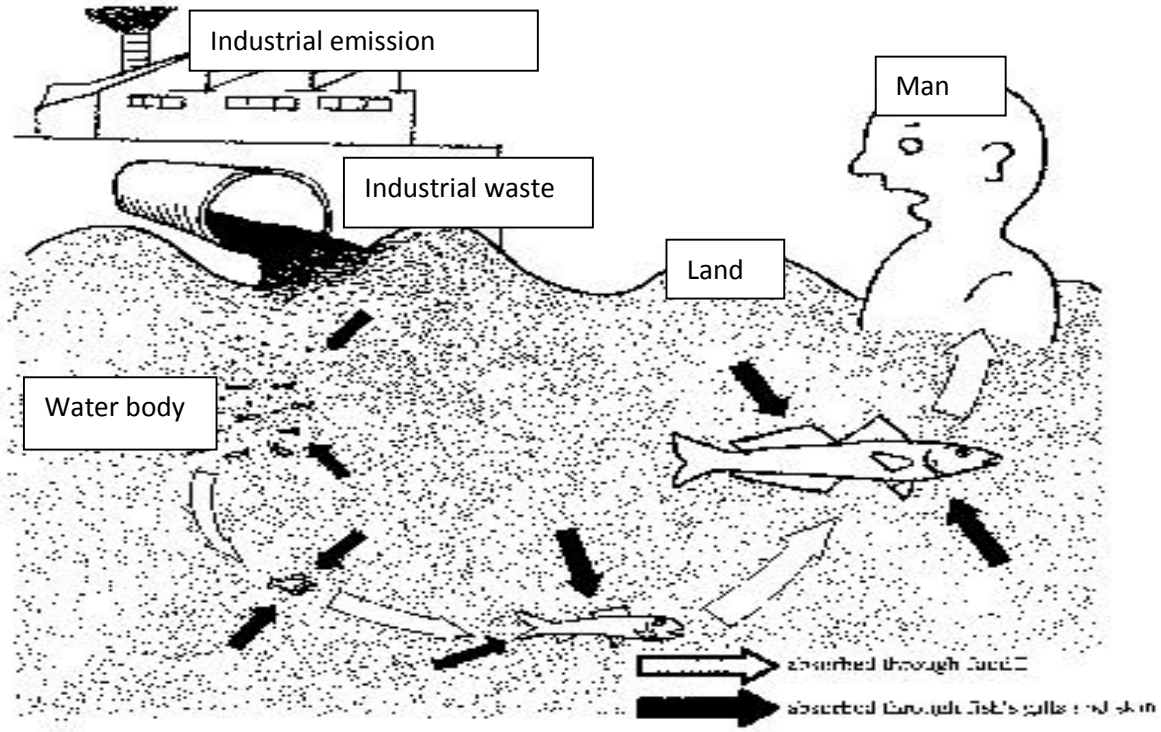


Figure 2.1.Sources of mercury exposure (Dewaily *et al.*, 2008).

2.2 TOXICOKINETICS OF MERCURIC CHLORIDE

a) **Absorption**; organic mercury are absorbed much more rapidly than inorganic forms, while approximately 80% of mercury vapor is absorbed following inhalational exposure (Hursh *et al.,* 1989). Metallic mercury and mercurous salts (Hg_2Cl_2) are poorly absorbed following oral exposure (Piotroski *et al.,* 1992). Absorption of mercury chloride was reported at 48 % in rats of the orally administered substance (Piotroski *et al.,* 1992; FAD, 1998). Gastrointestinal absorption of inorganic salt has shown to be readily absorbed by human and animals following oral exposure (ATDRS, 1989; Goyer, 1991; Yannai, 2002).

b) **Distribution**: Mercury vapor readily enters the red blood cells and central nervous system following inhalational exposure (Warfringe, 1995; Barregard *et al.,* 1992). The kidney and central nervous system exhibits greatest concentration of mercury following exposure to inorganic mercury salts (Neilsen, 1991). Both organic and inorganic mercury are readily distributed throughout the body, but tend to concentrate more in the brain and kidney (Barr *et al.,* 1973). Mercury is known to bind to microsomal and mitochondrial enzymes resulting in cell injury and death. Mercury in renal cells is localized in lysosomes (Neilsen *et al.,* 1991). However, the concentrations of organic and as well as inorganic mercury were shown to be increased in plasma and erythrocytes respectively (Goyer, 1991). Animal data indicates that all forms of mercury cross placenta barrier and are two folds greater than in maternal levels with fetal red blood cell containing mercury at 30% higher than maternal red blood cell (Goyer, 1991). The placenta provide no barrier for organic and inorganic mercury, thereby allowing easy access to the developing brain and development of subsequent neurological disorders characteristic of fetal exposure to mercuric compounds (Rice *et al.,*1990).

c) **Metabolism:** mercury is not destroyed by metabolism rather converted to different forms and oxidation states. The metabolism of mercury and mercury compounds appear to be similar in animals and humans (ATDRS, 1989). Inhaled mercury vapor is rapidly oxidized to divalent forms in red blood cells (Halbach and Clarkson, 2008). Oxidation of elemental mercury occurs in lungs of humans and animals

(Hursh *et al.,* 1989). And some evidence suggested hepatic-mediated oxidation of mercury in the body

d) **Excretion:** urine and feces is the primary route of excretion of inorganic mercury by humans (ATDRS, 1989). Following brief exposure of humans to inorganic mercury, urinary excretion accounts for 13% of the total burden, whereas this value increased to 58% for long term exposure (Betti, 1992; Berlin, 2003). There are three forms of mercury identified in the urine of the occupationally exposed individuals. These include elemental mercury, a reducible mercuric –cysteine complex and a large complex in which the mercury can only be released following organic destruction. Additional excretory routes include saliva, sweat, bile and mammary gland (ATDRS, 1989; Booth and Zeller,2005).

Mercury poisoning or toxicity is known as mercurialism in which the mercuric compound(s) is or are absorb via the skin and elementary canal, and the vapor is taken via the lungs (WHO, 1991; ATDRS, 1995; Thurtson *et al.*, 2007; EHD, 2000; Berlin, 2007).

2.3 MERCURIALISM AND ITS EFFECTS

Fetuses, children and females within the reproductive age are more susceptible than adults to mercury poisoning (Thomas, 2007; Winneke *et al.,* 2011). In children, mercury poisoning is known as acrodynia or pink disease which is characterized by neuropsychiatric disturbances, peripheral vascular effect and disturbances in sensation of the renal function within few minutes of poisoning and circulatory system collapse

(Choic *et al.,* 2011). The signs and symptoms of mercury poisoning can be seen based on the categories of the observed manifestations These signs and symptoms of mercury poisoning include, chronic or frequent headaches, numbness and tingling anywhere, dizziness, ringing or noises in the ear, tremors in hands, feet, lips, eyelids, tongue and lowering of pain threshold (ATDRS, 2003; Warkany, 1953; Murphy, 1979; Winship, 1985; Pamber, 1989; IARC, 1993; Clarkson, 2003; Onyeike *et al.,* 2002; W.H.O, 1999 and 2003; ATDRS, 2005; Musa and Sadeeq, 2009).

Irritability, nervousness, shyness or timidity, loss of memory, inability to concentrate, mental confusion, mood changes, lack of interest in life and hobbies. Attention Deficit Syndrome or similar problems, decline of intellect, loss of self-confidence, anger and loss of self control, depression, crying spells, anxiety, drowsiness and Insomnia are some of the neurological effects and disorders of mercury poisoning (ATDRS, 2005). Mercurialism can also cause bleeding gums, bone loss and loosening of teeth, foul breath, excessive salivation, metallic taste, white patches on the gums and cheeks, chronic inflammation of gums and mouth, ulcerative burning in the mouth or throat and tissue black pigmentation (Clarkson, 2003).

Gastrointestinal effects include**,** bloating and excessive gas, abdominal cramps, constipation or diarrhea, irritable bowel syndrome, colitis, nausea, loss of appetite, voracious appetite and obesity and excessive thirst (W.H.O, 1999 and 2003). Irregular heartbeats, feeble and irregular pulse, alterations in blood pressure, arterial plaque are some of the cardiovascular problems associated with mercury poisoning. Also mercurialism can result to chronic fatigue syndrome, fibromyalgia, rheumatoid,

arthritis, allergies, sinusitis, asthma, muscle weakness and joint pain (Thurtson *et al.,* 2007). Other problems associated with mercury poisoning include, excessive perspiration without fever, low body temperature, sometimes with clamminess, skin rashes, especially around face, head and neck, dim or double vision and Hypoxia (Booth, 2005).

2.4 MERCURY EXPOSURE AND TOXICITY

No information available regarding the effects of mercury and its compounds on cancer. This may be due to poor experimental design, lack of adequate and methodological description provided or no definitive evidence of carcinogenicity (ATDRS, 1989). Janicki et *al.,* (1987) and Choic *et al.,* (2011), has reported an association between mercuric compounds exposure and leukemia, but the work was not accepted by World Health Organization due to inadequate evidence and data for carcinogenicity of mercury and mercuric compounds on humans and animals. Hence, IARC classified elemental mercury and inorganic mercury compounds as category 3 carcinogens i.e. non-carcinogenicity to humans (IARC, 1993).

Evidence suggests that, exposure to mercury compounds in women may increase the frequency of menstrual disturbance by prolonging uterine cycle and spontaneous abortion and also pregnancy complication. (ATDRS, 1989; Rao and Sharma, 2001; Baltimore, 2006). Amin-Zaki *et al.,* (1974), has reported that; no evidence of teratogenicity, but found other developmental effects on impaired motor and mental function, hearing loss and blindness. Abnormal cytoarchitecture of the

brain in infants prenatally exposed to mercury was evident (Fuyuta *et al.,* 1978). A 100% incidence of neonatal death and failure to deliver was reported in mice (Bell, 2008).

Dermal contact is seen when mercury applied locally on skin and mucous membrane. Typically, there is manifestation of erythemas and contact dermatitis in hypersensitive individuals (Pambor, 2007; Goh, 2008). A special form of hyper sensitivity is found in children and this syndrome is called acrodynia or pink disease. Local application can result to rashes over the body, chills limitation and swelling of hand, cheek, feet and nose. Desquamation, lost of hair, photophobia, perspiration which can result in dehydration due to dilated and enlarged sweat glands, hyperplasia, hyperkeratosis and frequent urination (Charles, 2006; Goh, 2008). When mercury makes contact with eyes, they exhibit discoloration of cornea and lens, tremor of eyes lid, disturbance of vision, extra ocular muscle and conjunctivitis (Charles, 2006). Eyes contact can causes ulceration of conjunctiva and cornea (Altmann *et al.,* 1998). It was reported that 0.5g tablet of mercuric chloride is the toxic value for human to affect eyes (Altmann *et al.,* 1998; Crespo-lopez, *et al.,* 2009).

Inhalation of mercury vapor may result in corrosive bronchitis, intestinal pneumonitis, and death (Milne, 1970; Goyer, 1991; Meyer-Baron, 2002). Lungs are the primary organ to exhibit toxicity due to the reaction of the Hg and O_2 to give mercury oxide, (Hallee, 1969; Goyer,1991). Cough and chest pain may start few hours of exposure and last for days (Hu *et al.,* 2012).

The concentration of inorganic mercury of 0.4 to 2.2 ug/ml present in blood serum or plasma has been reported to cause death in humans (Winneke, 2011). Influence of mercuric chloride on the erythrocyte and leukocyte counts especially lymphocytes, easinophils and monocytes has been shown to increase due to mercury toxicity (Barregard, 1991). Slight change in hematocrits index accompanied the change in numbers of formed blood elements (Makikallio *et al.,* 2001). Auto-immune response manifested as a general stimulation of immune system with hyper immunoglobulaneamia, antinuclear fibrillarin, antibodies and glumerular disease, accompanied by vascular immune complex deposit (Warfvinge 1995; Vappurituni, 2005). A significant decrease in weight of the thymus and spleen in antibody response were also exhibited. Increase in B-lymphocyte mediated lymphocyte proliferation was also observed (Andres, 1984; Brockmeiser, 2010; Albalaghi *et al.,* 1996).

Saha *et al.,* (1986) has reported that, mercurialism induced chromosomal abnormalities, which induces stretching and elongation disperilization of chromatids, sticky bridge and elongation of X-chromosome, pulverization. It has been shown that there was prepundence of chromosome breaks over chromatics backs. (Betti, 1992). Data from 14 studies of cytogenetic effects, such as sister chromatid exchange, micronucleus formation, chromosomal aberrations, aneuploidy and polyploidy in peripheral blood lymphocytes of individuals exposed to elemental and inorganic mercury or mercury compounds were inconclusive (Cantoni, 1983; Betti, 1992). This is because the studies have provided little convincing evidence that exposure to mercury causes chromosomal damage (Clarkson, 2003).

Renal function may be disturbed within few minutes after mercury poison reaches the circulation. If circulation is adequate, the first response of the kidney may be diuresis caused by inhibition of tubular reabsorption and renal damage may be extensive that oligonuria results (Grandjean *et al.,* 2004; Kosan *et al.,* 2001; Halbach, 2008). Kidney is the critical organ of injury although site of diuretic action of mercuric compounds has not been demonstrated, but it is suspected to be in the proximal tubule (Cartoni, 1983; Bell, 2008; Coulibally *et al.,* 2012).

The effect of mercury chloride intoxication on the activity of acid and alkaline phosphatase and glucose-6- phosphatase was evident in the work of Haltbach (2008). The result show an elevation of acid phosphates and inhibition of alkaline phosphates activities, and Glucose -6-phosphatase activity was elevated and there was inhibition of the enzymes, alkaline phosphatase (Andres, 1984; Haltbach, 2008). Fuyuta., (2000) had reported that the activity acetyl cholinesterase was determined in RBC and bone marrow after exposure to high mercuric chloride which showed a marked declined in the activity of the enzymes

Tachycardia and hypertension have been reported following ingestion of mercuric chloride (Altman, 2008). In addition, tachycardia has been reported secondary to severe pneumonitis after acute exposure to mercury vapor. It has been shown that there was increase in blood pressure and heart rate (ATDRS, 1999; Altman, 2008). Cardiovascular toxicity in humans has also been observed following ingestion of mercuric chloride in a report of a suicide attempt through ingestion of

approximately 20 mg mercury/kg body weight (Makikallio, 2001; Oka *et al.,* 2002). Electrocardiogram of a 22-year-old male revealed no P-wave, prolongation of the QRS segment, and a high T-wave. Murata *et al.,* (2006) and Lim, (2009)., described multiple cases in which tachycardia and elevated blood pressure were observed in children treated with mercurous chloride tablets for worms or mercurous chloride-containing powders for teething discomfort. A number of children whose diapers had been rinsed in a mercuric chloride solution also experienced tachycardia and elevated blood pressure (Grandjen *et al.,* 2004).

The Gastro intestinal tract exhibits stomatitis, excessive salivation, difficulty in swallowing, abdominal pains, nausea, vomiting diarrhea, drilling and sore gums and ulceration of oral mucosa incase of exposure to mercury and their compounds. (ATDRS, 1999). Report also indicates that; there is oropharyngeal pains, hemorrhage throughout the length of the GIT (Murphy *et al*., 1976). Hepatotoxicity at low concentration shows mercury ions accumulation in the liver and at high concentration the ions rupture the lysosomes and release destructive acid hydrolysis (Rao *et al.,* 2010). Mercury toxicity in the liver can causes jaundice, hepatomegaly, and central tubular vacuolation at autopsy (ATDRS, 1999).

2.5 MEMORY

Memory is an organism's ability to store, retain and recall information and experience (Hackijng, 1996; Mark, 2007; Micheal, 2009). During the late Nineteenth

and early Twentieth century, scientists have put memory within the paradigm of cognitive psychology. In recent decades, it has become one of the principal pillars of a branch of science called cognitive neuroscience, an interdisciplinary link between cognitive psychology and neuroscience.

2.5.1 Types of Memory

2.5.2 Sensory Memory

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

2.5.3 Short-Term Memory

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

string of 10 digits. This is because of the ability to chunk the information into meaningful groups of numbers. Short-term memory is believed to rely mostly on an acoustic code for storing information, and to a lesser extent a visual code (Alberimi, 2001).

2.5.4 Long-Term Memory

Long-term memory involves the posterior cinglate gyrus and can store much larger quantities of information for potentially unlimited duration, sometimes a whole life span (Alberimi, 2005). Its capacity is immeasurably large. For example, given a random seven-digit number we may remember it for only a few seconds before forgetting, suggesting it was stored in our short-term memory. On the other hand, a person can remember telephone numbers for many years through repetition, this information is said to be stored in long-term memory (Tyler *et al.,* 1979). This type of memories, are maintained by more stable and permanent changes in neural connections widely spread throughout the brain. The hippocampus is essential for learning new information and the consolidation of information from short-term to long-term memory, although it does not seem to store information itself (Gross, 1993). Without the hippocampus, new memories are unable to be stored into long-term memory, and there will be a very short attention span (Gross, 1993; Mark, 2007)

2.6 THE CENTRAL NERVOUS SYSTEM

The Central Nervous System (CNS) is composed of the brain and spinal cord and is surrounded by bone mainly the skull and vertebrae. Fluid and tissue also

insulate the brain and spinal cord. The brain is composed of three parts: the cerebrum, the cerebellum, and the medulla oblongata.

2.6.1 Hippocampus

2.6.2 Morphology and Formation of the Hippocampus

Anatomically, the hippocampus is an elaboration of the edge of the cerebral cortex (Gray, 2000, Chris, 2008). In rodent, the hippocampus, a horseshoe shape paired structure, with one hippocampus located in the left brain hemisphere and the other in the right hemisphere (Gross, 1993). The hippocampus, the dentate gyrus and the sibiculum collectively make up the hippocampal formation, which is said to be part of the limbic system as shown in Figure 2.1. The hippocampus and the dentate gyrus belong to the allocortex and has simplified laminar pattern compared with the neocortex.

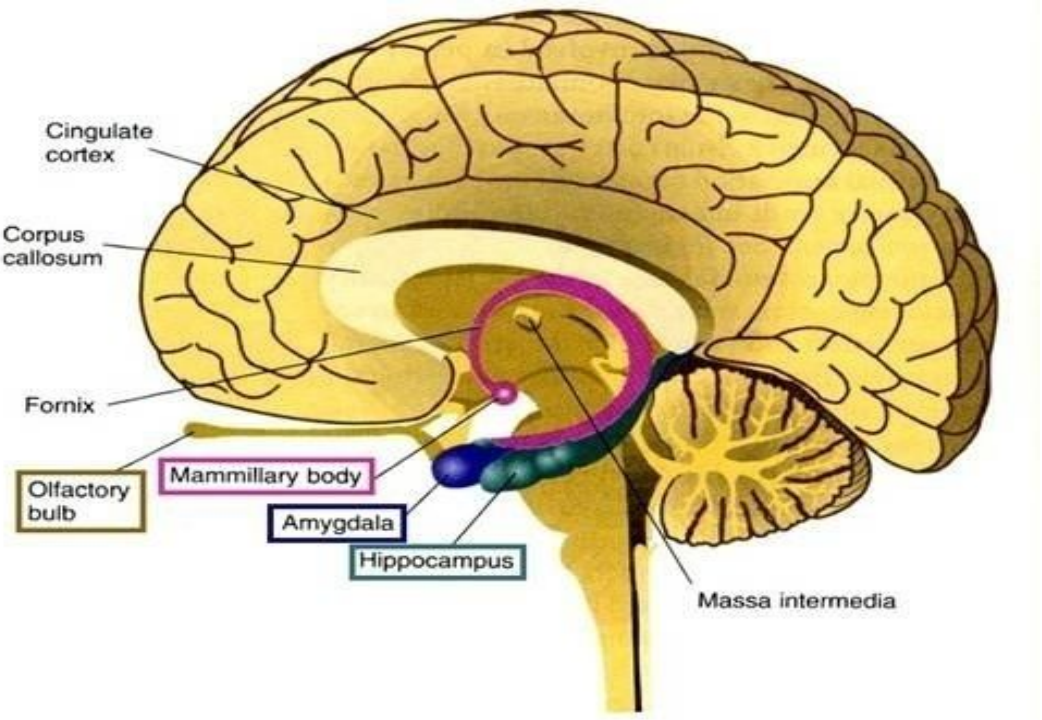


Figure 2.2: Hippocampus and associated structures of the limbic system (Chris, 2008).

2.6.3 The Cells and Layers of Hippocampus

The hippocampus is composed of multiple subfields. Though terminology varies among authors, the terms most frequently used are dentate gyrus and the cornu ammonis literally "Amun's horns", abbreviated CA. The dentate gyrus contains the fascia dentata and the hilus, while CA is differentiated into fields CA1, CA2, and CA3 (Chris, 2008). The horse shoe appearance of the hippocampus is caused by cell density differentials and the existence of varying degrees of neuronal fibers as shown in Figure

2.4. The CA regions are also structured depth-wise in clearly defined strata (Philippe, 2007; Hiroaki, 2007; Annabelle 2009).

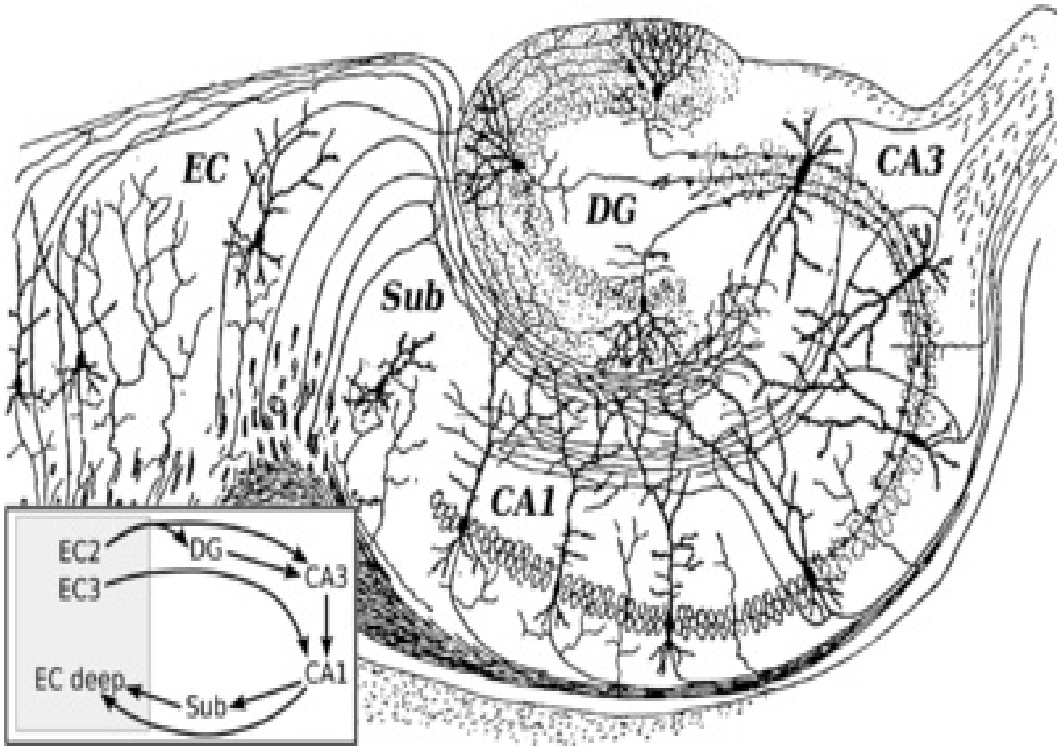


Figure 2.3 Cells and layers of the hippocampus (Annabelle, 2009).

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

hippocampus usually recurrent connections, especially in CA3 and CA2 regions (Annabelle, 2009).

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

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

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

2.6.4 The Dentate Gyrus

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

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

2.6.5 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. Hippocampus is a limbic system structure that is particularly important in forming new memories and connecting emotions and senses, such as smell and sound, to memories. The hippocampus acts as a memory indexer by sending memories out to the appropriate part of the cerebral hemisphere for long-term storage and retrieving them when necessary.

2.7 CEREBELLUM

2.7.1 Morphology of the Cerebellum

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

2.7.2 Cellular Layers of the Cerebellum

The cerebellar cortex is divided into three layers. The layers from the lower to the upper include the followings as shown in Figure 2.5.

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

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

iii. **The molecular laye**r is thick. It contains a sparse population of neurones, dendritic arborizations, non-myelinated axons and radial fibres of the neuroglial cells. (Clerke,

1996; Gray, 2000). Purkinje cell dendritic trees extend towards the surface and spread out in a plane perpendicular to the long axis of the cerebellar folia. Purkinje cell dendrites are flattened. This outermost layer of the cerebellar cortex also contains two types of inhibitory interneurons, stellate cells, and basket cells (Linas *et al.,* 2004).

2.7.3 Functions of the Cerebellum

The cerebellum performs the following function:

i. Coordination of Movement; the cerebellum controls the timing and pattern of muscle activation during movement.
ii. Maintenance of Equilibrium in conjunction with the vestibular system.
iii. Regulation of Muscle Tone and modulation of spinal cord and brain stem mechanisms involved in postural control.

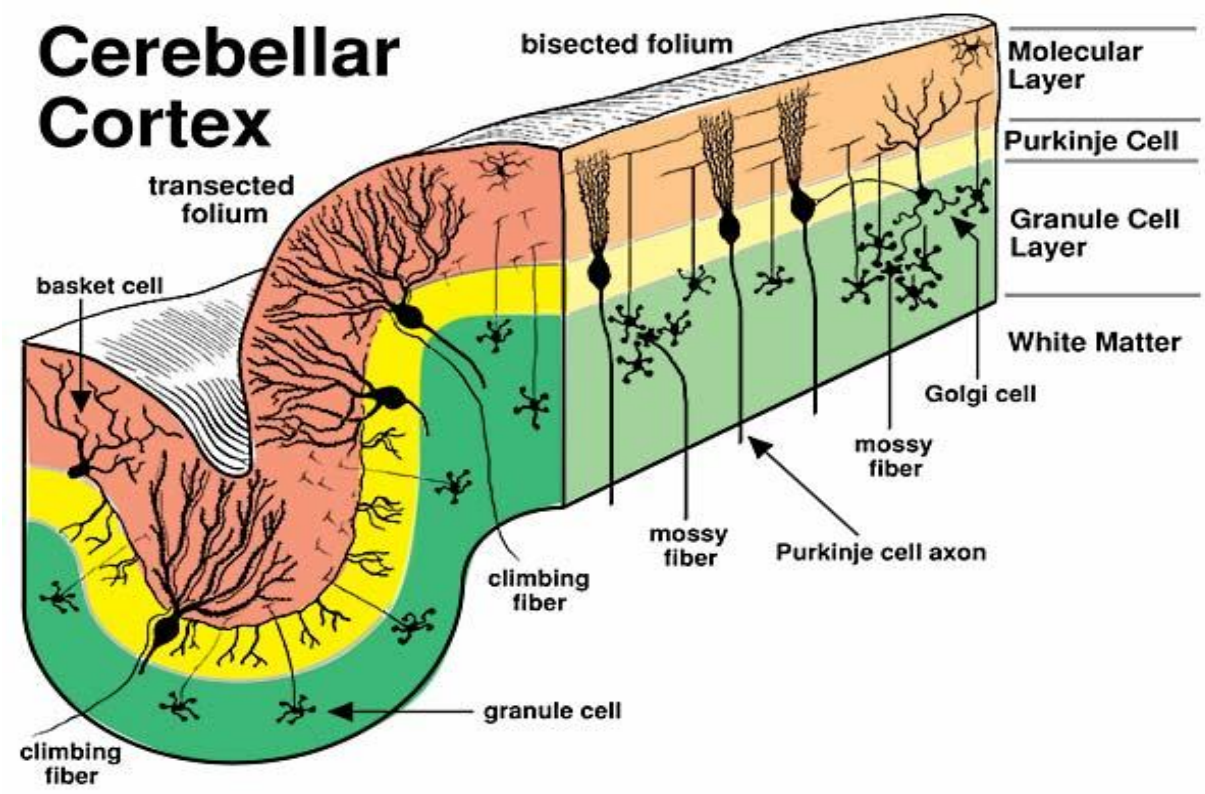


Figure 2.4: Cellular layers of the cerebellum showing the outer molecular layer **(brown**), the purikinje cell layer **(yellow)** and the granule cell layer **(green)** (Linas *et al.,*2004).

2.8 THE CEREBRUM

2.8.1 The Morphology of the Cerebrum

The cerebrum comprises a large portion of the brain. It lies in front or on top of the brainstem and in humans is the largest and best-developed of the five major divisions of the brain. The cerebral cortex is the largest part of the brain; it is formed by grey matter and cerebral hemisphere on the surface as shown in Figure 2.6. The

cortex is convoluted with projections called sulci and gyri. The cerebral cortex is about 1.5 - 4mm thick, and contains nerve cells, fibers, neuroglia and blood vessels. The cerebral hemisphere is supplied by cerebral branches of polygon of Willis i.e. the anterior, middle and posterior cerebral arteries (Kandel *et al.,*2000).

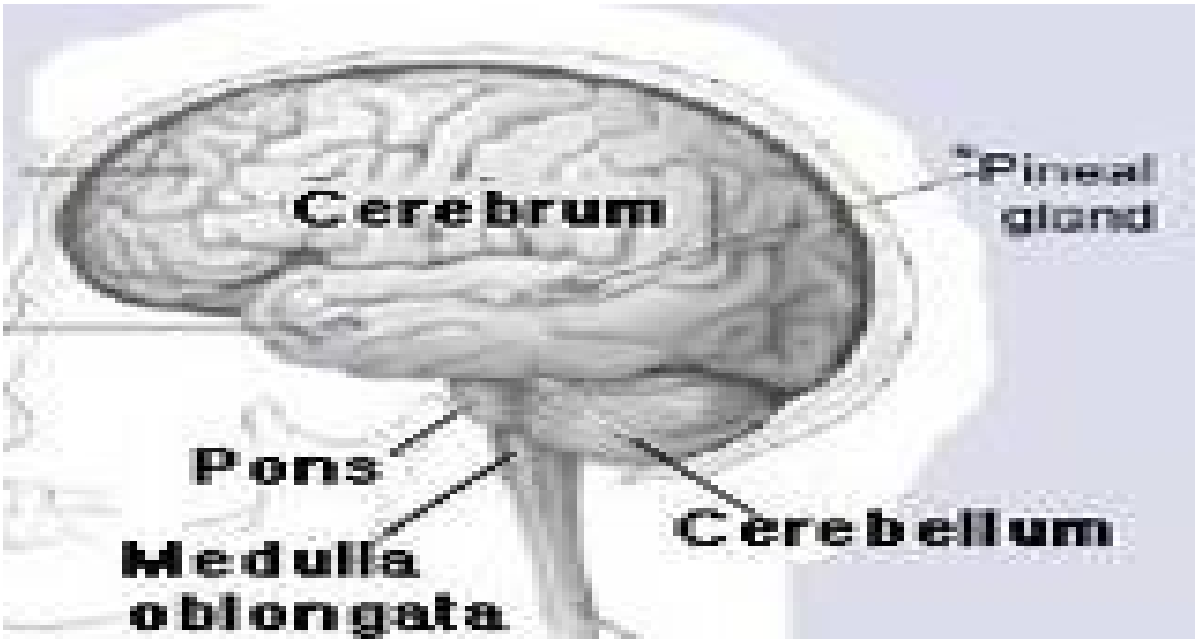


Figure 2.5. The structure of the brain showing the cerebrum (Kandel *et al.,* 2000)

2.8.2 Cellular Layers of the Cerebral Cortex

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

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

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

shown in Figure 2.7, according to Kandel *et al*. (2000); Shipp (2007) and Saladin (2010).

Layer I: the Molecular layer, which contains few scattered neurons and consists mainly of extensions of apical dendritic tufts of pyramidal neurons and horizontally-oriented axons, as well as glial cells. Some Cajal-Retzius and spiny stellate cells can be found in the molecular layer. Inputs to the apical tufts are thought to be crucial for the ‘‘feedback’’ interactions in the cerebral cortex involved in associative learning and attention (Saladin, 2010). While it was once thought that the input to layer I came from the cortex itself, it is now realized that layer I across the cerebral cortex mantle receives substantial input from ‘‘matrix’’ or M-type thalamus cells in contrast to ‘‘core’’ or C-type that go to layer IV.

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

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

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

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

Layer VI, the Polymorphic or Multiform layer contains few large pyramidal neurons and many small spindle-like pyramidal and multiform neurons. Layer VI sends efferent fibers to the thalamus, establishing a very precise reciprocal interconnection between the cortex and the thalamus. These connections are both excitatory and inhibitory. Neurons send excitatory fibers to neurons in the thalamus and also from collateral to other ones via the thalamic reticular nucleus that inhibit these thalamus neurons or ones adjacent to them. Since the inhibitory output is reduced by cholinergic input to the cerebral cortex, this provides the brainstem with adjustable "gain control for the relay of lemnsical inputs



Figure 2.6 The cellular layers of cerebral cortex (kandel *et al.*, 2000).

Variations of cortical structure

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

I. In the agranular cortex, the external and internal granular Laminae are inconspicuous. This type of cortex is refered to as precentral gyrus (area 4) and is therefore to be of typical area.

II. In the granular cortex, the granular cortex is highly developed while the pyramidal and ganglion layer are poorly developed or absent. This type of cortex is mostly present in sensory area including the post-central gyrus.

2.8.3 Functions Associated With Cerebrum

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

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

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

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

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Wistar rats, Mercury chloride, Bouin's fluid, Beakers, Specimen bottles, Weighing balance, Nikon digital camera with 12.0 megapixels, 1ml and 5ml syringes and injection needle, Dissecting tray. Animal feed, Chloroform and dissecting kit.

3.2 EXPERIMENTAL DESIGNED

Twenty four (24) adult Wistar rats of both sexes were obtained from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The weights of the animals were between 190 – 230g and were housed in polyester cages with wire gauze covering. The animals were allowed to acclimatize for two (2) weeks in the animal house of the Department of Human Anatomy, Ahmadu Bello University, Zaria. Animals were fed with Grower's mesh brand of the Animal feed purchased from Mae-Syl Agrochemical Company at 22 Sokoto road, Samara, Zaria, Kaduna State, Nigeria.

The animal feed was prepared in pellet form to reduce spillages and the animals were fed 3 times daily while clean water was provided in plastic drinking bottle and animals were allowed to feed and drink *ad-bilitum.*

Animals were weighed and randomly divided into four (4) groups with six animals per group. And the administration of the mercury chloride lasted for twenty one (21) consecutive days, which was done orally using 1ml syringe with butterfly rubber that was put on the cap of the syringe to serve as mean of administration. Mercuric

chloride (May and Bakers limited, Dagenham England) with batch number XN202. was obtained from Steve Moore chemicals limited Samaru, Zaria. Kaduna State, Nigeria.

The LD_{50} of Mercury chloride was adopted from (Berlin *et al.,* 2007) and ATDRS, (2011) as 166mg/kg body weight. The Concentration of Mercury chloride used was determined using 30%, 15% and 7.5% of the standard LD_{50} per kg body weight according to the methods of (Berlin *et al.,* 2007).

3.3 EXPERIMENTAL PROCEDURE

Animals were divided into four (4) groups of six (6) per group (GI, GII, GIII and GIV). GI was used as the control group and as such received normal saline, while groups GII, GIII and GIV were administered with mercury chloride at 7.5%, 15% and 30% respectively of the LD_{50} per kg body weight as shown in Table 3.1.

Table 3.1 Animal Grouping, concentration and frequency of administration of mercury chloride

S/no	Groups	Concentration	Frequency of administration
1	I	Normal saline	Once daily
2	II	12.45 mg/kg (7.5%)	Once daily
3	III	24.9 mg/kg (15%)	Once daily
4	IV	49.8 mg/kg (30%)	Once daily

3.4 NEUROBEHAVIORAL STUDIES

3.4.1 Spatial Learning and Memory Test Using Morris Water Maze.

Morris water maze was used to test spatial learning and memory in the test animals according to the methods of Morris (1981), which was further modified by Charles *et al.,* (2006), Mark *et al.,* (2007) and Liu *et al*, (2011). According to this method, a platform was submerged beneath the surface of the maze pool; the animal task is to find the hidden platform. The animal starting point was changed from time to time so as to build a cohesive spatial representation of the pool in order to find the platform during training trials and the latency to find the platform location was recorded both during the training and the experimental periods as shown in Figure 3.1. Animals were placed in a circular pool of clear water which was partitioned into four quadrants. Each animal's starting point was in a random position and each animal swam from one quadrant to the other searching for an escape route. The time taken by each animal to locate the platform which served as the escape route was recorded as the latency period.

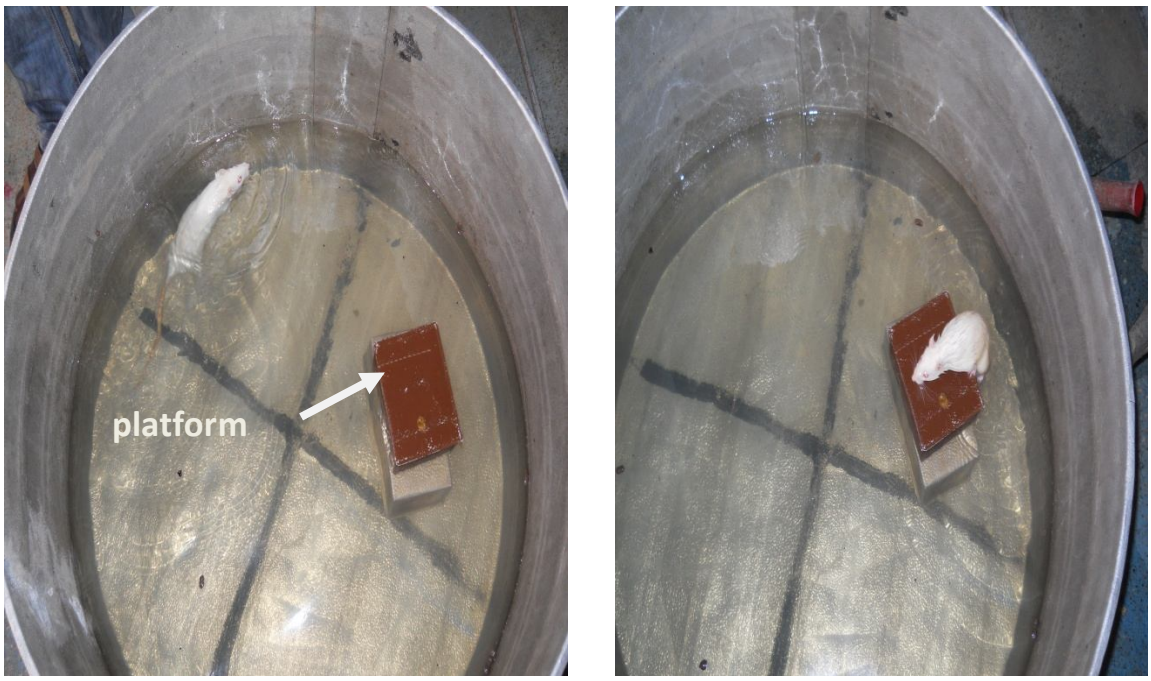


Figure 3.1 Morris water maze pool showing: (A) the platform and the rat looking for the platform (B) rat on the platform

3.4.2 Montoya Staircase Test Method

The Montoya staircase test method was used to study the locomotor function and skills in motor co-ordination. This method helps in determining the effects of a test substance on motor function abilities (Montoya *et al.,* 1990 and 1991). The tasks involved forepaw reaching for food pellets located at various steps along the staircase and the time taking to explore the activity cage (Abrous *et al.,* 1994; Whishaw *et al.,*, 1986 and 2000, Whishaw, and woodward,1997). The test was carried out as a measure of Motor coordination shown by muscle action in climbing and exploring the staircase and motor and locomotor function shown by the independent forelimb ability to reaching out and grasping food pellet in the staircase.

3.4.3 Training Time

At the outset of the test, animals were first trained to recognize that there are food pellets along the wells in Montoya staircase for three consecutive days. The animals were able to familiarize themselves with the Montoya staircase and then the animals were deprived of food for 18 hours in order to facilitate their search for food. During the test, each animal was placed on the foot of the staircase which will enable them to climb the stairs in order to reach out, grasp, or retrieve the food pellets and each test session lasted for 5 min, at the end of which the time taken to climb up and down from the basement was recorded and grasping abilities of each animal was also observed as shown in Figure 3.2.

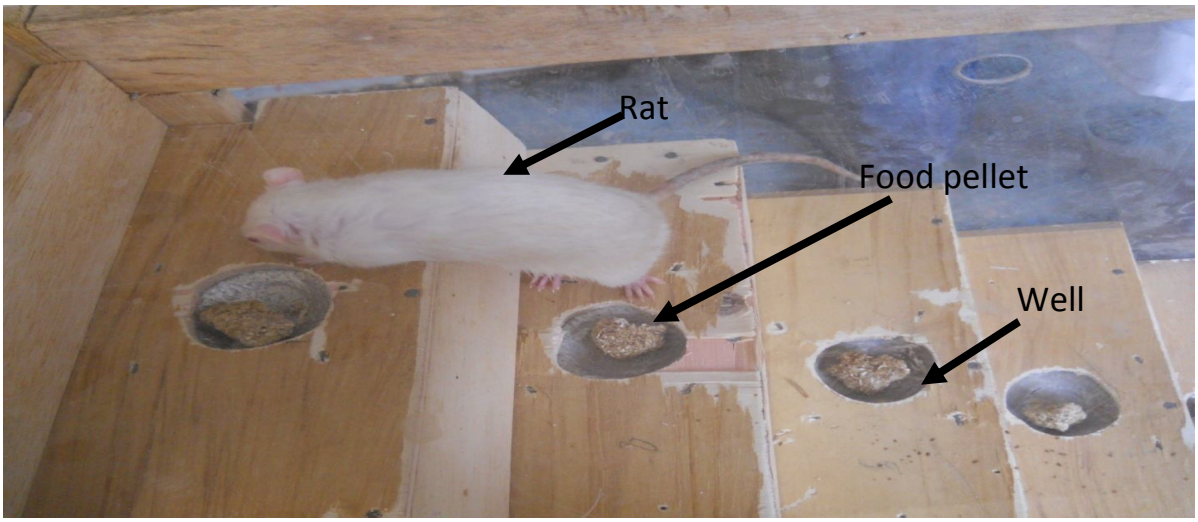


Figure 3.2. Montoya staircase test: Showing wells with food pellets inside and the animal trying to move-up the next step.

3.5 ANIMAL SACRIFICES

At the end of the 21 days of mercury chloride administration, the animals were anasthesize using chloroform and the brain tissues were removed by opening through the sutures of the skull using a brain opener obtained from the Department of Human Anatomy, Ahmadu Bello University, Zaria. The brain tissue were then transferred into specimen bottle, containing Bouin's fluid for fast fixation.

3.6 TISSUE PROCESSING

The tissues were fixed in Bouin's fluid for 48 hrs for proper fixation. The tissues were processed routinely and stained using Heamatoxyline and Eosin technique and special modified silver stain, Bielschowsky's methods.

3.6.1 H and E Staining Method

The methods of H and E staining technique was carried out by de-waxing the tissue in two changes of xylene for three (3) minutes each, hydrated by passing them through descending grades of alcohol (100%, 95%, 90%, and 70%) for three minutes each, and then stained in Harris haematoxyline for ten minutes, washed in tap water to remove excess stain. The slides were then flooded with acid alcohol for few seconds for differentiation and then washed in tap water again. The slides were then blued in Scott's tap water for five minutes and counter stained with Eosin for three minutes. The sections were rinsed in tap water, and then dehydrated in ascending grades of alcohol, cleared in xylene. Tissues were then mounted with cover slips using a

mounting media. Sections of the tissues were viewed under light microscope and photomicrographs were taken using digital Am scope (MD 900) photomicroscope.

3.6.2 Special Stain Using Bielschowsky Method.

The frozen tissue sections were immersed in distilled water and changed for 3 times over one hour and was treated with silver nitrate (AgNo3) solution for 4hrs in the dark at room temperature. The tissue sections were rinsed briefly in distilled water and treated with ammonia silver nitrate solution for 5 minutes until brown color appeared. The tissues were washed briefly in distilled water and treated with 4% formalin in tap water for 10minutes. The tissues were washed in water and toned with 0.2% gold chloride, washed and then treated with 5% hypo for 2 minutes each. The tissues were washed and mounted on a glass slide and blotted dry. Digital photomicrographs at the magnification of X250 were made from all the experimental groups with the aid of MD900 Am scope microscope digital camera.

3.7 NEUTRON ACTIVATION ANALYSIS NAA-1 METHOD

Neutron activation analysis (NAA-1) was used for the quantitative multi-element analysis which stands at the forefront of techniques for the quantitative multi-element analysis of major, minor, trace and rare elements. NAA-1 begins with neutron bombardment of a sample to convert stable isotopes to radioactive isotopes (IAEA, 2004). With sequential instrumental neutron activation analysis it is possible to measure quantitatively about 60 elements in small samples (5 to 100 mg). Element

sensitivities vary from 10^{-3} to 10^{-10} grams per gram of sample (IAEA, 2004; Jonah and sadiq (2006) Jonah and Sadiq (2008).

3.7.1 Preparation of Tissues for Organic Trace Element Analysis Method

Trace element analysis of the brain tissue was carried out with the aid of nuclear research reactor (NNIR-1) at the center for energy research and training Ahmadu Bello University, Zaria, Kaduna, Nigeria.

The brain tissue samples were dried at 100^{o}C using Memmert DINI ovum-280 model-KA. After successfully drying the tissues, they were crushed in Agate mortar and piston at zero contaminant level in order to have the tissues in powdered form which were then put in sealed polyethene bag and weighed using Metter AE 240 electronic digital balance to prepare tissue for irradiation.

The NNIR-1 has two methods of irradiation; the short-lived and long lived irradiation methods. The brain tissue samples were labeled with letter 'B' which is used to denote biological matrix or sample. The samples labelled B with even numbers e.g B2, B4, B6 and B8 stand for long-lived irradiation while those with odd numbers for example B1, B3, B5 and B7 stand for short lived irradiation. Each sample was irradiated for 5 minutes for short lived irradiation and exposed to a semi-conductor detector for characteristic gamma-rays detection for 10 minutes followed by another 10 minutes for 3 hours after the first count, while long lived irradiation lasted for 6 hours with a cooling time of 3 days, this is to allow for safe handling of the radio nuclei formed. (Akaho and Nyako, 2002).

The samples were both counted for all long –lived irradiation using Maestro software attached to an Analog to Digital Converter (ADC) card and Multi-channel Analyzer card (MCA). First count for the long –lived elements were done 3 days after irradiation for 30 minutes, while the count was repeated for 1 hours after 7 days, this is because some elements with shorter half life can decay away so that those with longer half-life will become prominent**.** The Trace elements present in the tissue were analyzed using Win span software.

3.8 STATISTICAL ANALYSIS

Data obtained was expressed as Mean ± SEM (Standard error of mean). One Way Analysis of Variance (ANOVA) was used to compare the Means difference between and within the groups and a P-Value less than 0.05 was considered to be statistically significant. Statistical analysis was performed using EZanalyze v3.0 and a post hoc test of Bonferroni was applied. Chart were produced using Microsoft$^{(R)}$ Excel 2007 for windows.

CHAPTER FOUR

4.0 RESULTS

4.1 PHYSICAL OBSERVATION

No visible physical change was noticed few hours after administration normal saline water to the control group, the animals were very active. Scratching of the nose was observed in animals of group II that received 12.45mg/kg of mercury chloride, after administration, the animals were more active. In group III that received 24.90 mg/kg orally, serious scratching of the nose was observed among animals of the group after administration. About five hours after administration, there was reduction in physical activities, mucus secretion from nostril, rising of body hair with frequent urination and watery feces. While in the high dose, group IV (that received 48.9mg/kg of mercury chloride) watery stool, deep yellow urination, gnawing, exopthalmu, restlessness, decreased physical activity, rising of body hair, less food consumption and mucus secretion were observed.

4.2 BODY WEIGHT OF THE ANIMALS

The results show non-significant increase in weight of animals in control group (Group 1), while there was non –significant decrease in weight of the experimental groups (II, III, IV) as shown in table 4.1

Table 4.1 Effect of oral administration of mercury chloride on body Weight of animals

		Weight (g)	Weight (g)	Weight (g)	Weight (g)	
Group	**n**	**1 Day**	**7 Days**	**14 Days**	**21 Days**	**% weight change**
I	**6**	200.00±4.50	211.68±4.77	215.68±4.55	220.20±4.90	**+23.73**
II	**6**	201.67±4.01	200.33±3.10	197.33 ±3.10	193.00 ±2.45	**-7.14**
III	**6**	220.00±6.86	215.00±5.63	200.33±4.22	191.00±3.16	**-19.90**
IV	**6**	200.00±4.58	196.68±3.33	190.50±2.50	180.00±1.16	**-17.40**

Data presented as Mean ±SEM

n=Number of rats in a group=6

4.3: PHYSICAL OBSERVATIONS OF ANIMALS IN MONTOYA STAIRCASE

The results of physical observation of animals in control group (Group I) showed that the animals were able to use their forepaw in displacing the food pellets along the staircase, grasped some of the food pellets. The animals were able to reach the topmost of the staircase of 6 steps within five minutes. The animals were also observed to have fine movements in Group I while Group II animals that were given with 12.45mg/kg of mercury chloride were able to displace few food pellets and no tremor was observed and the animals were able to move maximum distance and was able to reach the top of the stair at step number 6. There was no loss of balance in animals of group II. In group III animals, there was serious tremor, poor movement and stability and the maximum distance reached was step 4 out of 6 with no attempts to move further. Group IV animals that received the high concentration of mercury chloride of 49.8mg/kg, it was observed that animals falls back when trying to move-up to the next step, with maximum distance reached was at step 3 of 6, serious tremor was observed with poor movement and stability and no further attempt to move up for about five minutes, no food pellet was displaced.

4.4: TRACE ELEMENT ANALYSIS WITH NEUTRON ACTIVATION ANALYSIS-1 (NAA-1)

The results of trace element analysis using Neutron Activation Analysis (NAA-1) method showed the presence of copper and zinc in the brain samples. Figure 4.1 showed that there was decrease in the concentration of copper in group IV animals

while groups II and III animals did not show any decrease in this trace element. The concentration of Zinc in the experimental groups II, III and IV showed a decrease in the concentration of zinc particularly in group IV animals.

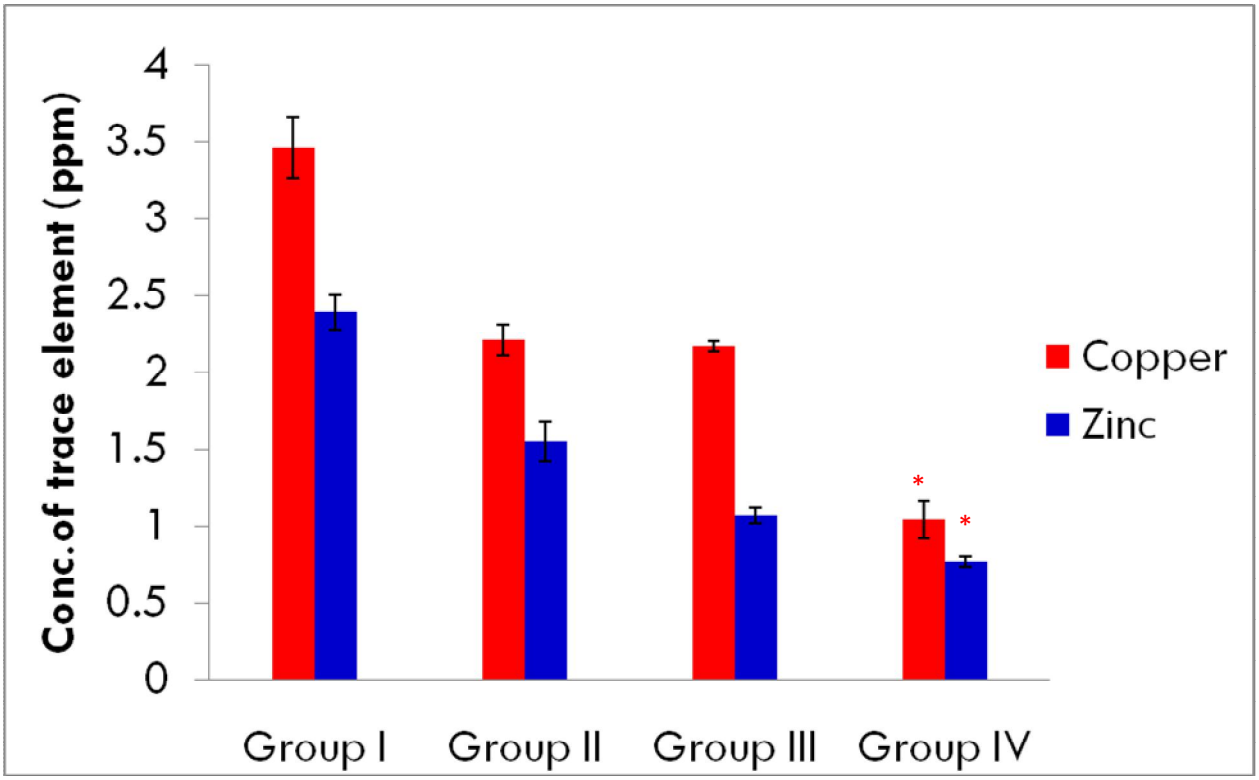


Figure 4.1: Show the concentration of trace elements in the Brain tissues of adult Wistar rats

* Symbol indicates significant difference at P≤ 0.05 compared to the Group I (control)

4.5 MOTOR ACTIVITY TEST USING MONTOYA STAIRCASE TEST

The results of motor activity test using Montoya staircase method are shown in Table 4.2. The results showed that there was a non-significant decrease in the mean time taken for the animals to reach the top of the six stairs throughout the 3 weeks of administration. While the animals in group II that received the minimum concentration (12.45mg/kg b.w) of mercury chloride had the mean time taken to reach the stairs increased ($P \geq 0.05$) between the 1^{st} and 2^{nd} week of administration. The increase in the mean time taken was statistically significant ($P \leq 0.05$) between the 1^{st} and 3^{rd} week, and between the 2^{nd} and 3^{rd} week, respectively as shown in Table 4.2. The results showed that the time taken for the animals to reach the top of the stairs was significantly increased in both groups III and IV animals. There was significant increase in time taken by the animals between week 1 and 2, between week 1 and 3 and between week 2 and 3, respectively in group III and IV animals.

Table 4.2: The mean time taken to explore Montoya staircase used for testing motor activity

		Group I (n)	Group II (n)	Group III (n)	Group IV (n)
Days	n	**Mean** ±SEM(s)	**Mean** ±SEM(s)	**Mean** ±SEM(s)	**Mean** ±SEM(s)
7 days	6	18.67±2.03	23.67±4.70	52.67±2.60	57.53±4.26*
14 days	6	20.33±4.50	24.67±7.51	55.33±5.84 *	83.67±9.03**
21 days	6	13.00±1.73	36.00±3.06*	76.33 ±6.60**	132.00 ±4.93***

* P ≤ 0.05; **P≤0.01; ***P ≤0.001; indicates significant difference compared to the Group I (control)

n=number of animals per group=6

s= mean time in seconds

4.6: THE EFFECT OF MERCURY CHLORIDE EXPOSURE ON MORRIS WATER MAZE TEST

The results of the effect of mercury chloride exposure on spatial learning and memory using Morris water maze test, showed that there was a decrease in the mean time taken for the animals to complete Morris water maze task in the control group though the decrease was not significant as shown in Table 4.3. The results show that animals in group II had an increased time in Morris water maze activity though the increase between week 1 and week 2 was not significant but significant increase was observed between week 1 and week 3. The result showed a significant increase in time in Morris water activity between week 1 and 2, between week 1 and 3 and between week 2 and 3 respectively in both groups III and IV as shown in Table 4.3.

Table 4.3: Effect of mercury chloride ingestion on spatial learning and memory using Morris water maze test

		Group I	Group II	Group III	Group IV
Days	n	**Mean** ±SEM(s)	**Mean** ±SEM(s)	**Mean** ±SEM(s)	**Mean** ±SEM(s)
7 days	6	12.43±2.71	17.53±1.77	20.70±2.20	37.30±1.75*
14 days	6	11.81±1.75	21.74±2.41	24.40±6.20*	41.71±3.60**
21 days	6	10.53±2.34	26.44±3.41*	36.00±5.32*	52.00±4.24**

*P≤0.05; **P≤0.01; indicates significant difference compared to the Group I (control)
n=number of animals per group=6;
s= mean time in seconds.

4.7: HISTOLOGICAL CHANGES

The results from histological observations showed histological changes in the tissues studied. The results of the histological changes showed that animals in control group have normal histology of the cerebral cortex as shown in Plate 1, while animals in group II show some degenerated cortical areas with some normal pyramidal and Stellate cells in their cerebral cortex (Plate 2). The animals in group III, showed area of neuronal degeneration, with some normal Pyramidal cells and stellate cells as in Plate 3, while animals in group IV, showed area of cellular degeneration, with neuronal aggregation in some areas and cells appeared smaller and decreased in number than normal with nuclear distortion as shown in Plate 4.

The cerebellum of the animals control group (group I) showed cytoarchitecture of the Molecular, Granular and Purkinje cell layer appearing normal as shown in plate 5.While group II animals showed similar cellular architecture with cells and layers looking normal in plate 6. The histological observations for the group III animals as shown in plate 7 depicts normal molecular and granular layer but there was degeneration of the purkinje cells within the Purkinje cell layer. The same pattern of histological changes was observed in group IV for the purkinje cells with cells showing mark degeneration and reduction in number as shown in plate 8 and table 4.4 respectively.

The histological studies showed that animals in the control group I have normal appearance of the hippocampus with normal pyramidal cell layer with pyramidal cells as shown in Plate 9 while group II animals showed disorientation of the pyramidal cell layer with loss of pyramidal cells (Plate 10). While Plate 11, showed the hippocampus of

group III animals, with degeneration of the pyramidal cell layer, loss of some of the pyramidal cells and clumping of pyramidal cell nuclei. And group IV animals show disorientation of the pyramidal cell layer and degeneration of some pyramidal cells with the pyramidal cells appearing to be smaller than normal as in Plate 12.

Table 4.4: Cell Count for the Hippocampus, Cerebellar and Cerebral cortices of adults Wistar Rats

Groups	n	**Hippocampus** (pyramidal cell)	**Cerebellum** (purkinje cell)	**Cerebral Cortex** (pyramidal cell)	**Cerebral Cortex** (Glial cell)
Group I	6	21.00±3.07	12.00±2.71	14.00±1.77	10.00±1.34
Group II	6	16.00±2.34	8.00±1.75	10.00±1.35	10.00±2.30
Group III	6	9.00±1.74*	3.00±2.01**	4.00±2.08*	6.00±2.75*
Group IV	6	5.00±2.20**	1.00±1.19**	2.00±1.01**	1.00±1.89***

*P≤0.05; **P≤0.01; ***P≤0.001 indicates significant difference compared to the Group I (control)

Data presented as Mean ±SEM n=sample size

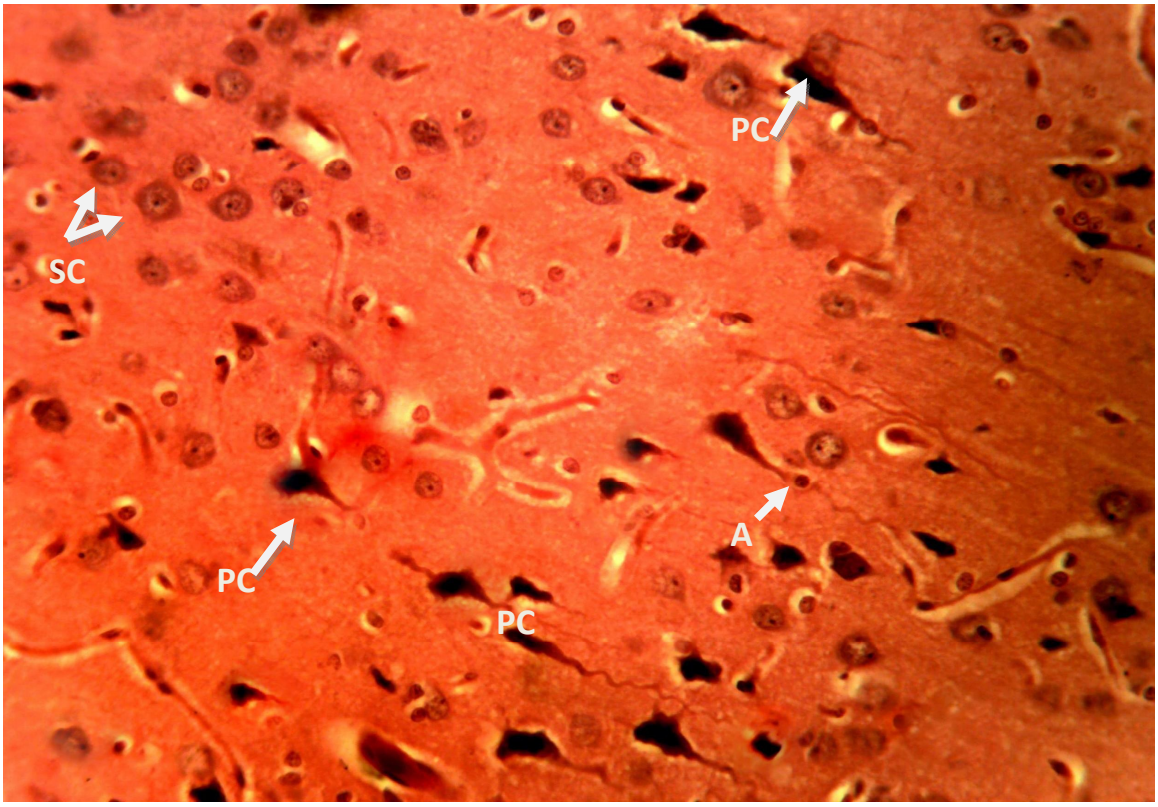


Plate 1: A section of the cerebral cortex of the control group (G1) showing normal architecture with Pyramidal cells (PC), Stellates cells (SC), Axon (A) of pyramidal cell (H and E X250).

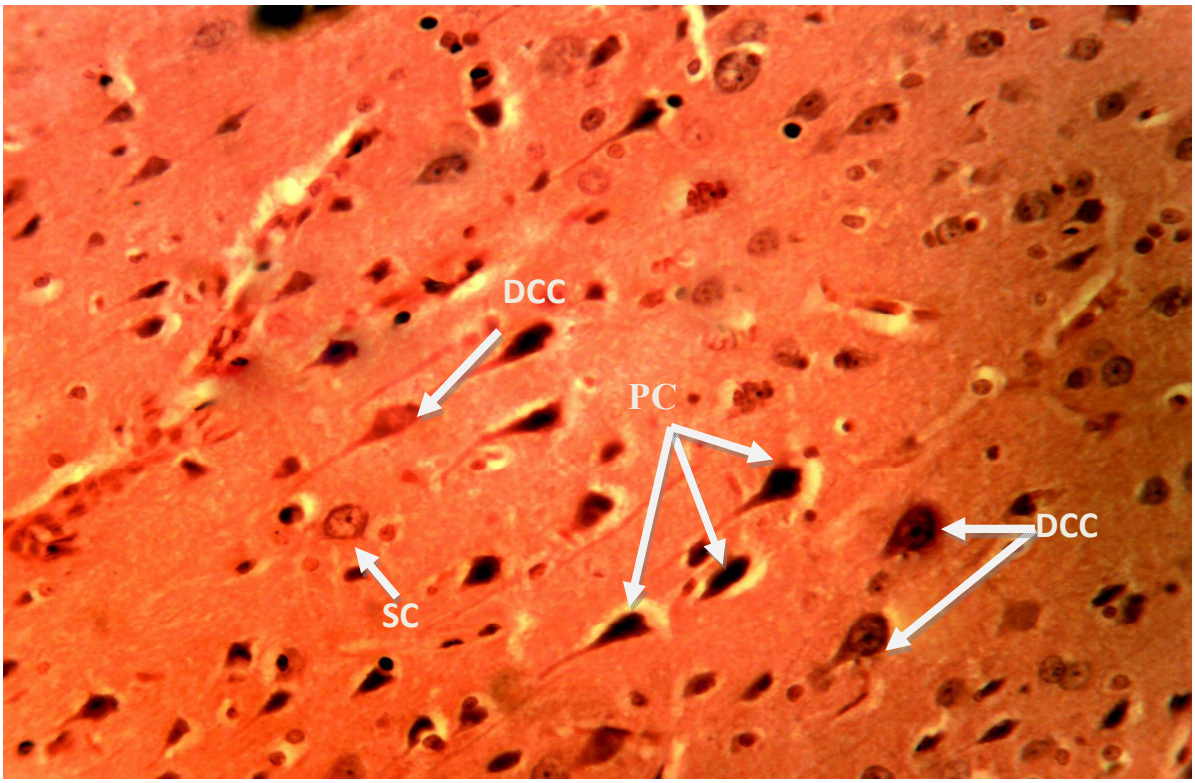


Plate 2: A section of the cerebral cortex of animals in group II showed some degenerating cortical cell (DCC) with some normal pyramidal cell (PC) and Stellates cells (SC) (H and E stain x250).

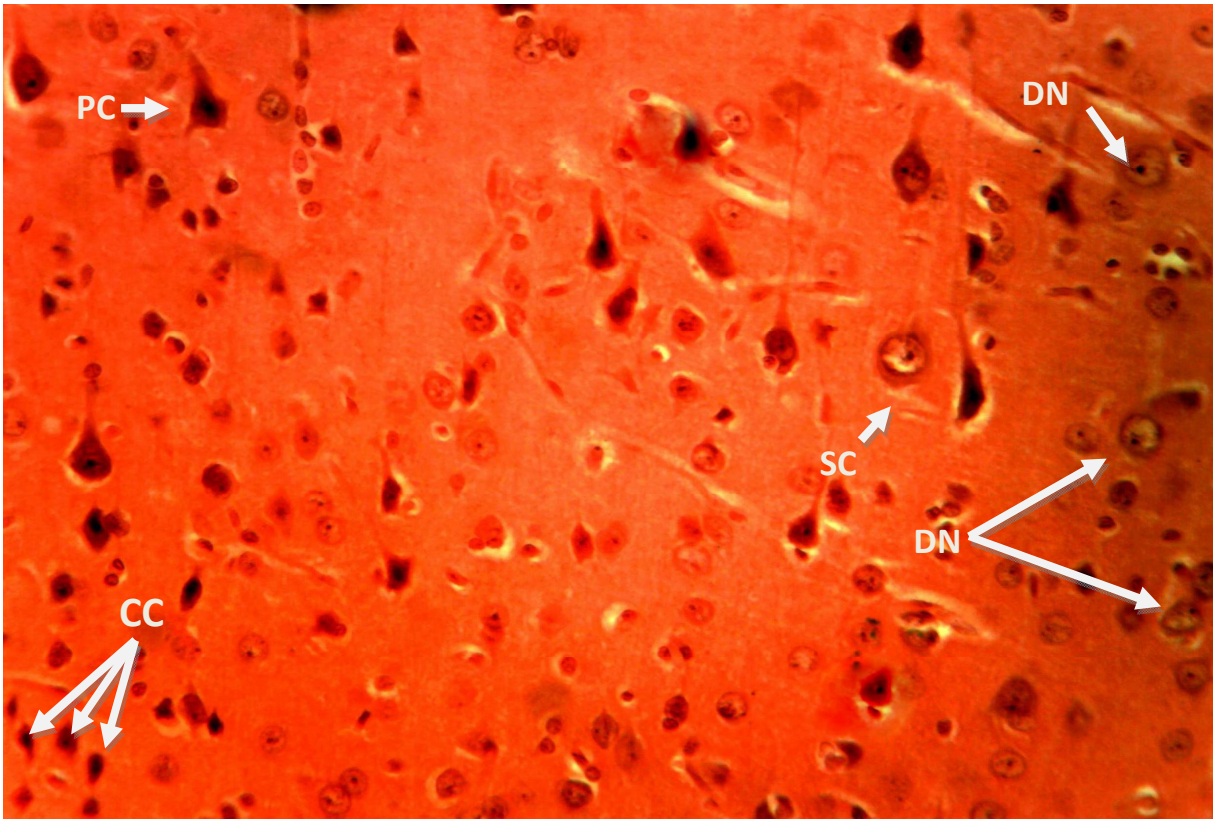


Plate 3: A section of the cerebral cortex of animals in group III, showing some clumping of cell (CC), degenerating neurons (DN), and some normal Pyramidal cells (PC) and stellates cells (SC) (H and E stainx250).

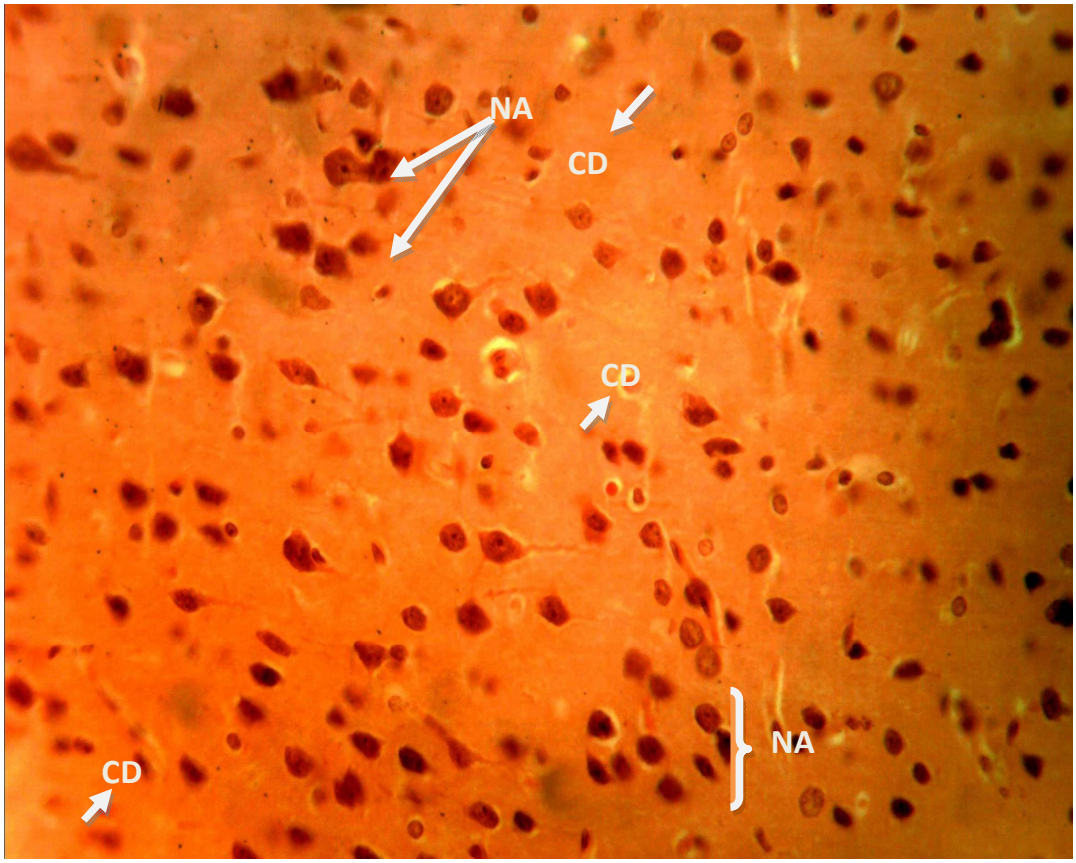


Plate 4: A section of the cerebral cortex of animals in group IV, showing areas of cellular degeneration (CD), with neuronal aggregation in some areas (NA) while cells appear smaller than normal with nuclear distortion (H and E Stain, x250)



Plate 5: 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&E Stain, ×250)

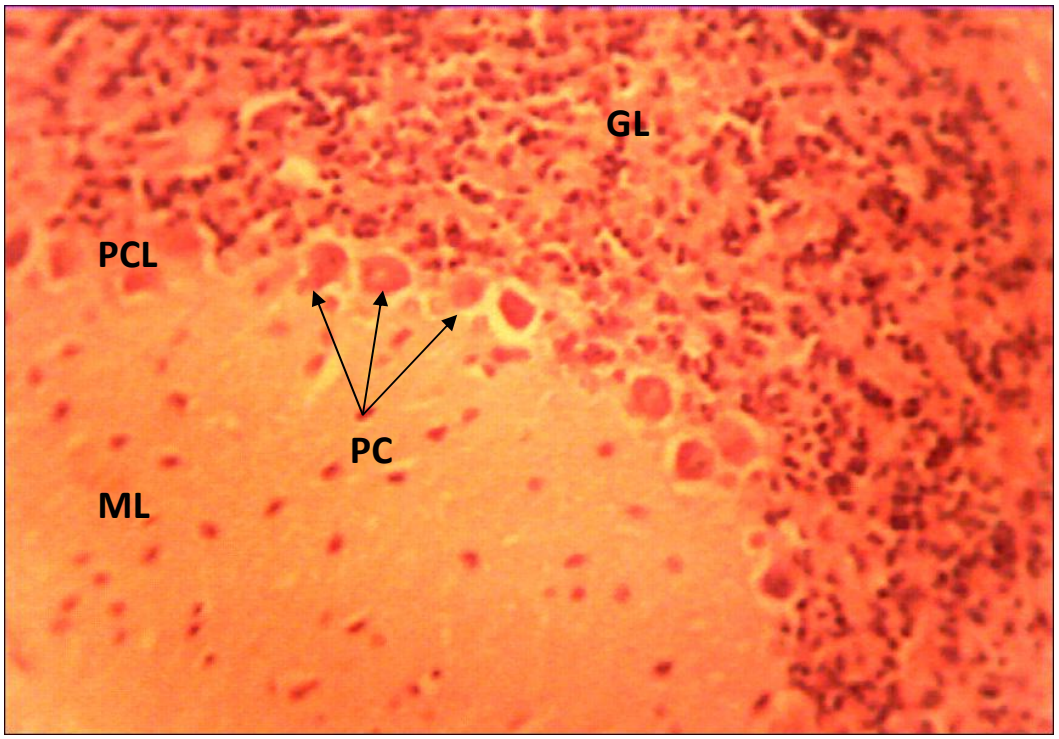


Plate 6; A section of the cerebellum of the group II showing normal orientation of the Molecular layer (ML), Granular layer (GL), and the Purkinje cell layer (PCL) with normal Purkinje cells (PC) (H&E Stain, ×250)

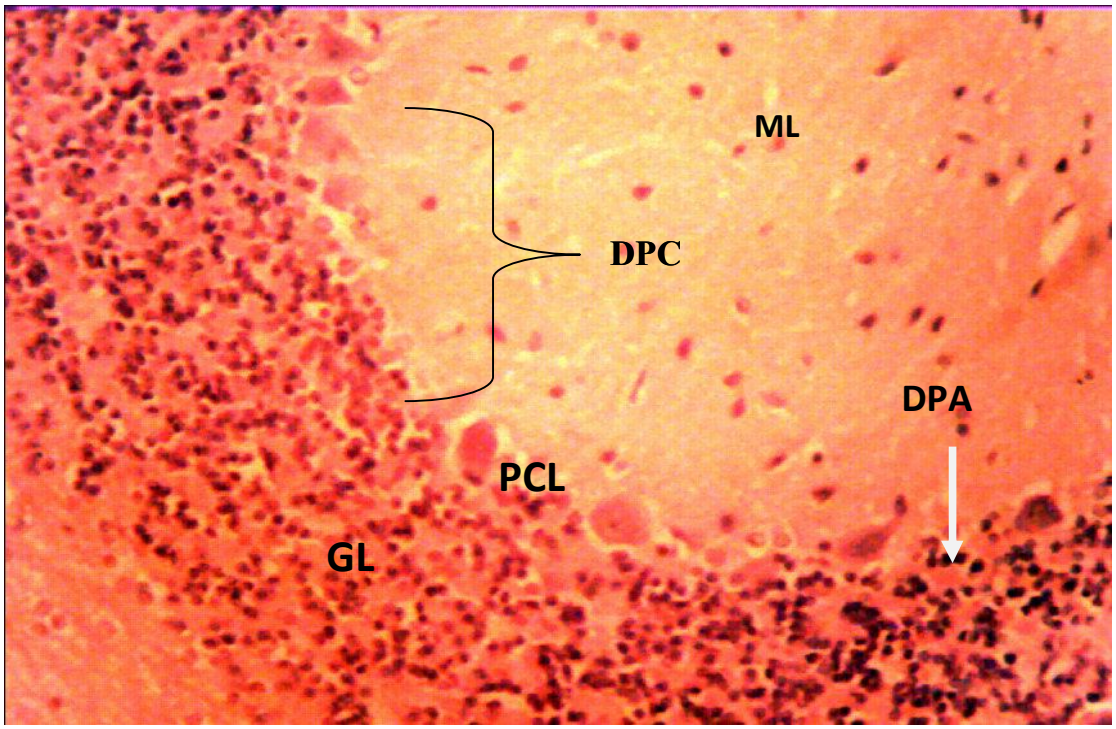


Plate 7 A section of the cerebellum of the Animals in group III showing the Molecular layer (ML), Purkinje cell layer (PCL) with degeneration of the Purkinje cells (DPC) and some densely populated areas in granular layer (DPA) in the Granular layer (GL) (H&E Stain,×250).

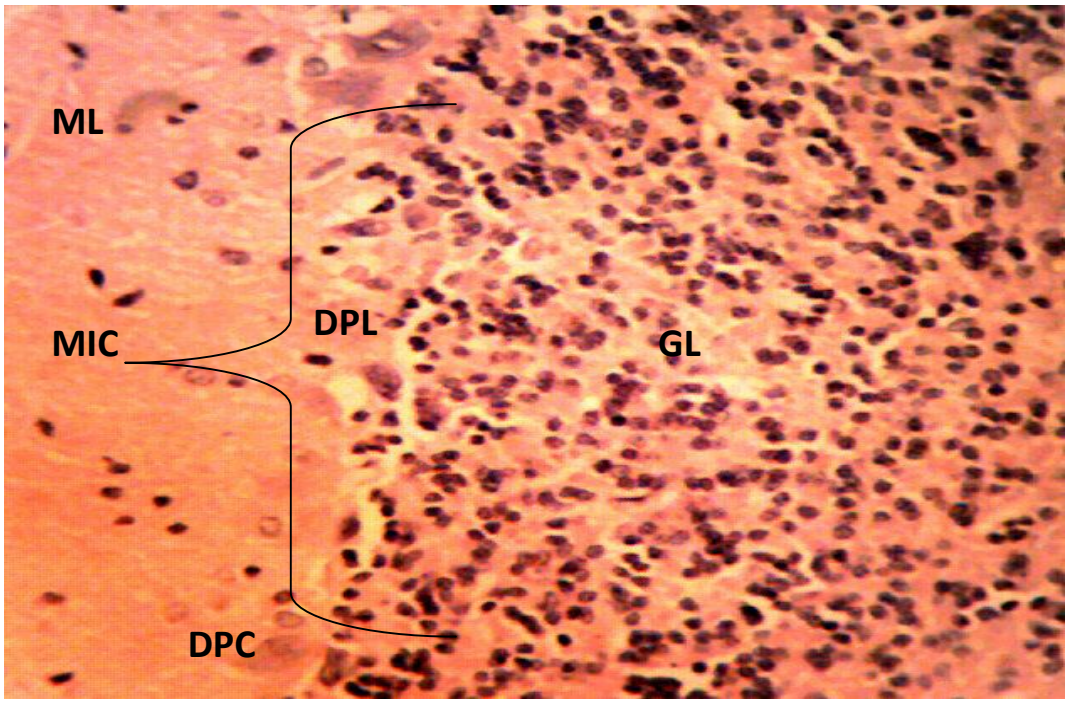


Plate 8: A section of the cerebellum of the animals in group IV showing the normal Molecular layer (ML), with degeneration of the Purkinje cell layer (DPL) and degeneration of the Purkinje cells (DPC) while the Granular layer (GL) showed massive infiltration of cells (MIC) (H&E Stain, ×250)

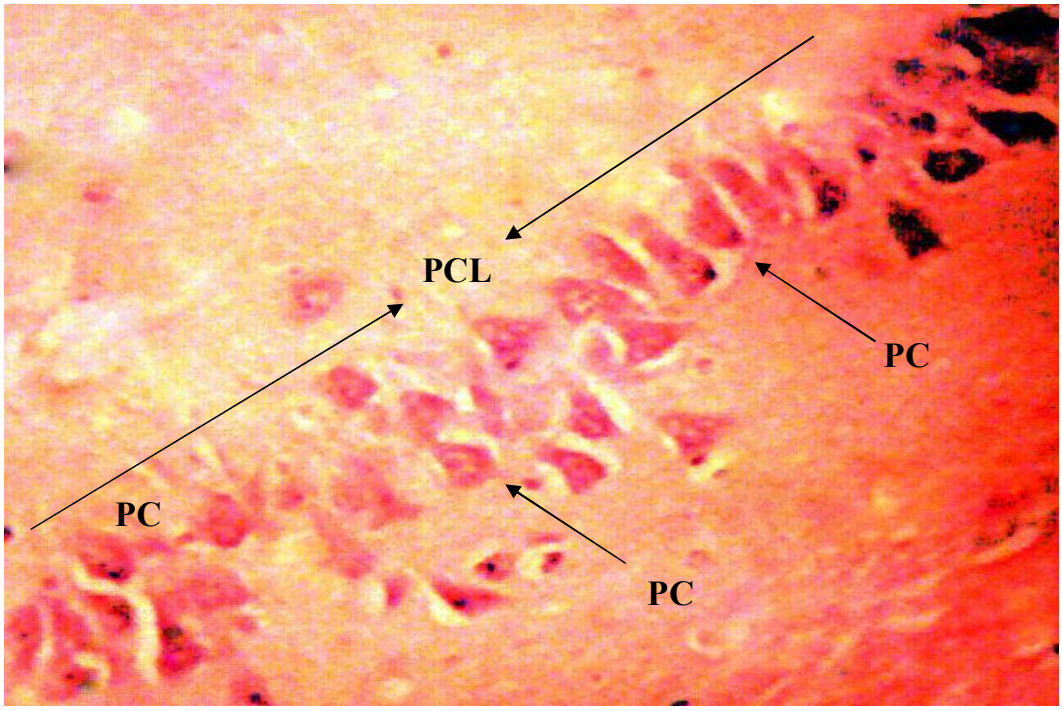


Plate 9: A section of the hippocampus of the control group (GI), showing normal pyramidal cell layer (PCL) with normal pyramidal cells (PC) (H&E Stain, ×250).

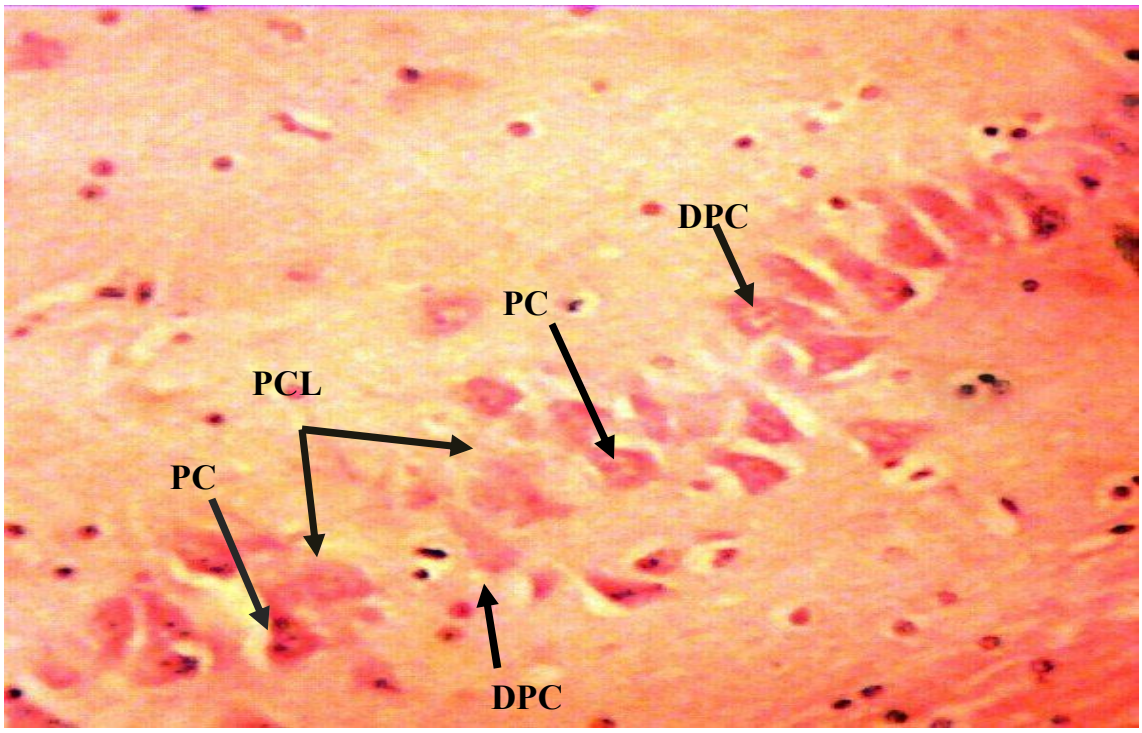


Plate 10: A section of the hippocampus of group II animals, showing disorientation of the pyramidal cell layer (PCL) with degenerating pyramidal cells (DPC) (H&E Stain, ×250)

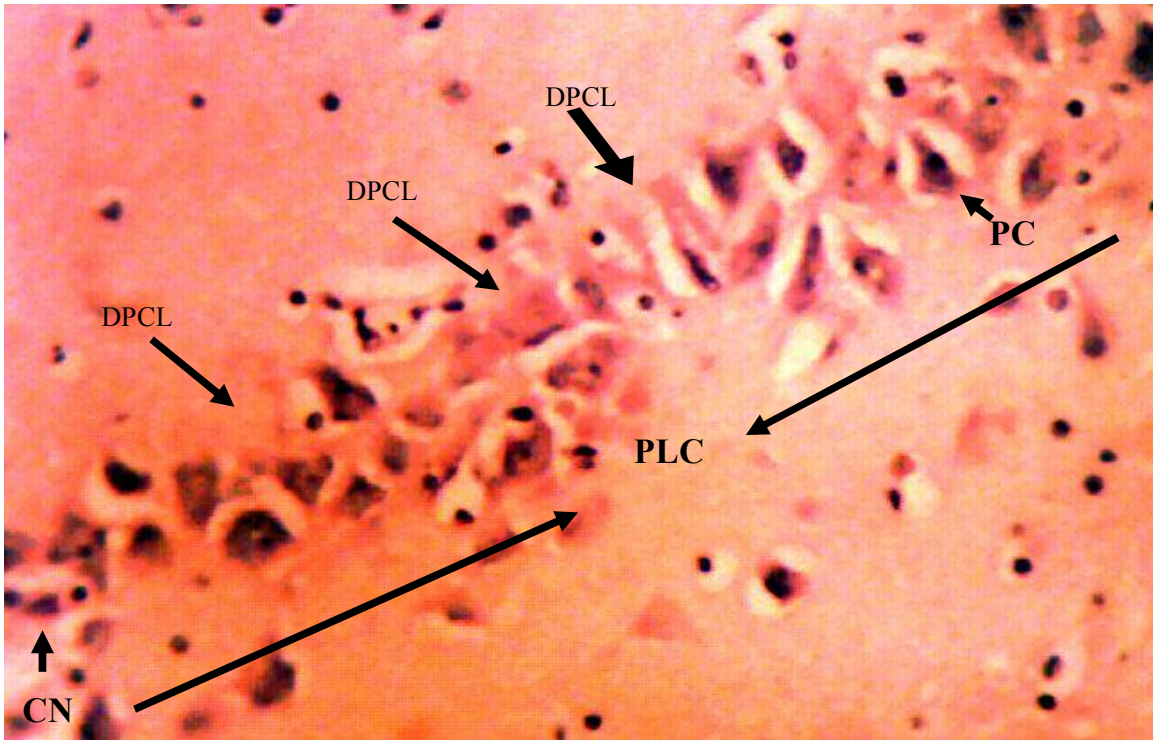


Plate 11: A section of the hippocampus of animals in group III, showing, some degeneration of pyramidal cell layer (DPCL) with loss of some pyramidal cells (PC), clumping of pyramidal cells nuclei (CN) (H&E Stain,×250).

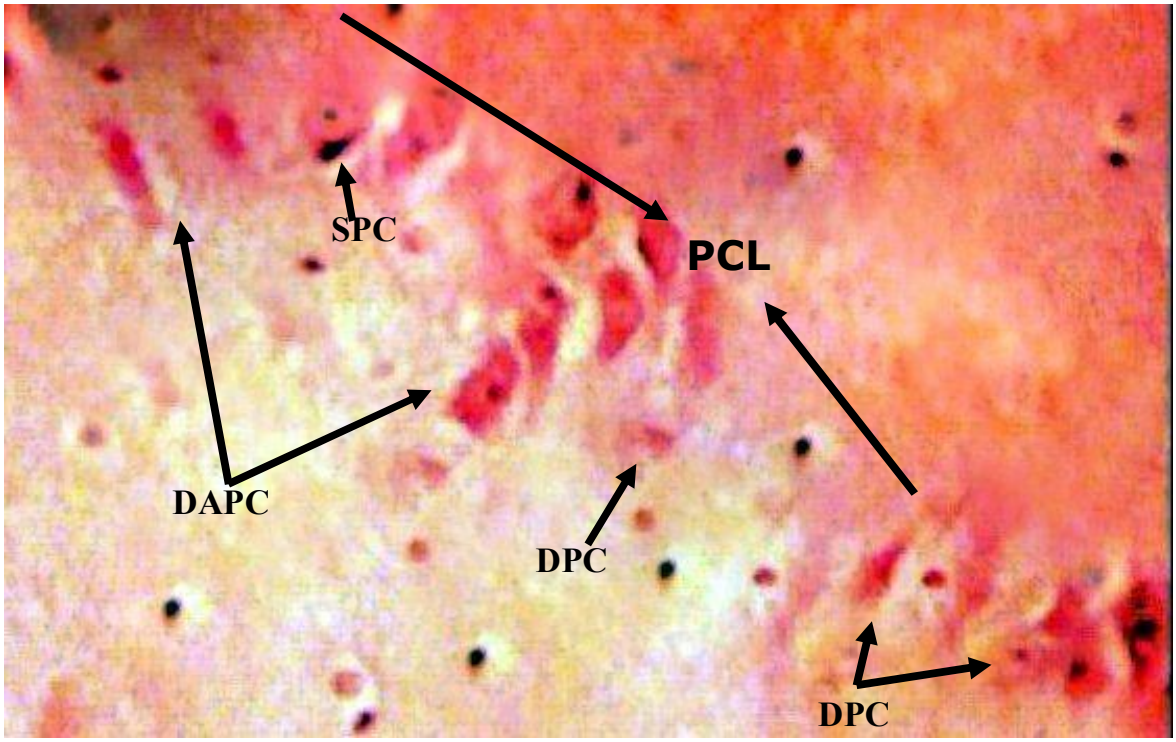


Plate 12: A section of the hippocampus of the animals in group IV, showing degenerated area of the pyramidal cell layer (DAPC), degenerating pyramidal cells (DPC).The pyramidal cells appear to be smaller than normal (SPC) (H&E Stain, ×250)

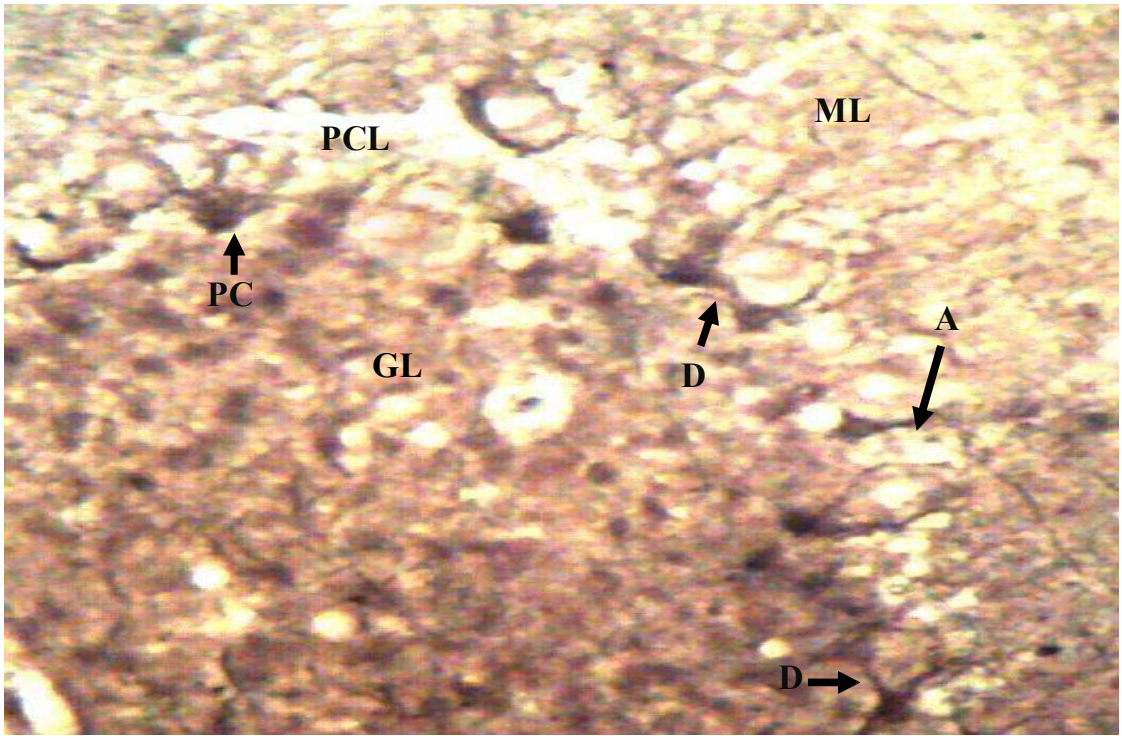


Plate 13: A section of the cerebellum of the control group showing normal molecular layer (ML), granular layer (GL) and Purkinje cell layer (PCL) with the purkinje cell body (PC) dendrite (D) axon (A) (Bielschowsky stain ×250).

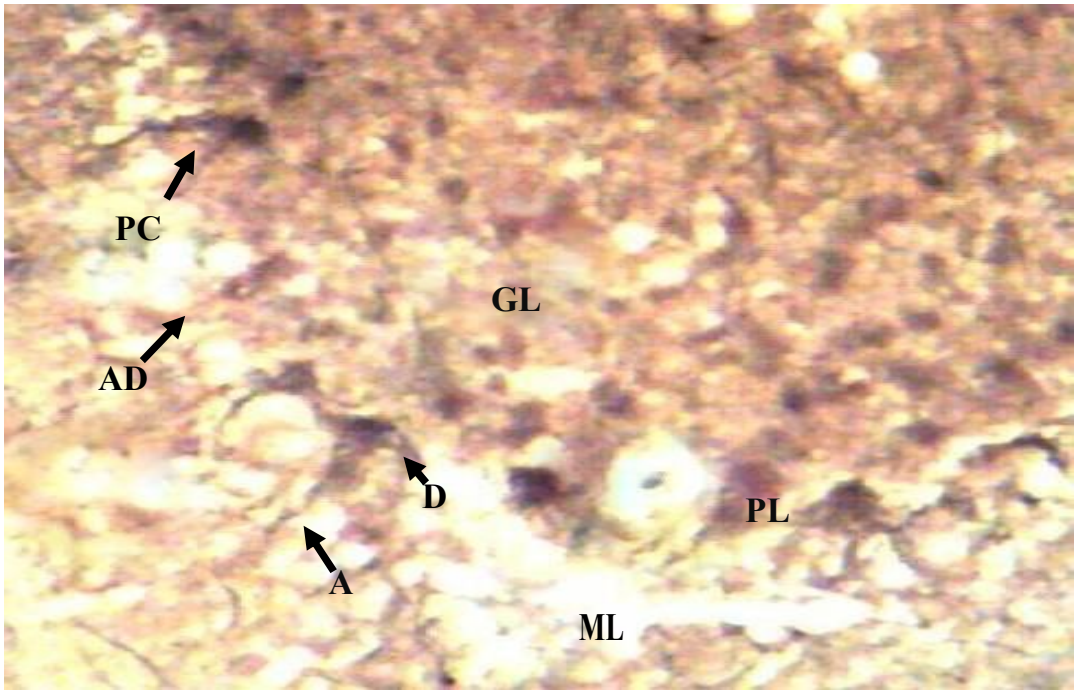


Plate 14: A section of the cerebellum of the animals in group II showing molecular layer (ML), granular layer (GL) and the purkinje layer (PL) with the Purkinje cell (PC), Dendrite (D), axon (A) and some areas of degeneration (AD) (Bielschowsky stain ×250)

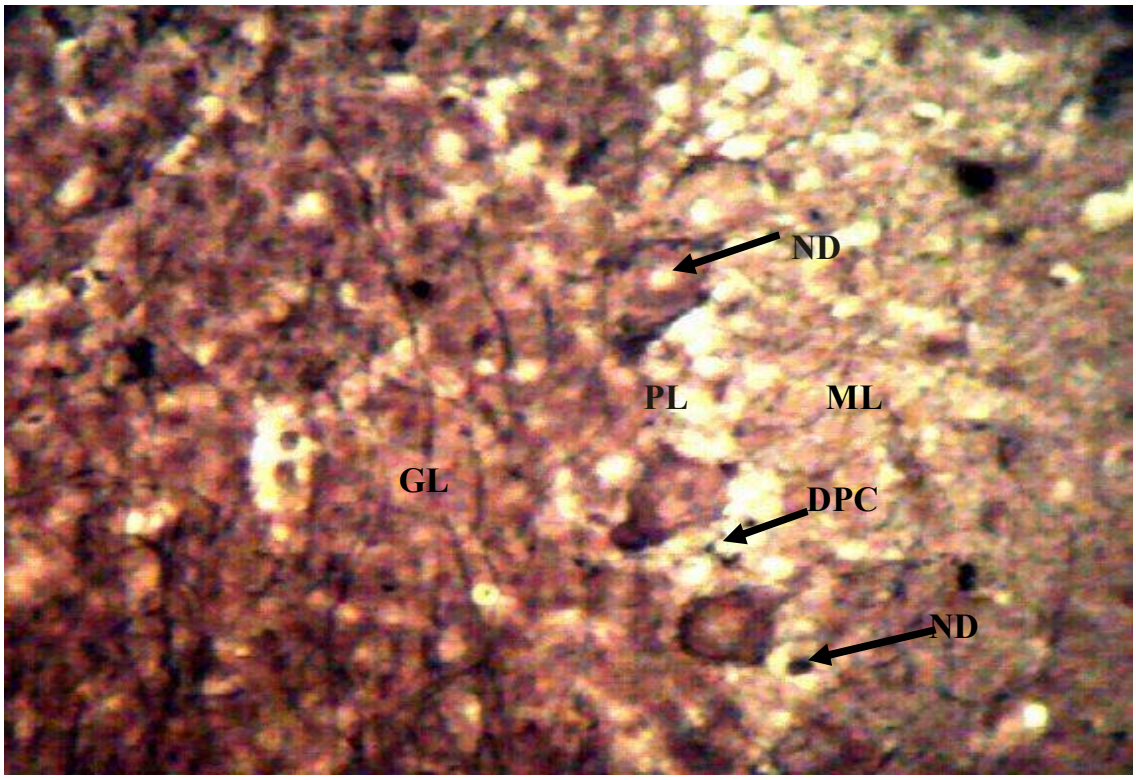


Plate 15: A section of the cerebellum of group III showing the molecular layer (ML), granular layer (GL) and the purkinje layer (PL) with some dead Purkinje cell (DPC) and nuclei displacement of Purkinje cell (ND) (Bielschowsky stain ×250).

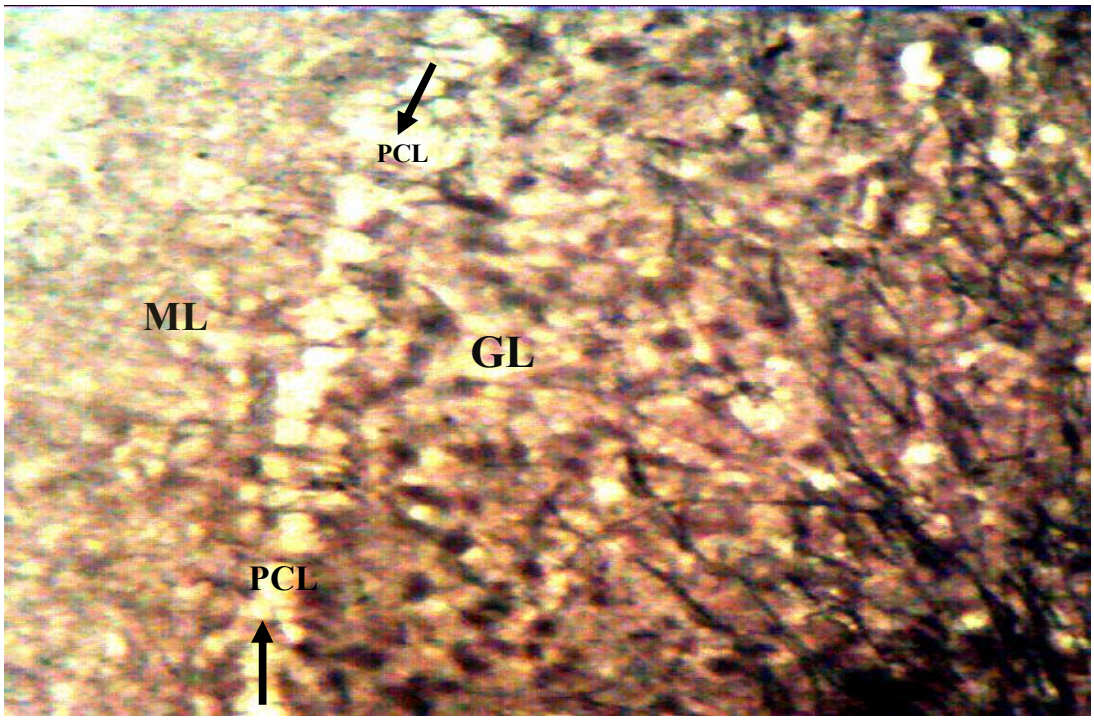


Plate16: A section of the cerebellum of the group IV showing molecular layer (ML), granular layer (GL) and Purkinje cell layer (PCL) with degenerated Purkinje cells (Bielschowsky stain ×250)

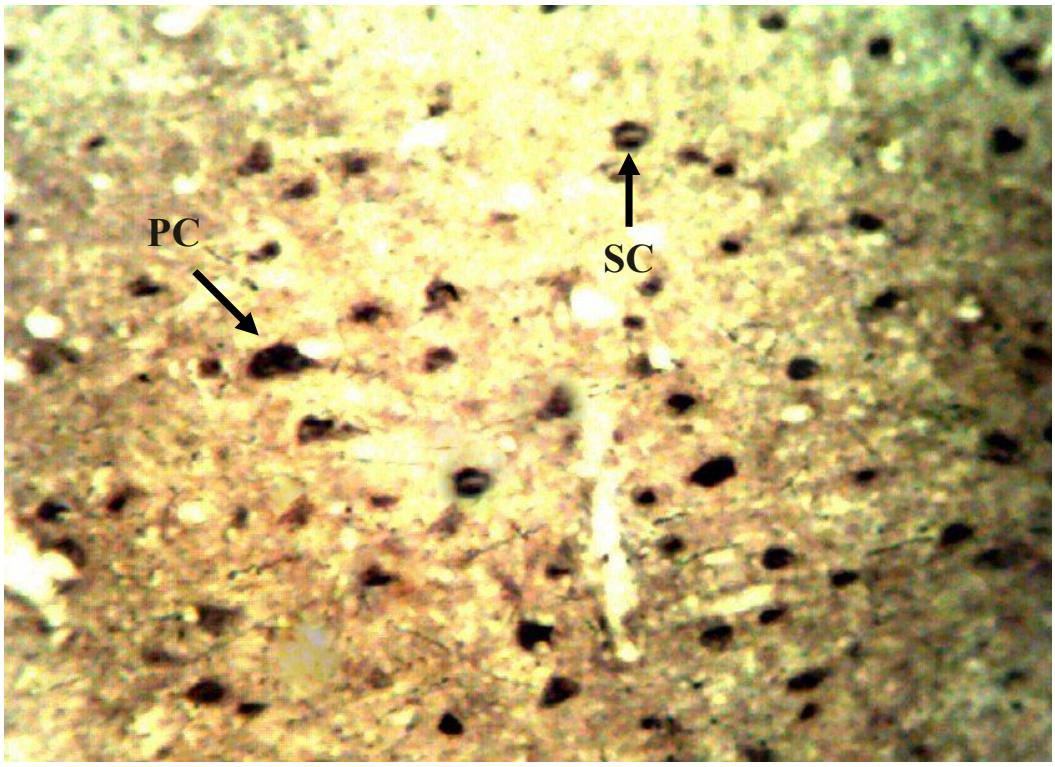


Plate17: A section of the cerebrum of the control group, showing normal appearance of pyramidal cells (PC) and stellates cell (SC) with dark cell bodies (Bielschowsky stain ×250).

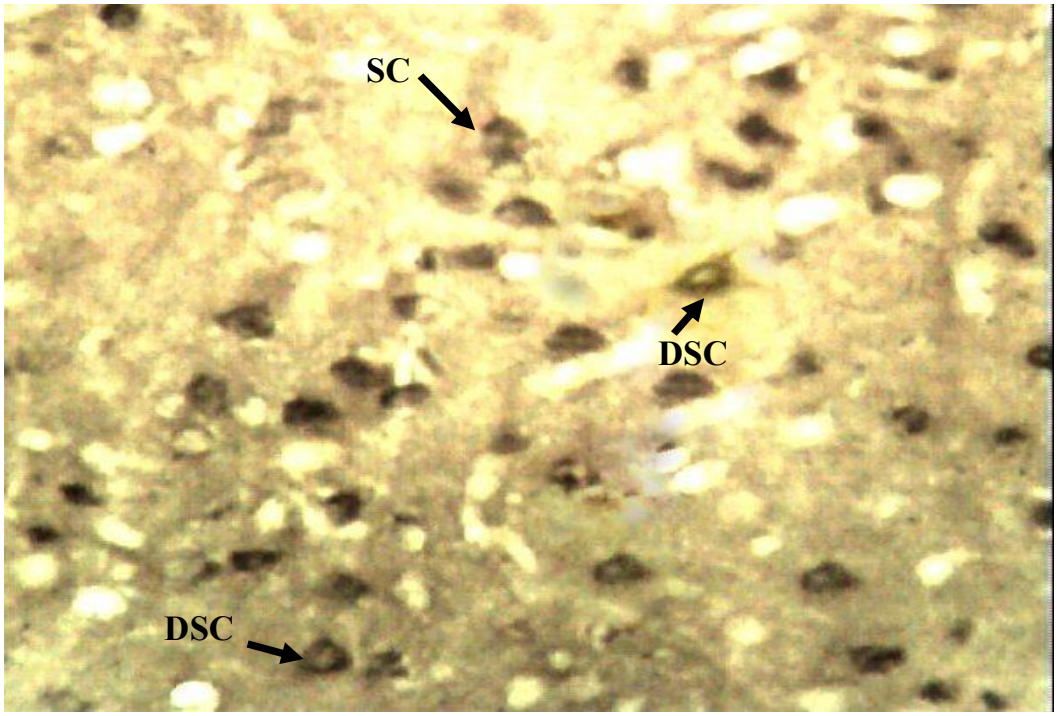


Plate18: A section of the cerebrum of animals in group II showing some normal stellate and some degenerated Stellate cells (DSC) (Bielschowsky stain ×250)

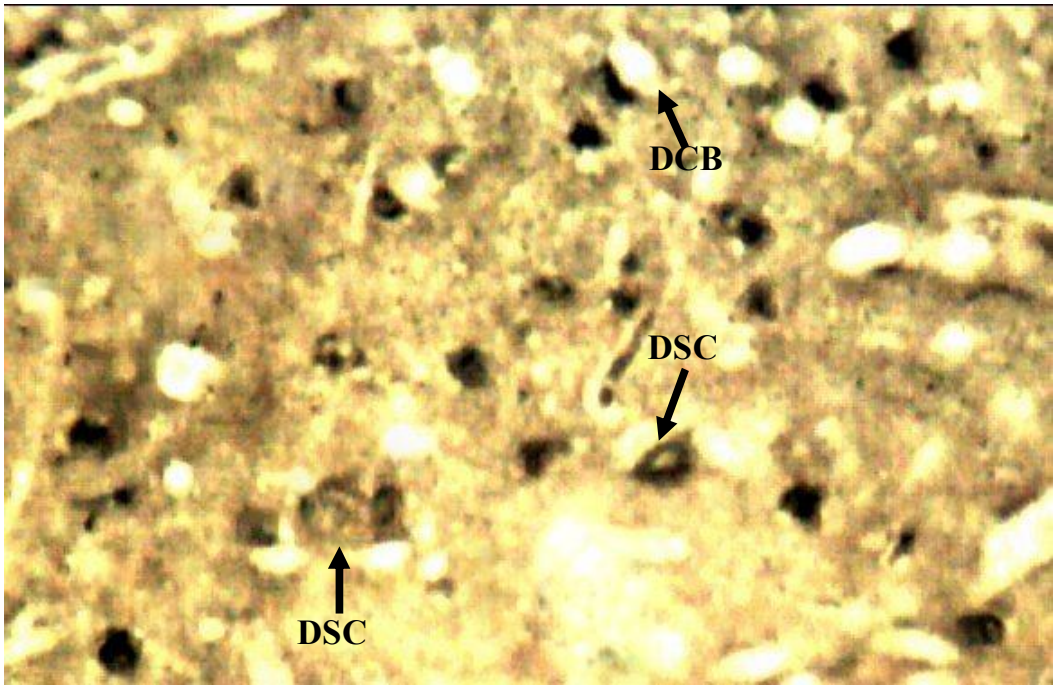


Plate 19: A section of the cerebrum of the animals in group III showing degenerated Stellate cells with vacuolated degenerated cell bodies (DCB) (Bielschowsky stain ×250).

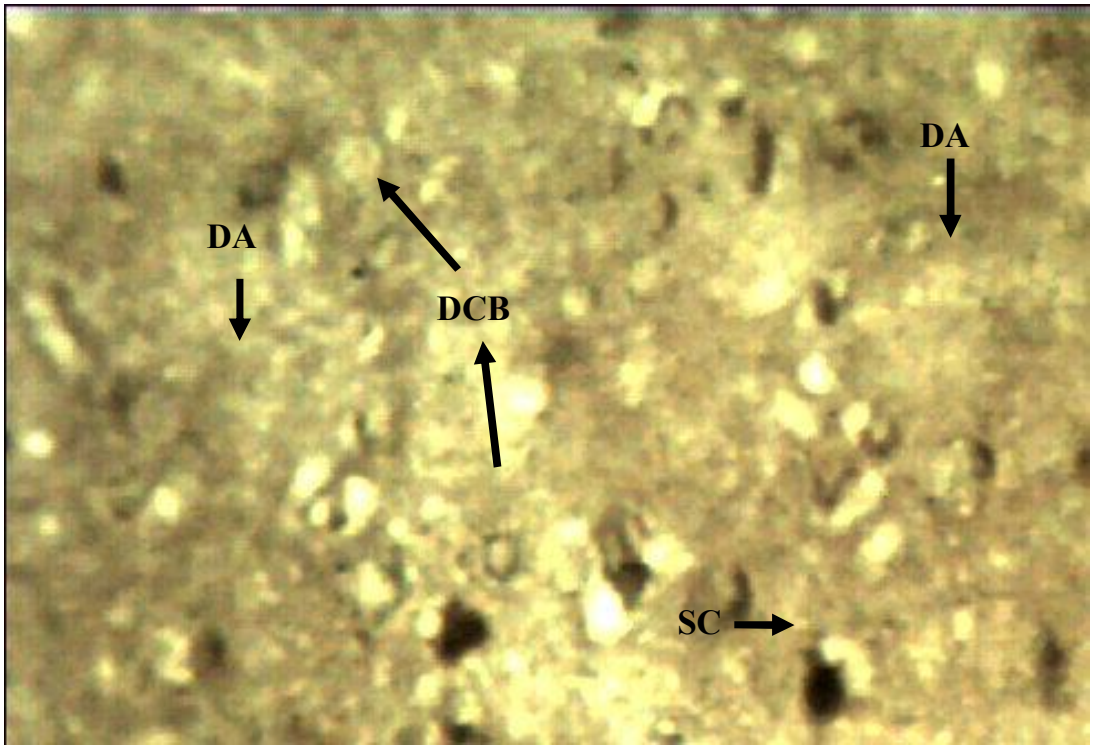


Plate 20: A section of the cerebrum of the animals in group IV showing Stellate cells (SC), some degenerated areas (DA) and vacuolated degenerated cell bodies (DCB) (Bielschowsky stain ×250)

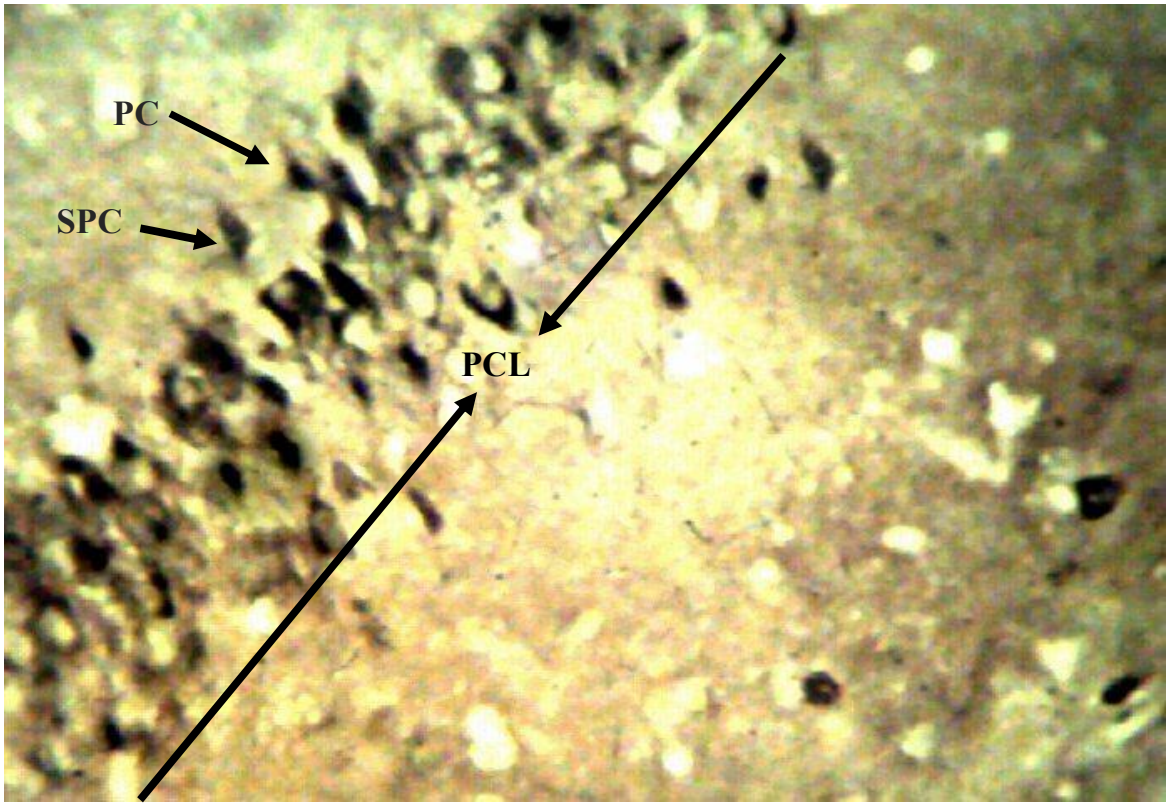


Plate 21: A section of the hippocampus of the control group (Group 1), showing pyramidal cell layer (PCL), with pyramidal cells and small pyramidal cells (SPC) (Bielschowsky stain ×250)

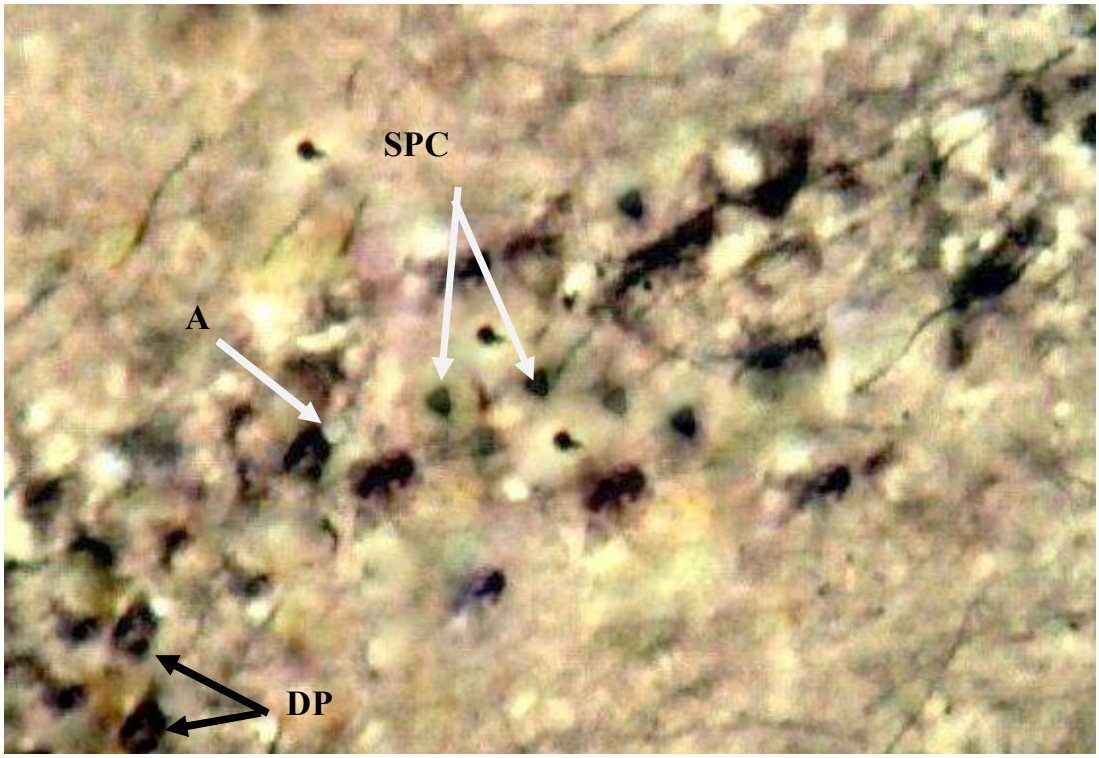


Plate 22: A section of the hippocampus of the animals in group II showing some degenerating pyramidal cells (DP), small pyramidal cell (SPC), Axon (A) and dendrite (D) (Bielschowsky stain ×250)

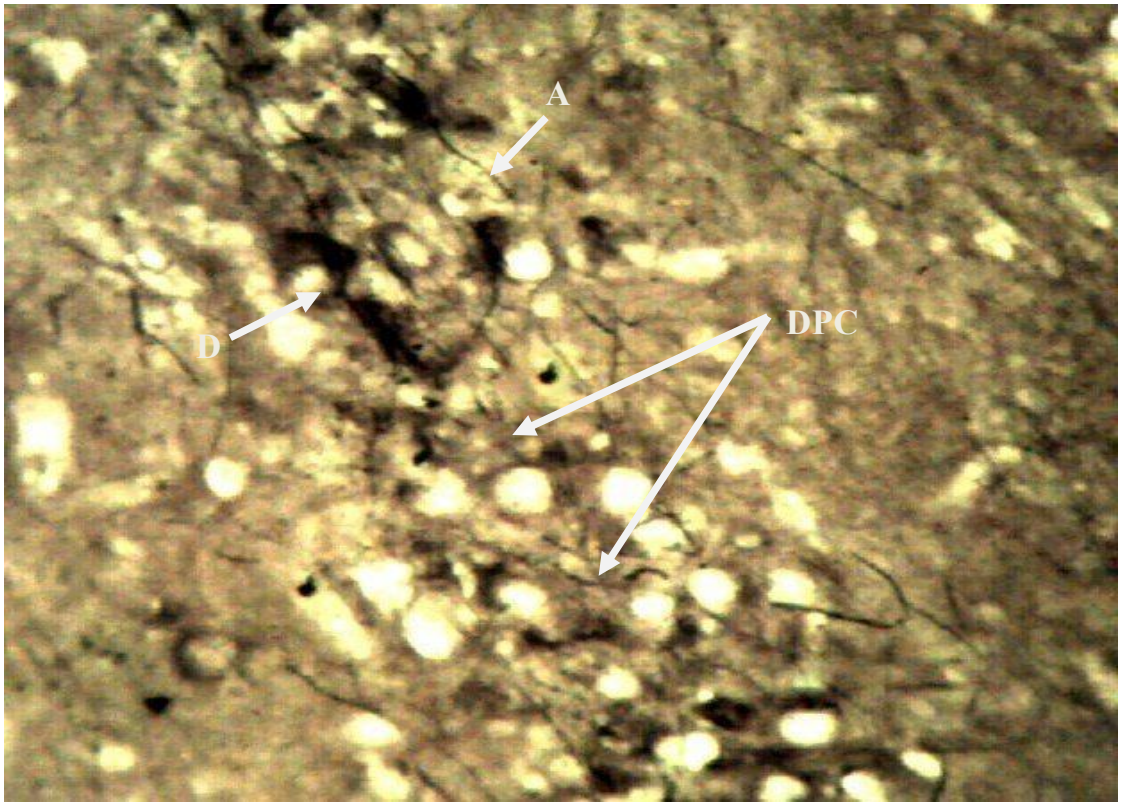


Plate 23; A section of the hippocampus of the animals in group III, showing degeneration of pyramidal cell layer (DPC), reduction of pyramidal cell bodies, and Dendrite (D) (Bielschowsky stain ×250)

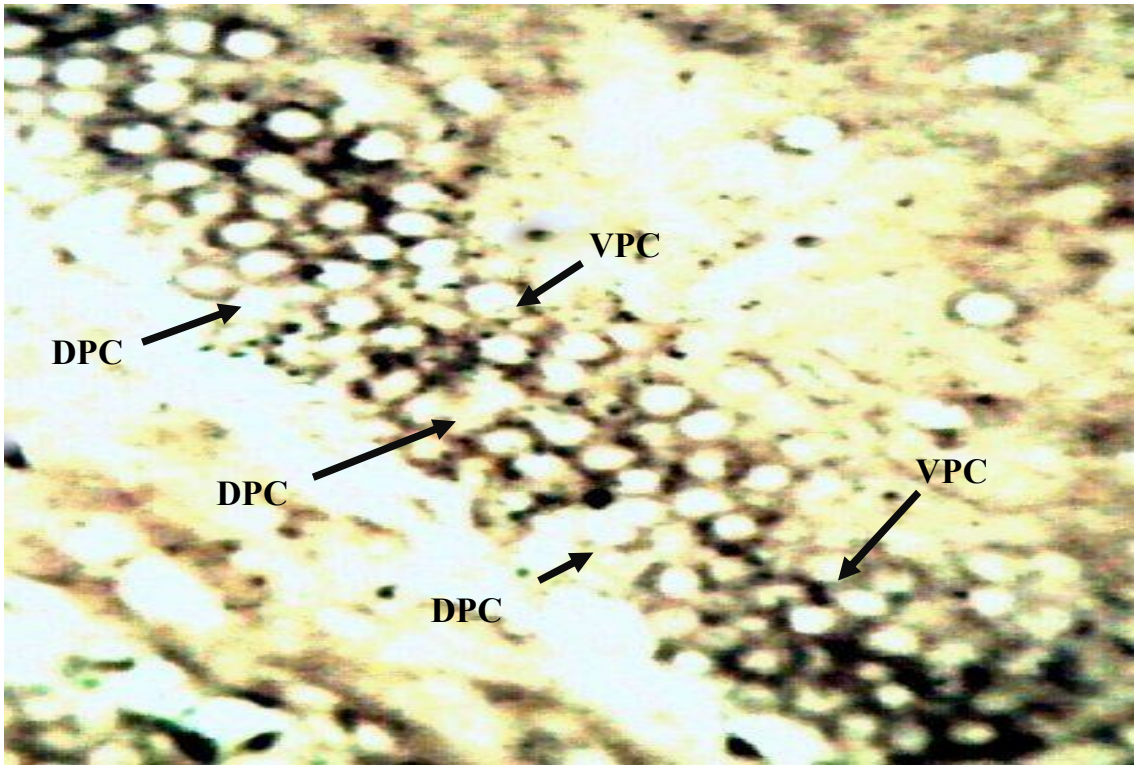


Plate 24: A section of the hippocampus of the animals in group IV, showing degenerated pyramidal cell layer (DPCL) with some vacuolated cells (VPC) and loss of neuronal fibers among the cell of pyramidal layer (Bielschowsky stain ×250).

CHAPTER FIVE

5.0 DISCUSSION

5.1 EFFECTS OF MERCURY ON BODY WEIGHT

The administration of Mercury chloride did not show any significant difference on the body weight throughout the period of administration even among the highest dose (48.9mg/kg) of mercury chloride but there was increase in mean body weight before the commencement of the administration at 0 (zero) week. During the onset of administration there was a decrease in the mean body weight across the weeks especially among the highest dose group though this decrease was not significant. This none significant change in the mean body weight could be attributed to the duration of the oral administration of mercury chloride which may be short to manifest any significant decrease in the body weight of the animals. This is closely related to findings of Hans, (2006) that showed that there was a decrease in relative body weight in rats fed diets containing mercuric chloride equivalent to 20 mg mercury/kg body weight per day for 4 weeks. In addition to the duration of administration, the form of mercury administered also play a role in causing changes in body weight (ATDRS, 2005). Exposure of rats, rabbits and dogs to metallic mercury vapor at concentrations of 0.1 mg/m3 for 7 h/day, 5 days/week for 72–83 weeks, resulted significantly in weight decrease (Thomas *et al.,* 2007).

5.2 TRACE ELEMENT ANALYSIS WITH NEUTRON ACTIVATION ANALYSIS-1 (NAA-1)

The investigation of trace elements present in the cerebellar and cerebral cortices of rats was done using Neutron activation analysis (NAA-1). The results show that there was a decrease in the concentration of Copper and Zinc in the brain of the experimental rats. The decrease in the level of copper and zinc could be attributed to the damage to the cerebellar and cerebral cortices caused by the mercury exposure to the animals.

The present studies have shown that mercury intoxication can reduce the concentration of copper with the lowest concentration of copper found in the group of animals that received the highest dose of mercury. The decrease in the level of copper concentration has been shown to interfere with the modulatory role of dopamine receptors in the brain which could in turn affect the functions of the dopamine neurotransmitter and can consequently lead to motor function deficits (Zacconi *et al.,* 2007). The decrease in the concentration of copper was observed in the cerebellum of animals treated with methyl mercury chloride for 72 week (Rasia *et al.,* 2005). Madsen and Giltmin *(*2007) had reported decreased levels of copper in patients with Parkinson's disease, in which there was alteration in dopamine levels. (Gagelli *et al.,* 2011) had also reported a low dopamine concentration associated with Parkinson's disease which was characterized by mild tremors and motor disorders. In the present study tremors and motor function disorder was observed in the experimental animals which can be related to the decrease in copper levels in the brain of the experimentally exposed rats with mercury chloride which supported the findings of Gagelli *et al.,* (2011).

The decrease in the concentration of zinc following oral administration of mercury chloride was observed particularly in group of animals administered with the

highest dose of mercury chloride. It has been shown that Zinc deficiency can alter the modulatory role of neurotransmitter glutamate from excitatory to inhibitory which is shown to be very crucial in learning and memory functions (Bhavnagar, *et al.,* 2010). Glutamate is the most abundant of the group of endogenous amino acids in the mammalian central nervous system which presumably functions as the excitatory neurotransmitters and under abnormal conditions may behave as neurotoxins. As neurotransmitters, these compounds are thought to play important roles in learning and memory. As neurotoxins, they are believed to be involved in the pathogenesis of a variety of neurodegenerative disorders in which cognition is impaired (Bhavnagar, 2010). The decrease in the levels of zinc has been shown to hinder the activity of hippocampal cells which are involved in memory formation, organization, storage and retrieval (Halas *et al.,* 2007). Zinc deficiency has been associated with numerous CNS diseases such as depression, cognitive impairment and Alzheimer's diseases and dementia (Halas *et al.,* 2007). The decreased levels of zinc have been shown to inhibit the levels of glutamate production in the brain and as such, there is a positive association between the levels of zinc and glutamate. This means that as level of zinc decreases, glutamate also decreases. This relationship between zinc and glutamate has been reported among Alzheimer disease patients and among workers exposed to mercury vapor for 20 years (Mutter *et al.,* 2010).

5.3 HISTOLOGICAL ANALYSIS

The general degenerative changes in the hippocampus, cerebellar and cerebral cortices was observed as degenerated cortical areas, necrotic cells and distortion of cellular layers of the hippocampus, cerebellar and cerebral cortices. The pyramidal cells in the hippocampus manifested some changes ranging from degeneration and reduction in number of pyramidal cells to the loss of neuronal cell fiber due to the reduced

number of cells when compared to the control group which could be as a result of the exposure of mercury chloride. 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. The findings in this study agree with the studies of Wolf *et al.*, (2009), who reported that rats exposed to high concentration of mercury vapor, showed neurodegenerative changes in the hippocampus which was responsible for memory deficit in such animals. While (Gagelli *et al.,* 2007) had shown that cell sizes and cell numbers were observed to be decreased in mice treated orally with inorganic mercury at high doses for a week. In the present study, the results show that cell sizes and cell numbers were observed to be decreased which agrees with the findings of (Gagelli, *et al.,* 2007). Onwureme and Oladimeji (2010) found out that mercury exposure causes histopathological changes in the hippocampus of *Oreochromis niloticus* exposed to waste products from Nigeria National Petroleum Company (NNPC) Kaduna refinery.

The cerebellum showed evidence of mercury chloride exposure by manifesting some histopathological changes and these changes include the destruction of the Purkinje cell layer, infiltration of cells in the granular cell layer and the loss of the architecture of the Purkinje cell layers. There was sparse distribution of the Purkinje cells of the Purkinje cell layer. These could result in interference with the motor activity and other motor functions such as loss of fine movement, lost 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. Moreover, it has been shown that neuronal degeneration in the cerebellum can affect the level of copper concentration in the cerebellum and this can affect the action of the neurotransmitter, Dopamine which is very crucial in motor activity. This is because copper serve as a modulator for the neurotransmitter, dopamine activity and if there is a decreased in copper level, dopamine activity will be reduced. (Mutter *et al.,* 2010) had reported decrease in dopamine concentration in the cerebellum of mice ingested with cadmium chloride for a week which resulted in motor function disorder. It has been shown that mercury intoxication does not show any significant changes in the granular layer of chicken treated with mercury chloride in their food (Wolf *et al.,* 2009; Quirino *et al.,*

2012). This is not in agreement with the findings of the present study which showed infiltration of granular cells of the granular layer into the molecular layer.

The cerebral cortex showed neuronal changes by clumping of the cortical cells and the loss of pyramidal and stellate cells. Neurodegenerative and histomorphological changes in the cerebral cortex can affect the functional areas of the cerebral cortex which deals with skilled movement, ability to speak, ability to appreciate pains and temperature. Musa and Sadeeq (2009) had reported that administration of mercury for eight days caused cell death in the cerebrum. Saladin, (2010) and Shipp (2007) revealed that exposure to mercury dust among workers in cement industry for a decade manifested with inability to speak which was associated with damage to the motor area of the cerebral cortex. The present study shows that there was loss of fine movement, loss of grasping and tremor as a result of mercury chloride administration.

5.4 NEUROBEHAVIORAL STUDY

The result from the present study show that, there was a significant increase in the time taken by the experimental rats to find the hidden platform in Morris water maze test, for memory and learning. This may be due to decrease in concentration of zinc that tends to control the population of the cells of the hippocampus. It could also be due to the decrease in the modulatory role of zinc on the neurotransmitter, Glutamate that plays a vital role in memory and learning. Zinc deficiency was associated to memory loss among Alzheimer's disease patients and also among workers exposed to mercury vapor for 20 years (Mutter *et al.,* 2010). Conversely, the pyramidal cell layer of the hippocampus appears to be damaged with dead cells, and vacuolated spaces and

distortion in the general morphology of the pyramidal cells that appear smaller than normal. These alterations can consequently result to memory impairments which could be as a result of neuronal degeneration, decrease in zinc concentration which could lead to decrease in glutamate neurotransmitter as observed from the present study. Also the role of hippocampus to detect or extract significant information for further memory consolidation and from repetitive activity that is already learned or remembered may be lost. The destruction of the 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). Mutter (2010) had reported that short term occupational exposure to high levels of mercury induced slight cognitive deficits. A memory deficit among animals exposed to methyl mercury was not significant in latency time or swim length between the different groups of animals according to Olson (2005). Mutter (2010) had conducted a research on cognitive test using Y-maze for memory and showed that mercury has no effect on memory which disagrees with the results of the present work.

The results in the present study showed a significant decrease in motor activity which was shown by the increase in the time taken to reach the maximum attainable distance in the Montoya staircase method. This could be attributed to low concentration of copper that modulates the activity of the neurotransmitter, dopamine which is important in motor function activity. The deficiency of copper can also be related to the degeneration of the cerebellar cortical neuronal cells. (Gagelli *et al.,* 2011) had reported an association between copper and dopamine concentration that was positive in patients with Parkinson's disease which was characterized by motor function disorders. The decrease in the level of dopamine was observed in patients with Parkinson's disease, in

which there was loss of smooth and controlled movements (Rasia, 2005; Madsen, 2007). Histomorphological changes in the cerebellum were observed which can in turn interfere with the motor ability of animals in the experimental group. It has been shown that ingestion of mercury of 220ml affected muscle coordination and physical movement which caused fatigue and tremor. (Dolbec, 2000). It has been shown that neurobehavioral manifestation, affected motor functions which was associated with high and medium dose of mercury exposure especially in movements, grip and balance (Mutter, 2010). It has been shown that exposure to high concentration of mercury vapor causes tremor initially affecting the hand and then spread to the other part of the body (Dolbec *et al.,* 2000; Yoshida *et al.,*2011). These findings agree with the findings of the present study which showed a decrease in the level of copper and zinc concentration in the brain which initially leads to decrease in memory and learning and motor function activity in the experimental animals.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

It can be concluded from the present study that mercury intoxication has no significant decrease in weight as a result of oral administration of mercury chloride for the period of 21 days. The main finding of the present study was on spatial learning and memory using Morris water maze test which was induced as a result of administration of mercury chloride orally. The results showed an increase in time taken to find the hidden platform which was an indication of memory loss. Spatial learning and memory impairments could be attributed to be dose and time dependant and the level of damage on the hippocampal cells. Complex motor behavior including motor balance, grasping and retrieval of food pellets as observed using Montoya stair case was significantly increased but there was decrease in the time in the control group. The motor activity deficit was as a result of damage to the cerebral motor area and the cerebellar motor coordination and balance area. The histopathological changes range from mild to severe degeneration of hippocampal, cerebellar and cerebral cells, other include clumping and congestion of cells, loss of branching patterns and eventually cell death. These histopathological changes were more severe in high dose groups among the experimental animals. These changes in the histological analysis as a result of mercury chloride intoxication in rats affect the neuronal cells in the cerebrum, cerebellum and hippocampus which were related to neurobehavioral deficiencies.

Mercury exposure affects the level of trace elements (Zinc and copper)) in the brain of rats which causes which are essential trace elements that play significant roles

in the modulation of dopamine and glutamate neurotransmitters respectively, which in turn results to motor, spatial learning and memory impairments.

The various observations do, however, justify some conclusions about the possible consequences of exposure to mercury chloride as dose and time dependent, which may lead to memory impairments, motor function deficits, histopathological changes and alteration in concentration of essential trace elements, most especially copper and zinc.

6.2.1 RECOMMENDATIONS FOR FURTHER RESEARCH

1. It is recommended that further work should be carried out in order to quantify the amount of mercury that could possibly be deposited in the respective tissues of the brain.

2. Investigations using histochemical methods should be carried out to show the deposits of mercury in the brain tissues.

3. Further studies are recommended on neurochemistry in order to quantify or ascertain the level of neurotransmitter Dopamine and Glutamate depletion and activity as a result of exposure to mercuric compounds.

REFERENCE

Abrous, D.N., (1994) .Skilled paw reaching in rats: the staircase test. *Neuroscience Protocol.* 3: 1-11

Akagi, A. (1995). Human exposure to mercury due to gold mining in the Tapajo river basin,amazon, Brazil: Specification of mercury in human hair, blood, and urine. *Water, Air and Soil Pollution*. 80: 85–94.

Akaho.E.H., Nyako B.J. (2002). Characteristic of neutron flux in irradiation site of MNSR reactor using Westcott formalism for the neutron activation analysis.Appl radiat isot 57,265-273

AlBalaghi.,S.Möller,E.,Möller,G.,Abedi-Valugerdi.,M.(1996).Mercury induces polyclonal B-Cell activation, autoantibody production and renal immune complex deposits in young (NZB x NZW) F1 hybrids', *Environmental Journal of Immunology.*26(7):p.15.

Alberimi.,C.M.(2001).Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes. *Trends in Neuroscience*. 28, 51-56.

Altmann L (2008). Visual functions in 6-year-old children in relation to lead and mercury levels. *Neurotoxicology Teratology* 20(1)9-17.

Amin-Zaki, L.,El-hassani.,S.Majeed, M.A.(1974).Intra-uterine methyl mercury poisoning in Iraq.*Pediatrics.* 84:587-595.

Andres, P. (1984). Brief communications: IgA-IgG disease in the intestine of Brown-Norway rats ingesting mercuric chloride. *Clinical Immunology- Immunopathol.*30:488-494.

Annabelle C.(2009). Rewarded Outcomes Enhance Reactivation of Experience in the Hippocampus. *Neuron* 64:6, 910-921

ATSDR.(1989). Decision guide for identifying substance-specific data needs related to Toxicological profiles. Agency for Toxic Substances and Disease *Registry, Division of Toxicology,* Atlanta.

ATSDR. (1992). Case studies in environmental medicine -mercury toxicity. *US Department of Health and Human Services Public Health Service*

ATSDR. (1995). US Department of Health & Human Services, 'ATSDR/EPA *Priority List*

(ATDRS) (1999) Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Mercury.*U.S Department of Health and Human Services. Atlanta, U.S.A

ATDRS (2005). Mercury neurotoxicity: Mechanisms of blood-brain barrier transport *Neuroscience Biobehavor Review* 14(2):169-176.

ATSDR. (2011). Exposure to hazardous substances and reproductive health. *American Family Physician* 48(8):1441-1448.

Baltimore, A and Williams.J. (2006).Developing fetus *Clinical Toxicology of commercial products*th Eds. New York, NY: Elsevier Press.

Barr R.D.(1973). Levels of mercury in urine correlated with the use of skin lightening creams. *American Journal of Clinical Pathology*.59:pp.36– 40.

Barregard L (1991). Effects of occupational exposure to mercury vapor on lymphocyte micronuclei. *Scand Journal Work Enviromental Health* 17(4):263-268.

Barregard L., Sallsten G., Schutz A.(1992). Kinetics of mercury in blood and urine after brief occupational exposure. *Archives of Enviromental Health.* 47(3):176-184.

Bell C.C (2008). "Cerebellum-like structures and their implications for cerebellar function". *Annual Review Neuroscience.* 31: 1–24.

Berlin M. (2003).Mercury in dental-filling materials – an uppdated risk analysis in environmental medical terms. In: Lidmark A, ed. *Dental materials and health. Stockholm: SOU* 53:17-57.

Berlin M. (2006).Mercury. In: Friberg L, Nordberg G.R, Vouk V.B, eds. *Handbook on the toxicology of metals.* 2nd ed. New York, NY: Elsevier Press.

Berlin, M .Zalups, R.K and B. A. Fowler (2007), "Mercury," in *Handbook on the Toxicology of Metals*, G. F. Nordberg, B. A. Fowler, M. Nordberg, and L. T. Friberg, Eds., chapter 33, Elsevier, New York, NY, USA, 3rd edition

Betti C (1992). Genotoxic activity of methyl mercury chloride and dimethyl mercury in human lymphocytes. *Mutation Research* 281(4):255-260.

Bhavnagar S. and Taneja S. (2010) Zinc and cognitive development. *British. Journal of. Nutrition.* 85:S139-S145

Bjornberg.A., M. Vahter, B., Berglund, B., Niklasson, M., Blennow., and G. Sandborgh-Englund, (2011). "Transport of methylmercury and inorganic mercury to the fetus and breast- fed infant," *Environmental Health Perspectives*, vol. 113, no. 10, pp. 1381–13

Bodek I., Lyman W.J., Reehl W.F. (1988). Mercury. In*: Environmental inorganic chemistry*. NewYork NY: Pergamon Press

Booth S and D. Zeller. (2005). "Mercury, food webs, and marine mammals: implications of diet and climate change for human health," *Environmental Health Perspectives,* vol. 113, no, pp. 521–526

Brockmeier J. (2010). "After the Archive: Remapping memory". *Culture & Psychology* 16 (1): 5–35.Cadmium, mercury and exposures in the glass manufacturing industry.

Bruce L. McNaughton, Francesco P. Battaglia, Ole Jensen, Edward I Moser, May-Britt Moser. (2006) Path integration and the neural basis of the 'cognitive map'. *Nature Reviews Neuroscience*.7:8,663-678

Burger, J.,C. Jeitner, and M. Gochfeld, (2011)"Locational differences in mercury and selenium levels in 19 species of saltwater fish from New Jersey," *Journal of oxicology and Environmental Health,* vol. 74, no. 13, pp. 863–874.

Cantoni O. (1983). Correlations of DNA strand breaks and their repair with cell survival following acute exposure to mercury (II) and X-rays. *Molecular Pharmacology. 24*:84-89.

Charles V (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory; *Nature Protocols* 1, Pp 848 – 858

Choic W.S., Kim S.J., Kim J.S. (2011). Inorganic lead (Pb)- and mercury (Hg)-induced neuronal cell death involves cytoskeleton reorganization. *Laboratory Animals Research.*21; 345-349

Chris M. (2008) The hippocampus and memory: insights from spatial processing. *Nature Reviews Neuroscience* 9:3, 182-194

Clerke. E. (1996). "Ch. 11: Cerebellum". *The Human Brain and Spinal Cord*. Norman Publishing.

Clarkson T.W, Hursh J.B, Sager P.R. (1989). Mercury. In: Clarkson TW, Hursh JB, Sager P.R. eds. *Biological monitoring of toxic metals.* New York: Plenum Press, Pp.199-246.

Clarkson T.W (2003). Mercury. *Journal of American College Toxicology* 8(7):1291-1296.

Coulibaly S., Atse B.C., Koffi K.M., Sylla S., Konan K.J., Kouassi N.J.(2012). Seasonal Accumulations of Some Heavy Metal in Water, Sediment and Tissues of Black-Chinned Tilapia Sarotherodon melanotheron from Biétri Bay in Ebrié Lagoon, *Ivory Coast.Bulletin Enviromental Contaminant Toxicology*.88(4):571-6.

Crespo-López M.E., Macêdo G.L., Pereira S.I., Arrifano G.P., Picanço-Diniz D.L., do Nascimento J.L., Herculano A.M. (2009). Mercury and human genotoxicity: critical considerations and possible molecular mechanisms.*Pharmacology Research*; 60 (4):212-20.

Dewailly E., Chateau-Degat L., Suhas E. (2008). Fish consumption and health in French Polynesia. *Asian Pacific Journal of Clinical Nutrition*17:86–93.

Dolbec J., Mergler D., Sousa Passos C.J., Sousa de Morais S., Lebel J. .(2000). Methylmercury exposure affects motor performance of a riverine population of the Tapajós river, Brazilian Amazon *international Arches of Occupational Environ Health*

Environmental Health Department (EDH) (2002).Ministry of the Environment, Minimata Disease: The History and Measures, *Ministry of the Environment, Government of Japan,* Tokyo, Japan.

F.A.O (Food and Agriculture Organization of the United Nations) (1994). *Manual of methods in aquatic environment research,* part 10. Short term static bioassays. FAO fisheries technical paper, 247.92

Food and drugs agency. (1998). Mercury in fish: cause for concern? *U.S. Food and Drug Administration. FDA* Consumer updated 2/26/96.

Fuyuta M (1978). Embryotoxic effects of methylmercuric chloride administrated to mice and rats during organogenesis. *Teratology* 18:353-366.

Gagelli E., Kozlowski H., Valensin D., Valensin .G.(2011) .Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). Chem 106:1995–2044.

Ghez C (1985). "The cerebellum". In Kandel ER, Schwartz JH. *Principles of Neural Science, 2nd edition*. New York: Elsevier. pp. 502–52Z

Goh C. L. (2008). Occupational allergic contact dermatitis from metallic mercury. Contact ;*Dermatitis* 19(3):232-233.

Goyer. A.H .(1986*).Toxic effects of metals.* Casaret and Doull's toxicology- *the basic science of poisons.* ed. 3 New York. Macmillan Publishing. Pp. 582-609 .

Goyer; R. (1991). Toxic effect of metals In: Amdur M.O; J.D Doul and C.D Klassen; Eds Casarett and Doulls. *Toxicology*. 4th ed. Peganison press New York.P.567.

Grandjean P., Murata K., Budtz-Jorgensen E., Weihe P. (2004). Cardiac autonomic activity in methylmercury neurotoxicity: 14-year follow-up of a Faroese birth cohort. *The Journal of pediatrics*; 144:169–176.

Gray, J.A(2000). *The Neuropsychology of Anxiety:* An Enquiry into the Functions of the Septo-Hippocampal System.Oxford University Press,P.44.

Gross., Charles G. (1993). "Hippocampus Minor and Man's Place in Nature: A Case Study in the Social Construction of Neuroanatomy". *Hippocampus 3 (4): 403–416*

Gunderson E.L. (1988). FDA total diet study, April 1982-April 1984, dietary intakes of pesticides, selected elements, and other chemicals. *Journals Association of Analogue Chem*istry71(6):1200-1209

Hacking, I. (1996). *Memory science, memory politics*. In P. Antze & M. Lambek (Eds.), Tense past: Cultural essays in trauma and memory. New York & London: Routledge.pp.67-87

Halas, E.S., and Eberhardt, M. J. (2007) A behavioral review of trace element deficiencies in animals and humans. *Nutritional. Behavior.* 3: 257-271.

Halbach S (2008). Enzymatic oxidation of mercury vapor by erythrocytes. *Biochemistry and Biophysiology Acta* 523:522-531.

Hallee T.J. (1969). Diffuse lung disease caused by inhalation of mercury vapor. *American Review of Respiratory Disease* 99:430-436.

Hans, W. (2006). The hazards of Cadmium in man and animals. *Journal of applied toxicology,* 2,61-67.

Hiroaki. W (2007). Neural dynamics of the cognitive map in the hippocampus. *Cognitive Neurodynamics* 1:2, 119-141

Hu. X., Tulsieram K.L., Zhou. Q., Mu. L., Wen .J. (2012). Polymeric nanoparticle-aptamer bioconjugates can diminish the toxicity of mercury in vivo *Toxicology Letter* 5;208(1):69-4

Hursh. J.B., Clarkson T.W., Miles. E.F.(1989). Percutaneous absorption of mercury vapor by man. *Archives of Environmental Health.* 44:120-127.

IAEA (2004) WINSPAN 2006 a multipurpose gamma ray spectrum analysis software CIAE ,Beijing china.

IARC (1993).Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry:Evaluation of carcinogenic risks to humans. *International Agency for Research On Cancer.* Vol 58.

Janicki. K., Dobrowolski. J, Drasnicki. K. (1987). Correlation between contamination of the rural environment with mercury and occurrence of leukemia in men and cattle. *Chemosphere* 16:253- 257.

Jonah S.A. Sadiq. U. (2006).Efficiency for curve NNIR-1 gamma ray spectrometry system at near and far geometries by empirical and semi emperical method *Nuclear instruments methods* 463,321-323.

Jonah S.A Balogun G. I.,Umar., I Maiyaki .M.C. (2008). Neutron spectrum parameters in irradiation channels of Nigerian research reactor -1(NNIR-1) for NAA standardization *Journal of radio anal nuclear chemistry* 266(1) 83-88

Kandel, Eric R.; Schwartz, James H.; Jessell, Thomas M. (2000). *Principles of Neural Science Fourth Edition*. United State of America: McGraw-Hill. p. 324. ISBN 0-8385-7701-6

Kosan,C, A. K. Topaloglu, and B. Ozkan, (2001)"Chronic mercury intoxication simulating pheochromocytoma: effect of captopril on urinary mercury excretion," *Pediatrics International,* vol. 43, no. 4, pp. 429–430,

Lim S (2009).Low dose mercury and heart rate variability among community residents nearby to an industrial complex in Korea. *Neurotoxicology.* 31:10–16.

Liu. Li., Jiong D., Charles M., Junying G., Gang., Hu, M., Xiao.K. (2011). Pretraining affects Morris water maze performance with different patterns between control and ovariectomized plus d-galactose-injected mice. *Behavioural Brain Research* 217:1, 244-247

Linas. R.R., Walton. K.D., Lang E.J. (2004). "Ch. 7 *Cerebellum*". In Shepherd GM. *The Synaptic Organization of the Brain*. New York: Oxford University Press.

Madsen E., Gitlin J.D. (2007) Copper and iron disorders of the brain. *Annual Revision Neurosci*ence 30:317–37.

Mahaffey, K. (2004). Update on Recent Epidemiologic Mercury Studies. *Proceedings, National Forum on Contaminants in Fish.* San Diego, CA. 25-28

Makikallio. T.H, Huikuri.H.V., Makikallio A., Sourander .L.B., Mitrani. R.D., Castellanos A., Myerburg R.J.(2001). Prediction of sudden cardiac death by fractal analysis of heart rate variability in elderly subjects. *Journal American Cardiology*.;37:1395–1402

Mandler, G. (1967). "Organization and memory". In K. W. Spence & J. T. Spence (Eds.), The psychology of learning and motivation: *Advances in research and theory*. Vol. 1, pp 328–372. New York: Academic Press.

Mark C (2007). Context Learning in the Rodent Hippocampus. *Neural Computation* 19:12, 3173-3215

Meyer-Baron (2002) "A meta-analysis for neurobehavioural results due to occupational mercury exposure," Archives of Toxicology, vol. 76, no. 3, pp. 127–136,

Michael E. Hasselmo. (2009).A model of episodic memory: Mental time travel along encoded trajectories using grid cells. *Neurobiology of Learning and Memory* 92:4, 559-573

Milne J (1970). Acute mercurial pneumonitis. *Brritish Journal Industrial Medicine.*27:334-338.

Mohan M (2011). Accumulation of mercury and other heavy metals in edible fishes of Cochin backwaters, Southwest India. *Environmental Monitoring Assessment* .

Montoya, C.P.; Astell, S.; Dunnett, S.B. (1990).Effects of nigral and striatal grafts on skilled forelimb use in the rat. *Program. Brain Reearhs*. 82: 459-466;

Montoya, C.P.; Campbell-Hope, L.J.; Pemberton, K.D.; Dunnett, S.B. (1991).The staircase test: a measure of independent forelimb reaching and grasping abilities in rats. *Journal of. Neuroscience Methodology*. 36: 219-228

Morris W (1981) Test For Spatial Learning And Memory.*Science.*136,

Musa .S.A; Sadeeq A.A(2009).Effects of mercury chloride on the cerebral cortex of adults wistar rats. *Journal of experimental and clinical anatomy*.Vol 23 13;6-10

Murata K, Sakamoto M, Nakai K, Dakeishi M, Iwata T, Liu XJ, Satoh H. (2006).Subclinical effects of prenatal methylmercury exposure on cardiac autonomic function in Japanese children. *International Archives of Occupation Environment Health*. 79:379–386.

Murphy MJ, Culliford EJ, Parsons V. (1976). A case of poisoning with mercuric chloride. *Resuscitation.*7:35-44.

Mutter J, Curth A, Naumann J, Deth R, Walach H.(2010) Does inorganic mercury play a role in Alzheimer's disease? A systematic review and an integrat molecular mechanism *Journal Alzheimers Disease.* 22(2):357-74.

Nadorfy-Lopez,E, S. H. Torres, H. Finol, M. Mendez, and B. Bello,(2000) "Skeletal muscle abnormalities associated with occupational exposure to mercury vapors *Histology and Histopathology*, vol. 15, no. 3, pp. 673–682,

Neilsen J.B, Andersen H.R, Andersen O (1991). Mercuric chloride-induced kidney damage in mice: Time course and effect of dose. *Journal Toxicology Environmental Health* 34(4):469-483.

Nirenberg R.E. (2010). Delayed cerebellar disease and death after accidental exposure to dimethylmercury. *National England Journal of Medicine*.**338**: 1672–1676

Oka T, Matsukura M, Okamoto M, Harada N, Kitano T, Miike T, Futatsuka M. (2002) Autonomic nervous functions in fetal type Minamata disease patients: assessment of heart rate variability. Tohoku *Journal of Experimental Medicine*.;198:215–221.

Oliveri, G (2000). "Mercury induces cell cytotoxicity and oxidative stress and increases β- amyloid secretion and tau phosphorylation in SHSY5Y neuroblastoma cells," *Journal of Neurochemistr*y, vol. 74, no. 1, pp. 231– 236,

Olson K (2005). Decreased learning capacity in rats exposed prenatally and postnatally to low doses of mercury. *Bulletin of Environmental Contaminant Toxicology* 13:73-79.

Onyeike, E. N (2002). Inorganic ion levels of soils and streams in some areas of Ogoni land, Nigeria as affected by crude oil spillage. *Journal. Environmental. Monitoring. Assessments. 73:191-205*

Onwureme, B. (2010). Accumulation of metals and histopathology in *Oreochromis niloticus* exposed to treated NNPC Kaduna (Nigeria) Petroleum refinery effluent – *Ecotoxicology and Environmental* . *Safety*– 19:123

Pambor M, Timmel A.(2007). Mercury dermatitis. *Contact Dermatitis* 20(2):157.

Park, S.H, S. Araki, A. Nakata (2000). "Effects of occupational metallic mercury vapor exposure on suppressor-inducer (CD4+CD45RA+) T lymphocytes and CD57+CD16+ natural killer cells," *International Archives of Occupational and Environmental Health*, vol. 73, no. 8, pp. 537–542,

Penland J.G. and Prohaska J.R. (2004). Abnormal motor function persists following recovery from perinatal copper deficiency in rats. *Journal of Nutrition.* ; 134 -184

Philippe Taupin (2007). *The Hippocampus: Neurotransmission and Plasticity in the Nervous System*. Nova Pub

Piotrwski J.K, Szymanska J.A, Skrzypinska-Gawrysiak M, et al. 1992. Intestinal absorption of inorganic mercury in rat. *Pharmacology Toxicology* (Copenhagen) 70(1):53-55.

Quirino Cordeiro Júnior, M.D.; Marcília de Araújo Medrado Faria, M.D.; Renério Fráguas Júnior, M.D. (2012) Depression, Insomnia, and Memory Loss in a PatientWith Chronic Intoxication by Inorganic Mercury *The Journal of Neuropsychiatry and Clinical Neurosciences* 191-205

Rao M.V and P. S. N. Sharma,(2001) "Protective effect of vitamin E against mercuric chloride reproductive toxicity in male mice*," Reproductive Toxicology,* vol. 15, no. 6, pp. 705–712,.

Rasia R.M, Bertoncini C.W, Marsh D (2005). Structural characterization of copper(II) binding to alpha-synuclein: insights into the bioinorganic chemistry of Parkinson's disease. Proc National Academy of Science U S A;102:4294–9

Rice D.C, Gilbert S.G. (1990). Effects of developmental exposure to methyl mercury on spatial and temporal visual function in monkeys. *Toxicoogyl Applied Pharmacology*. 102(1):151-163.

Saha A .M (1986).compound bio chem.Physicalpharmacology *Toxicology (85) 1253-4*

Saladin, K (2010). Anatomy and Physiology: The Unity of Form and Function, 5th Ed. New York: McGraw-Hill Companies Inc, Print

Shipp, Stewart (2007). "Structure and function of the cerebral cortex". *Current Biology* 17 (12): R443–9

Sorensen N, Murata K, Budtz-Jorgensen E, Weihe P, Grandjean P. (1999). Prenatal methylmercury exposure as a cardiovascular risk factor at seven years of age. *Epidemiology*. 10:370–375

Thurston SW, Bovet P, Myers GJ, Davidson PW, Georger LA, Shamlaye C, Clarkson T.W. D (2007) prenatal methylmercury exposure from fish consumption affect blood pressure in childhood? *Neurotoxicology*.;28:924–930

Tyler, SW, Hertel, PT, McCallum, MC & Ellis, HC. (1979). "Cognitive effort and memory". Journal of Experimental Psychology: Human Learning & Memory, 5, 607-617.

Valera B, Dewailly E, Poirier P. (2008) Cardiac autonomic activity and blood pressure among Nunavik Inuit adults exposed to environmental mercury: a cross-sectional study. *Environmental Health*. 28:924

Vimercati, L,L. Santarelli, G. (2001) Pesola"Monocyte-macrophage system and polymorphonuclear leukocytes in workers exposed to low levels of metallic mercury," *Science of the Total Environment*, vol. 270, no. 1–3, pp. 157–163,

Vupputuri S, Longnecker MP, Daniels JL, Guo X, Sandler DP. (2000) Blood mercury level and blood pressure among US women: results from the National Health and Nutrition Examination *Environmental research*.;97:195–200

Wang J.S, Huang PM, Liaw WK, (2007). Kinetics of the desorption of mercury from selected fresh water sediments as influenced by chloride. *Water, Air, Soil Pollution* 56:533-542.

Warfringe K. (1995). Mercury distribution in the mouse brain after mercury vapour exposure. *International Journal of Expiremental Pathology* 76:29-35.

Warkany J, Hubbard DM. (1953). Acrodynia and mercury. *Journal of Pediatric* 42:365-386.

Wegwu, M. O. (1999). Seasonal Variations of Pollutants in some areas of the Niger Delta and their toxicity on some Aquatic fauna. *Ph.D. thesis*, University of Port Harcourt, Nigeria.

Whishaw, I.Q.; O'Connor, W.T.; Dunnett, S.B. (1986).The contributions of motor cortex,nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat. *Brain* 109:805-843

Whishaw, I.Q.; Woodward, N.C.; Miklyaeva, E.; Pellis, S.M. (1997). Analysis of limb use by control rats and unilateral DA-depleted rats in the Montoya staircase test: movements, impairments and compensatory strategies. *Behav. Brain Res*. 89: 167-177;

Whishaw, I.Q.; Tomie, J.A.; Ladowsky, R.L.(2000).Red nucleus lesions do not affect limb preference or use, but exacerbate the effects of motor cortex lesions on grasping in the rat. Behavior. *Brain Research*. 40: 131-144;

W.H.O. (1989). Mercury - environmental aspects. Vol. 86. Geneva, Switzerland: World Health Organization, *International Programme on Chemical Safety*.

W.H.O.(1999).InorganicmercuryGeneva,Switzerland:WorldHealth Organization,*International Programme on Chemical Safety*, Vol. 118. 168

W.H.O (World Health Organisation) (2003). Elemental mercury and inorganic mercury compounds: Human health aspects. Concise International Chemical Assessment Document. CICAD 50. Geneva.

W.H.O (2005) Mercury Training Module. WHO Training Package for the Health Sector. Geneva, *World Health Organization*

Winneke G. Mieiro CL, Bervoets L, Joosen S, Blust R, Duarte AC, Pereira ME, (2011.) Developmental aspects of environmental neurotoxicology: lessons from lead and polychlorinated biphenyls. *Journal of Neurological Science*.15;308(1-2):9-15.

Winship K.A. 1985. Toxicity of mercury and its inorganic salts. Adverse Drug React Acute *Poisoning Review* 4(3):129-160.

Wolf U, Rapoport M.J, Schweizer TA (2009). "Evaluating the affective component of the cerebellar cognitive affective syndrome". *Journal Neuropsychiatry Clinical . Neuroscience.* 21 (3) 245–53.

Yoshida M, Suzuki M, Satoh M, Yasutake A, Watanabe C.(2011) Neurobehavioral effects of combined prenatal exposure to low-level mercury vapor and methylmercury. *Journal of Toxicological Science.* 36(1):73-80.

Zucconi GG, Cipriani S, Scattoni R, Balgkouranidou I, Hawkins D.P, Ragnarsdottir KV.(2007) Copper deficiency elicits glial and neuronal response typical of neurodegenerative disorders. Neuropathology Apply Neurobiol;33:212–25.

Zhang, M.Q., Zhu, Y.C., Deng, R.W.(2002). Evaluation of mercury emissions to the atmosphere from coal combustion, *China. Ambiosphere* 31 (6),482–484.

APPENDIX I

Chemical Substance = Mercury Chloride $HgCl_2$

LD_{50} of mercury chloride = 166mg/kg body weight

30%, 15%, and 7.5% of the LD_{50} was used as high, medium, and low dose respectively.

Dose of mercury chloride used

$\frac{30 \times 166}{100}$ = 49.8 mg/kg was used as the highest Dose of mercury chloride

$\frac{15 \times 166 \text{ mg/kg}}{100}$= 24.9mg/kg was use as the medium Dose of mercury chloride

$\frac{7.5 \times 166\text{mg/kg}}{100}$ = 12.45 mg/kg was the low Dose mercury chloride used.

1000g _____ 49.8mg

1 _____ x = 0.0499mg

200g animal will receive 49.8mg/1000 x2

4,98x2 mg = 9. 96mg

Group I animals was used as control and was administered normal saline orally

Mercury chloride administration base on body weight of animals used

4.98/200rat/0.2ml

Stock preparation/ working solution

Therefore working solution will be 4.98mg / 0.2 ml= 24.9mg/ml

APPENDIX II

Table 1;The Mean and Standard error of Mean of Copper and Zinc in the brain tissue of adults Wistar rats after Neuron Activation Analysis.

S/no	Group	Copper Mean ± SEM (Ppm)	Zinc Mean ± SEM (Ppm)

1	Group I	3.46 ± 0.2	2.39±0.11
2	Group II	2.21±0.1	1.55±0.13
3	Group III	2.17±0.031	1.07±0.05
4	Group IV	1.04±0.12	0.77±0.03