# THE EFFECT OF ETHANOLIC EXTRACT OF *Psidium guajava* LEAVES ON MERCURIC CHLORIDE INDUCED NEUROBEHAVIOURAL, BIOCHEMICAL AND HISTOARCHITECHURAL CHANGES IN THE CEREBRUM, CEREBELLUM AND HIPPOCAMPUS OF ADULT WISTAR RATS

**BY**

**Joy OCHAI**

**DEPARTMENT OF HUMAN ANATOMY,**
**FACULTY OF BASIC MEDICAL SCIENCES,**
**COLLEGE OF HEALTH SCIENCES,**
**AHMADU BELLO UNIVERSITY,**
**ZARIA, NIGERIA**

**MAY, 2018**

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**BY**

**Joy OCHAI**
**(B.Sc, Human Anatomy, ABU, 2012)**
**(P14MDHA8013)**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc) DEGREE IN HUMAN ANATOMY**

**DEPARTMENT OF HUMAN ANATOMY,**
**FACULTY OF BASIC MEDICAL SCIENCES,**
**COLLEGE OF HEALTH SCIENCES,**
**AHMADU BELLO UNIVERSITY,**
**ZARIA, NIGERIA**

**MAY, 2018**

## DECLARATION

I declare that the research work in this dissertation entitled "**Effect of Ethanolic Extract of *Psidium guajava* Leaves in Mercuric Chloride Induced Neurobehavioral, Biochemical and Histoarchitectural Changes on the Cerebrum, Cerebellum and Hippocampusof Adult Wistar Rats"** has been carried out by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree at this or any other Institution.

Joy OCHAI _____ _____

Sign Date

## CERTIFICATION

This dissertation entitled "**Effect of Ethanolic Extract of *Psidium guajava* Leaves in Mercuric Chloride Induced Neurobehavioral, Biochemical and Histoarchitectural Changes on the Cerebrum, Cerebellum and Hippocampus of Adult Wistar Rats"** by**Joy OCHAI** meets the regulations governing the award of the degree of Master of Science (M.Sc.) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

**Prof. S. S. Adebisi (B.Sc., M.Sc., Ph.D, FASN)** _____ _____
Chairman Supervisory Committee Sign Date
Department of Human Anatomy,
Faculty of Basic Medical Science,
College of Health Sciences,
Ahmadu Bello University, Zaria.

**Dr. A .O. Ibegbu (B.Sc., M.Sc., Ph.D)** _____ _____
Member Supervisory Committee Sign Date
Department of Human Anatomy,
Faculty of Basic Medical Science,
College of Health Sciences,
Ahmadu Bello University, Zaria.

**Dr.Z.M. Bauchi (BSc., MSc., Ph.D)** _____ _____
Head of Department, Sign Date
Department of Human Anatomy,
Faculty of Basic Medical Science,
College of Health Sciences,
Ahmadu Bello University, Zaria.

**Prof. S. Z. Abubakar** _____ _____

Dean School of Post Graduate Studies, Sign Date

Ahmadu Bello University, Zaria

## DEDICATION

This dissertation is dedicated to my darling Husband (Engr. Innocent Okor) and wonderful Parent (Mr & Mrs Chris Ochai)

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## ABSTRACT

Mercury is a ubiquitous and toxic heavy metal which has caused extensive environmental contamination and significant public health problems in many parts of the world and there is increasing evidence that the central nervous system is its main target. The present study evaluated the neurobehavioral, biochemical, and histo-architectural changes following oral administration of ethanolic extract of *Psidium guajava* leaves on the cerebrum, cerebellum and hippocampus of Mercuric chloride treated adult Wistar rats. Thirty-Five (35) adult Wistar rats were distributed randomly into seven groups with each group containing 5 rats. Group 1, served as the Control and was administered with distilled water, Group 2 was administered with 41.5 mg/kg bwt of mercuric chloride for 21days, Group 3 was administered with 1000 mg/kg bwt of ethanolic extract of *Psidium guajava* leaves for 21 days, Group 4 was administered with 41.5 mg/kg bwt of mercuric chloride for 21 days and distilled water for another 21days. Group 5was administered with 41.5 mg/kg bwt of mercuric chloride for 21 daysand 500mg/kg bwt of ethanolic extract of *Psidium guajava* leaves for another 21 days. Group 6 was administered with 41.5mg/kg bwt of mercuric chloride for 21 daysand 1000 mg/kg bwt of ethanolic extract of *Psidium guajava* leaves for another 21 days and Group 7 was administered with 41.5 mg/kg bwt of mercuric chloride for 21daysand1190mg/kg body weight of ascorbic acid for another 21 days. All the administrations were done orally via orogastric tube for a total of 42 days. Neurobehavioral tests such as Moris Water Maze to test for spatial learning and memory, beam walking for Motor coordination and Elevated Plus Maze test for anxiety related behaviours were done during and after the administration. The animals were sacrificed 12 hours after the last administration and the cerebrum, cerebellum and hippocampus were excised and some of the tissues were homogenized and was used for biochemical analyses of the oxidative stress markers such as Catalase (CAT), Superoxide Dismutase (SOD), Malondaldrehye (MDA) and Glutathione (GSH) concentration. While some of the brain tissues were fixed with Bouin's fluid for histological studies. The result of Moris water maze test showed asignificant increase in the mean time taken by the animals exposed to mercuric chloride to locate the hidden platform (47.33±5.61 sec) when compared to the Control Group (3.33±0.33 sec), where as a decrease in the mean time taken to locate the hidden platform were observed in Groups treated with *Psidium guajava* (14.0±2.89 sec) and ascorbic acid (15.33±3.18 sec). The beam walking test showed the

highest mean time taken to cross the beam to the safety hamster box was observed in groups exposed to mercuric chloride (25.55±6.52 sec) when compared to the Control group (3.15±0.54 sec). Treatment with *Psidium guajava* (7.08±1.03 sec) and ascorbic acid (9.84±0.70 sec) after mercury exposure showed significant decrease in the time taken by the experimental animals to cross the beam to the safety hamster box. The result of Oxidative stress study showed a significant increasein Malondialdehyde concentration and decrease in Catalase (CAT), Superoxide dismutase (SOD) and reduced glutathione (GSH) in groups exposed to Mercuric chloride, however, treatment with *Psidium guajava* and Ascorbic acid following mercury exposure did not show any significant difference when compared to the Control group. The result of the histological studies showed normal cytoarchtecture of cells in the Control group whereas alterations such as Vacuolation of cell bodies and degeneration of cells in groups exposed to mercuric chloride. Treatment with *Psidium guajava* and ascorbic acid ameliorated the changes observed in groups exposed to mercury only. It may be concluded that *Psidium guajava* leaves extract possess vital antioxidant properties that could ameliorate the toxic effect of mercury in the Cerebral Cortex, Cerebellum and Hippocampus of adult Wistar rats and as such could be used for treatment of mercury poisoning or heavy metal poisoning.

Table of content

Title page ..... i

Declaration ..... iii

Certification ..... iv

Dedication ..... v

Acknowledgement …….. vi

Abstract …… vii

CHAPTER ONE

1.0 INTRODUCTION ..... 1
1.1 Background ..... 2
1.2 Statement of Research Problem ..... 3
1.3 Justification of the Study..... 4
1.4 Aim and Objectives of the Study..... 4
1.4.1 Aim of the study ..... 4
1.4.2 Objectives of the study..... 5
1.5 Scope of the Study..... 5
1.6 Study Hypothesis ..... 5

CHAPTER TWO

2.0 LITERATURE REVIEW ..... 6
2.1 Mercury..... 7
2.1.1 Forms of mercury..... 7
2.1.2 Properties of mercury..... 8
2.1.3 Sources of mercury and its compounds..... 9
2.1.4 Exposure to mercury compound..... 9
2.1.5 Mercury exposure, effects and toxicity..... 10
2.1.6 Toxicokinetics of mercuric chloride..... 12
2.2 *Psidium guajava*..... 13
2.2.1 Taxonomy of *Psidium guajava*..... 14
2.2.2 Medicinal uses of *Psidium guajava*..... 16
2.2.3 Phytochemicals in*Psidium guajava*..... 18
2.3 The Central Nervous System..... 22
2.3.1 The cerebral cortex..... 22
2.3.2 Areas of the cerebral cortex..... 24
2.3.3 Layers of the cerebral cortex..... 28
2.3.4 Cell of the cerebral cortex..... 29
2.3.5 The hippocampus..... 30

2.3.6 The cerebellum..... 34
2.3.7 Histology of the cerebellum..... 35

CHAPTER THREE

3.0 MATERIALS AND METHODS .....38
3.1 Materials..... 38
3.2 Experimental Animal.....38
3.3 Chemical Substances..... 39
3.4 Preparation of *Psidium guajava*..... 39
3.5 Acute Toxicity Study.....40
3.6 Experimental Protocol..... 40
3.7 Experimental Procedure..... 42
3.8 Neurobehavioural Studies..... 43
3.8.1 Spatial learning and memory test using morris water maze (MWM)..... 43
3.8.2 Elevated plus maze test..... 47
3.8.3 Beam walking test..... 49
3.9 Morphological Assessment..... 51
3.9.1 Change in weight..... 51
3.10 Animal Sacrifice..... 51
3.10.1 Estimation of oxidative parameter..... 51
3.10.2 Preparation of tissue homogenates..... 52
3.10.3 Malondialdehyde assay..... 52
3.10.4 Superoxide dismutase activity assay..... 52
3.10.5 Catalase activity assay..... 52
3.11 Tissue Processing..... 52
3.11.1 Haematoxylin and Eosin (H and E) staining Method..... 53
3.11.2 Special Stain using Cresyl Violet Method..... 54
3.13 Data Analysis..... 54

CHAPTER FOUR

4.0 RESULTS ..... 55
4.1 Acute Toxicity Study..... 55
4.2 Physical Observation of Animals During Administration..... 55
4.3 Body Weight Before and During the Experiment..... 56
4.4 Morris Water Maze..... 59
4.4 Elevated Plus Maze Test..... 61
4.5 Beam Walking Test Result..... 64
4.6 Biochemical Studies..... 66
4.7 Histological Studies..... 71

CHAPTER FIVE

5.0 DISCUSSION..... 114

5.1 Body Weight Assessment of Animals..... 114
5.2 Neurobehavioral Study..... 115
5.3 Beam Walking Test..... 116
5.4 Elevated Plus Maze Studies..... 117
5.5 Oxidative Stress Studies..... 118
5.6 Histological Studies..... 120

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION..... 123
6.1 Summary..... 123
6.2 Conclusion..... 124
6.3 Recommendations for Further Studies..... 124
Reference.....126
Appendix.....140

List of Figures

Figure 2.3: Layers of the cerebral cortex..... 27

Figure 2.5: Layers of the Cerebellar cortex..... 37

Figure 4.1: CAT concentration in the brain tissues of animals exposed to mercuric chloride.... 66

Figure 4.2: MDA concentration in the brain tissues of animals exposed to mercuric chloride.... 67

Figure 4.3: GSH concentration in the brain tissues of animals exposed to mercuric chloride..... 68

Figure 4.6: SOD concentration in the brain tissues of animals exposed to mercuric chloride.....69

List of tables

Table 2.1 Properties of mercury ..... 8

Table 2.2 Taxonomy of *Psidium guajava* leaves ..... 14

Table 2.3 Photochemical in ethanolic extract of *Psidium guajava* leaves ..... 18

Table 3.1 Treatment Schedule ..... 42

Table 4.1: Effect of mercuric chloride exposure, *Psidium guajava* leaves and ascorbic acid on weight of Wistar rats ..... 57

Table 4.2: Effect of mercuric chloride exposure, *Psidium guajava* leaves and ascorbic acid on spatial learning and memory ..... 59

Table 4.3 Effect of mercuric chloride exposure, *Psidium guajava* leaves and ascorbic acid on anxiety related behaviour using Elevated plus maze test ..... 61

Table 4.4 Effect of mercuric chloride exposure, *Psidium guajava* leaves and ascorbic acid on anxiety related behaviours using Elevated plus Maze test ..... 62

Table 4.5: Effect of mercuric chloride exposure, *Psidium guajava* leaves and ascorbic acid on motor coordination using beam walking test ..... 64

List of Plates

Plate 2.1: *Psidium guajava*leaves and fruit ..... 15

Plate 2.2: The three major parts of the brain..... 23

Plate 3.1 showing: Morris Water Maze Experiment..... 46

Plate 3.2: Shows the elevated plus maze setup..... 48

Plate 3.3: showing the beam walking test setup..... 50

Plate I: Section of the cerebellar cortex of Wistar rats in Group 1 (Control) (H and E; X250). 73

Plate II: Section of the cerebellar cortex of Wistar rats in Group 2 (41.5mg/kg bwt $HgCl_2$) (H and E; X250). ..... 74

Plate III: Section of the cerebellar cortex of Wistar rats in Group 3(1000mg/kgbwt ethanolic extract of *Psidium guajava* leaves) (H and E; X250). ..... 75

Plate IV: Section of the cerebellar cortex of Wistar rats in Group 4 (41.5mg/kg bwt$HgCl_2$ + distilled water) (H and E; X250). ..... 76

Plate V: Section of the cerebellar cortex of Wistar rats in Group 5(41.5mg/kg bwt Hgcl2 + 500mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) (H and E; X250). ..... 77

Plate VI: Section of the cerebellar cortex of Wistar rats in Group 6 (41.5mg/kgbwt Hgcl + 10 00mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) (H and E; X250). .....78

Plate VII: Section of the cerebellar cortex of Wistar rats in Group 7(41.5mg/kgbwt $Hgcl_2$ + 1000mg/kgbwt ascorbic acid) (H and E; X250). ..... 79

Plate VIII: Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 1 (control) (H and E; X250). ..... 80

Plate IX: Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 2(41.5mg/kg bwt $HgCl_2$) (H and E; X250). .....81

Plate X: Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 3(1000mg/kg bwt ethanolic extract of *Psidium guajava* leaves) (H and E; X250)..... .....82

Plate XI: Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 4(41.5mg/kg bwt $Hgcl_2$ + Distilled $H_2O$) (H and E X250). ..... 83

Plate XII: Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 5 (41.5mg/kgbwt $Hgcl_2$ + 500mg/kg bwt ethanolic extract of *Psidium guajava*) (H and E; X250). ..... 84

Plate XIII Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 6 (41.5mg/kgbwt Hgcl + 10 00mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) (H and E; X250). ..... 85

Plate XIV: Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 7(41.5mg/kgbwt Hgcl + 1000mg/kg bwt ascorbic acid) ) (H and E; X250). ..... 86

Plate XV: A section of the hippocampus (CA3) of Group I of Wistar rats (Control) (H and E; X250). ..... 87

Plate XVI: A section of the hippocampus (CA3) of Group 2 Wistar rats (41.5mg/kg mercuric chloride) (H and E; X250). ..... 88

Plate XVII: A section of the hippocampus of Group 3 Wistar rats (1000mg/kgbwt ethanolic extract of *Psidium guajava*) (H and E; X250). ..... 89

Plate XVIII: Section of the hippocampus (CA3) of Group 4 Wistar rats (41.5mg/kg mercuric chloride + Distilled $H_2O$) (H and E; X250). ..... 90

Plate XIX: Section of the hippocampus (CA3) of Group 5 Wistar rats (41.5mg/kg mercuric chloride + 500mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) (H and E; X250). ..... 91

Plate XX: Section of the hippocampus (CA3) of Group 6animals (41.5mg/kg mercuric chloride + 1000mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) (H and E; X250). .. 92

Plate XXI: A section of the hippocampus (CA3) of Group 7 Wistar rats (41.5mg/kg mercuric chloride + 1000mg ascorbic acid) (H and E; X250). ..... 93

Plate XXII: Section of cerebellar cortex of Group 1 Wistar rats (control) (Cresyl violet Stain; X250). ..... 94

Plate XXIV: Section of cerebellum Group 3 Wistar rats (1000 mg/kgbwt) (Cresyl violet Stain; X250). ..... 95

Plate XXV: Section of the Cerebellar Cortex of Wistar rats in Group 4 (41.5mg/kgbwt $Hgcl_2$+ Distilled) (Cresyl violet Stain; X250). ..... 96

Plate XXVI: Section of the Cerebellar Cortex of Wistar rats in Group 5 (41.5mg/kgbwt $Hgcl_2$+ 500mg/kgbwt of *Psidium guajava*leaves extract) (Cresyl violet Stain; X250). .... 97

Plate XXVII: Section of the Cerebellar Cortex of Wistar rats in Group 6 (41.5mg/kgbwt $Hgcl_2$+ 1000mg/kgbwt of *Psidium guajava*leaves extract) (Cresyl violet Stain; X250). ..... 98

Plate XXVIII: Section of the Cerebellar Cortex of Wistar rats in Group 6 (41.5mg/kgbwt $Hgcl_2$+ 1190mg/kgbwt of Ascorbic Acid) (Cresyl violet Stain; X250). ..... 99

Plate XXIX: Section of layer 5 of the cerebral cortex of Group I (Control) (Cresyl violet Stain; X250). ..... 100

Plate XXX: Section of cerebral cortex (internal pyramidal layer) of Group 2 Wistar rats (41.5mg/kg mercuric chloride) (Cresyl violet Stain; X250). .....101

Plate XXXI: Section of cerebral cortex (internal pyramidal layer) of Group 3 Wistar rat (1000mg/kg bwt of ethanolic extract of *Psidium guajava*) (Cresyl violet Stain; X250).102

Plate XXXII: Section of cerebral cortex (internal pyramidal layer) of Group 4 Wistar rat (41.5mg/kg mercuric chloride + distilled $H_2O$) (Cresyl violet Stain; X250). ..... 103

Plate XXXIII: Section of cerebral cortex (internal pyramidal layer) of Group 5 Wistar rats (41.5mg/kg mercuric chloride + 500mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) (Cresyl violet Stain; X250). ..... 104

Plate XXXIV: Section of cerebral cortex (internal pyramidal layer) of Group 6 Wistar Rat (41.5mg/kg mercuric chloride + 1000mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) (Cresyl violet Stain; X250). ..... 105

Plate XXXV: Section of cerebral cortex (internal pyramidal layer) of Group 7 Wistar rat (41.5mg/kg mercuric chloride +1000mg/kgbwt of ascorbic acid) (Cresyl violet Stain; X250). ..... 106

Plate XXXVI: Section of the hippocampus (CA3) of Group I Wistar rats (Control) (Cresyl violet Stain; X250). ..... 107

Plate XXXVIII: Section of the hippocampus (CA3) of Group 3 Wistar rat (1000mg/kg bwt of ethanolic extract of *Psidium guajava*) (Cresyl violet Stain; X250). ..... 108

Plate XXXIX: Section of the hippocampus (CA3) of Group 4 animals (41.5mg/kg mercuric chloride and distilled water) (Cresyl violet Stain; X250). ..... 109

Plate XXXX: Section of the hippocampus (CA3) of Group 5 Wistar rat (41.5mg/kg mercuric chloride and 500mg/kg ethanolic extract of *Psidium guajava* leaves) (Cresyl violet Stain; X250). ..... 110

Plate XXXXI: Section of the hippocampus (CA3) of Group 6 Wistar rat (41.5mg/kg mercuric chloride and 1000mg/kg ethanolic extract of *Psidium guajava* leaves) (Cresyl violet Stain; X250). ..... 111

Plate XXXXII: Section of the hippocampus (CA3) of Group 7 Wistar rat (41.5mg/kg mercuric chloride and 1000mg/kg of ascorbic acid) (Cresyl violet Stain; X250). ..... 112

## List of Abbreviations

i. WHO: World Health Organisation.....12

ii. ATSDR:Agency for Toxic Substances and Disease Registry.....11

iii. MSDS:Material Safety Data Sheet.....8

iv. DEFRA:Department for Environmental Food and Rural Affairs.....9

v. IPCS:International program on chemical safety.....9

vi. EPA: Environmental Protection Agency.....9

vii. CA: Cornus Ammonis .....31

viii. SEM: Standard Error Of Mean…….....54

ix. EEPGL: Ethanolic Extract of *Psidium guajava*Leaves…….59

x. CAT: Catalase.....66

xi. MDA: Malondialdehyde .....67

xii. SOD:Superoxide dismutase .....69

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Human and animal population interactwith their environment on a daily basis and as such are exposed to a range of chemicals and heavy metals (Wade *et al.,* 2002; Burger *et al.*, 2011). These interactions with the environment occur through food, air and water (Burger *et al.,* 2011). Heavy metals enter into the human and animal bodies through the lungs, stomach or skin and their presence may cause serious toxicity (Jarup, 2003; Alissa and Ferns, 2011). Some of these metals can cross the blood brain barrier and accumulate in the brain tissues causing damages (Langford and Ferner, 1999; Valko*et al.,* 2005).

Mercury is one among the most widespread heavy metals which is found extensively in many environmental and certain occupational settings (Bridges *et al.,* 2014). Mercury occur in the environment as a result of natural processes such as degassing from the earth crust, emissions from volcanoes, evaporation from water bodies, anthropogenic processes from coal-fires, power stations, residential heating systems and waste incinerators (Cox, 1997; WHO, 2006; Burger and Gochfeld, 2011). Mercury widely used in agriculture, pharmacology and industries (Agha *et al.,* 2014). Mercury is the third most dangerous heavy metal after Arsenic and Lead and its toxicity causes serious risk to health through unfavourable pathological and biochemical effects (Othman *et al.,* 2014). The central nervous system has been shown to be its main target (Musa *et al.,* 2009; Ibegbu *et al.,* 2013).

Mercury poisoning can result from inhalation, ingestion and absorption through the skin and may be highly toxic and corrosive once absorbed into the blood stream. High exposures to inorganic

mercury may result in damage to gastrointestinal tract, nervousand renal systems (US EPA, 2010). Symptom of high exposure to inorganic mercury includes: skin rash and dermatitis, mood swings, memory loss, mental disturbances and muscle weakness (US EPA, 2010). Pregnant women and children are most vulnerable to mercury exposure (De Bont*et al.,* 1989; EC, 2005). Children affected by mercury poisoning may have long-term stigmata, including motor impairment, visual loss, hearing loss, developmental delay, and seizure disorders (Medscape, 2015). Mercuric chloride is one of the most toxic forms of mercury and is primarily nephrotoxic (Lorschieder*et al.,* 1995; Aslanturk*et al.,* 2014; Moraes- Silva 2014), hematotoxic (Durak*et al.,* 2010), hepatotoxic (El- Shenawy and Hassan, 2008; Joshi *et al.,* 2014; Othman *et al*. 2014), neurotoxic (Moraes-Silva *et al.,* 2014) and genotoxic (Rozgaj*et al.,* 2005).

Studies have shown that mercuric chloride exerts negative effects on the reproductive system in male rats (Kalender*et al.,* 2013). Also, consumption of high mercury contaminated fish changes the blood pressure and cardiac autonomic activity (Varella*et al.,* 2011). On the other hand, chronic exposure by inhalation even at low concentration has been shown to cause effect such as tremor, impaired cognitive skill and sleep disturbance in workers (Liang *et al*., 1993). Mercury toxicity in Nigeria was reported in some fishes from Lagos Lagoon (Wegwu, 1999). In Sates like Katsina, Sokoto, Gombe and other Northern States, the use of kohl; a traditional cosmetic had been reported to predispose people to mercury toxicity (Onyeike, 2002). The United Nations Environmental Programme (UNEP) has sounded alarm about the potential increase in the risk of exposure to mercury in African Countries, including Nigeria. This will be a consequence of environmental pollution due to higher level of mercury emissions from a variety of sources. The increased risk of poisoning in Nigeria is as a result of prolonged exposure to mercury during

goldmining and consumption of contaminated fish and shell fish by inhabitants of coastal towns in riverine areas like the Niger Delta, Nigeria (WHO, 2011).

The National Agency for Food and Drug Administration and Control (NAFDAC) expressed concern over the alarming use of bleaching creams and skin lightening soaps. Theactive ingredient in some skin lighteners is mercury and therefore bleaching can lead to mercury poisoning. In Mali, Nigeria, Senegal, South Africa and Togo, 25%, 77%, 27%, 35% and 59% of women, respectively, are reported to use skin lightening products on a regular basis (WHO, 2011).

*Psidium guajava*commonly known as guavais a medium sized tree belonging to the family Myrtaceae which is native to tropical and subtropical countries. It is popular in an indigenous system of folk medicine (Geidam*et al.,* 2007; Sanda*et al.,* 2011). Traditionally it is used for the treatment of various ailments like diarrhoea, wounds, rheumatism, lung problems and ulcers. *Psidium guajava*contains a number of major pharmacologically active ingredients such as: flavinoid, saponins, tannins, alkanoids and anthraquinones (Sanda*et al.,* 2011). It was reported that *Psidium guajava* leaf extract has a wide spectrum of biological activities such as anticough, antibacterial, (Jaiarj*et al.,* 1999) and narcotic properties (Lozoya*et al.,* 1990), anti-inflammatory (Gutierrez*et al.,* 2008) and antioxidant properties (Kimura *et al.,* 1985; Limm*et al.,* 2006; Qian and Nihorimbere, 2004; Adeyemi*et al.,* 2009).

## 1.2 Statement of Research Problem

Mercury is one of the heavy metals that have been reported to cause devastating health problems worldwide (Musa *et al*., 2009). Progressive studies have shown that mercury is a neurotoxin implicated in a wide range of neurological or psychiatric disorders including autism spectrum

disorders, Alzheimer's disease, Parkinson's disease, epilepsy, depression, mood disorders and tremor (Fenglian*et al.,* 2012). However, mercury usage in agriculture, industries and consumption of contaminated aquatic animal (fish) is still on the rise thereby predisposing Human and animal populations to mercury toxicity.

Skin lightening products containing mercury are hazardous to health and as a result have been banned in many countries. However, there are reports of such products still being available to consumers and also advertised on the internet. Currently in Nigeria, 77% of women are reported to use skin lightening products on a regular basis (WHO, 2011). Therefore, mercury exposure still remains a considerable occupational and public health problem.

## 1.3 Justification of the Study

Recent increase in Mercury exposure in our environment has placed heavy burden on health of both adult and children which may result to oxidative stress and severe behavioural anomalies (Valko*et al.,* 2005). *Psidium guajava* leaves possess vital antioxidantsand has been usedin treatment of the most common worldwide diseases. The results to be obtained from this study could be of importance inthe identification and evaluation of available natural remedy in the treatment of mercury-induced toxicity.

## 1.4 Aim and Objectives of the Study

### 1.4.1 Aim of the study

The aim of the study is to evaluate the effect of ethanolic extract of *Psidium guajava* leaves on mercury-induced neurobehavioral, biochemical and histo-architectural changes in theCerebrum, Cerebellum and Hippocampus of adult Wistar rats.

### 1.4.2 Objectives of the study

i. To evaluate the effect of ethanolic extract of *Psidium guajava* leaves on mercuric chloride induced changes on the histo-architecture of the Cerebrum, Cerebellum and Hippocampus of adult Wistar rats using H&E and Cresyl fast violet stain.

ii. To evaluate the effect of ethanolic extract of *Psidium guajava* leaves on mercuric chloride induced change on weights of adult Wistar rats.

iii. To evaluate the effect of ethanolic extract of *Psidium guajava* leaves on mercuric chloride induced change in spatial learning and memory, motor coordination, anxiety and exploratory behaviour in adult Wistar rat.

iv. To evaluate the effect of ethanolic extract of *Psidium guajava* leaves on mercuric chloride induced changes in the biochemical parameters (CAT, SOD, GSH and MDA) of adult Wistar rats.

## 1.5 Scope of the Study

The study was limited to the general histo-architectural, neurobehavioral, and biochemical changes in the brain tissue of adult Wistar rats.

## 1.6 Study Hypothesis

Ethanolic extract of *Psidium guajava* will ameliorative the effect on mercuric chloride on neurobehavioral, biochemical and histological changes in the brain tissues mainly the cerebrum, cerebellum and hippocampus of adult Wistar rats.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Mercury

Amongst the several heavy metals that pose some risk to our environment, thirty- five of them have been listed or known to have significant occupational problems in man. These compounds include Mercury, Arsenic, Lead, manganese, thallium, Cadmium, Trichloroethene, 5-fluorouracil, Aluminium and dichlorodiphenyltrichloroethane. The toxic effects of these compounds are variable and diffuse, involving different part of the nervous system as well as other organ systems (Maurice and James, 1972; Volko*et al*., 2005). These elements are common in our environment and diet, but large amounts of any of them may cause acute or chronic toxicity (Glanze, 1996).

Mercury is a heavy, silvery white transition metal which belongs to group twelve, period six and P-block of the periodic table. It has the symbol Hg, atomic number 80, it is liquid at room temperature and relatively a poor conductor of electricity (WHO, 2003 and 2005; Burger *et al*., 2011). Mercury can be found in the environment in three forms namely; the elementary mercury, inorganic mercury and organic mercury (Bernhoft*et al.*, 2012). The properties of mercuric chloride are shown in the table below (2.1).

### 2.1.1 Forms of mercury

The three forms in which mercury exist are; elementary mercury, organic mercury and inorganic mercury.

i. **Elemental mercury:** This is the most volatile form of mercury that can occur both in liquid and gaseous form in the environment, elementary mercury can combine with chlorine,sulphur, and other elements to form inorganic compounds. Elemental mercury is traditionally used in thermometers and some electrical switches (WHO, 2003 and 2005).

ii. **Organic mercury:** Organic mercury is the most toxic forms of mercury and it is formed as a result of mercury combination with carbon or other elements. Example includes phenyl mercury acetate, methyl mercury and dimethyl mercury. Amongst these organic mercury, methyl mercury is most commonly found in the environment (ATDRS, 1995; WHO, 2005).

iii. **Inorganic mercury:** Inorganic mercury compounds also referred to as mercury salts are commonly found in nature as white powders or crystals and black powders. Examples of inorganic mercury are; mercuric chloride, mercuric oxide and mercuric sulphide (WHO, 2003).

### 2.1.2 Properties of mercury

**Table 2.1 Properties of mercury**

| PROPERTY | VALUE |
|---|---|
| **Atomic number** | 80 |
| **Atomic weight** | 200.59 |
| **Physical state** | White crystal |
| **Boiling point** | 356.6$^{o}$C |
| **Colour** | White |
| **Chemical formula** | Hg |
| **Density** | 13.55g/ml |
| **Half life** | 6 hours |
| **Latent heat of fusion** | 11.80 J/g or 2.8 cal/g |
| **Latent heat of vaporization** | 271.96 J/g or 65.0 cal/g |
| **Melting point** | -38.9Oc |
| **Odour** | Odourless |
| **PH** | 3.3(10g/l aq.sol |
| **Solubility** | 7.4g in 100g |
| **Thermal conductivity** | 0.092 |
| **Vapour density** | 7.0 |
| **Vapour pressure** | 2x10-3mm Hg |
| **Valency** | 1.2 |

**(MSDS, 2008)**

### 2.1.3 Sources of mercury and its compounds

There are different sources of mercury and its compound in the environment and these includes;

a. **Natural sources**: Mer6cury occurs naturally and it is widely distributed in the environment owing to natural and anthropogenic processes. The major natural sources of mercury in the environment are degassing from the earth crust, emissions from volcanoes, evaporation from water bodies and weathering of rocks (IPCS,1991).
b. **Diffusion and combustion sources:** Burning of fossil fuels (petrol and gas), battery disposal and burning mercury painted wood are all sources of mercury exposure (WHO, 2005).
c. **Consumer products**: This is another source of mercury, eating of contaminated fishes (bio-accumulation) is the main source of inorganic mercury exposure. Photographic toners, cosmetics (Creams, Perfumes, Soaps) and pigmented paints contain mercuric compounds.Mercury has the ability to be absorbed by the digestive tract and enter the blood stream, which, over time, can result in damage to the nervous system (EPA, 2006).
d. **Industrial sources**: Mercury is also released into the air following man-made activities such as mining ore containing mercury and burning fossil fuels. Mercury also enters the environment from fertilizers, fungicides and form solid waste (DEFRA,2002)

### 2.1.4 Exposure to Mercury Compound

There are many routes of exposure to mercuric compound but the evidence of exposure depends on the level of toxicity.

a. **Oral route:** Consumption of contaminated foods such as fishes (Tilapia,Shark and whales) and other marine mammals or grains preserved with mercuric compound can predispose humans to mercury and its compounds (Matheson, 1979;WHO, 2007).
b. **Occupational exposure:** Occupational exposure to mercury may be a major source of exposure. Individuals working in industries where electrical equipments, thermometers or barometers are used and those working in chemical processing plants or construction sites may all be exposed to mercury vapour via inhalation (WHO, 2005).
c. **Dermal exposure:** The use of mercury containing ointments, creams and soaps can result in dermal conditions(Altmann *et al.*,2008).
d. **Inhalation exposure:** Inhalation of air is also another pathway of exposure, especially for workers in mercury-related occupations. Mercury vapour is easily absorbed when inhaled and can result to mercury poisoning (WHO, 2003).

### 2.1.5 Mercury exposure, effects and toxicity

a. **Nervous system effects:** Mercury has been a major nervous system problem over decades (Brain and Fred 1995). Mercury is a potential factor in brain damage(Ibegbu *et al.*, 2014), mental impairment, behavioural anomalies(Sadeeq*et al*., 2013), neuromuscular weakness, hearing problem, impaired cognitive function and coma (Liuji*et al*., 2002). Children treated with tablets containing mercurous chloride exhibited irritability, fretfulness, sleeplessness, weakness, photophobia and confusion (WHO, 2005).
b. **Reproductive effect:** Progressive studies showed that exposure to mercury compounds can be embryo-toxic and teratogenic while others suggested complications such as increased menstrual disturbances, prolonged uterine cycle, spontaneous abortion as well as other

pregnancy related complications (Berlin, 2007). Inanother study, subcutaneous injectionof 9.5mg mercury/kg body weight administered as mercuric acetate to dams, produced a variety of malformations, including cleft palate, hydrocephalus and heart defects in mice (Gale and Ferm, 1971).

c. **Genotoxic effect**: There is little information concerning the potential geno-toxicity of mercury compounds (Wulf*et al*.,1986). However, heavy metals like mercury and lead has been associated with chromosomal aberrations, elongation of X-chromosome, pulverization and birth defects (Valverde*et al*., 2002; Hassan *et al*., 2010).

d. **Gastrointestinal effects**: Ingestion of mercuric chloride is highly irritating to the tissue of the gastro intestinal tract (ATSDR, 1999). Blisters and ulcers on the lips and tongue as well as vomiting were observed in a 19 months old baby who ingested an unknown amount of mercuric chloride powder(Samuels *et al*.,1982) while vomiting, diarrhoea, salivation, swollen gum, ulceration and many other gastrointestinal infections were recorded (WHO, 2005).

e. **Hepatic effect**: Children and experimental animals exposed to mercury presented hepatomegaly, jaundice (WHO, 2005), elevated liver enzyme, hepatic enlargement and softened liver (Ibegbu *et al*., 2014).

f. **Respiratory effect**: Respiratory symptoms are the prominent effect of short term high level exposure to mercury vapours. The most common reported symptoms include cough, chest tightness, chest pain, impairment of pulmonary function (WHO, 2005), pulmonary Oedema (Murphy *et al*., 1979), airway obstruction and desquamation of bronchiolar epithelium and death (ATSDR, 1999).

### 2.1.6 Toxicokinetics of mercuric chloride

a. **Absorption:** The rate of transportation of mercuric chloride across the intestinal tracts may depend on its solubility(Friberg and Nordberg, 1973) or how easily it dissociates in the lumen to become available for absorption. However, absorption decreases with decreasing solubility (Endo *et al*.,1990;Glynn, 1991). The rate of oral absorption of mercuric chloride in rodents is dependent on intestinal pH, age and diet (Glynn1991; WHO, 2005). Mercuric salt can be absorbed through the skin of humans via mercuric ointments (Berlin *et al.*,2007)

b. **Distribution:** Inorganic mercury distributes to all tissues in the body, it crosses the blood brain barrier by the L-type neutral amino acid carrier transport system to accumulate in the brain tissue (Farina *et al*., 2011) while the liver and kidney accumulate inorganic mercury readily(Ibegbu *et al*,2014). Mercury deposits readily in the placenta and fetal tissue and is found in breast milk(Berlin *et al.,* 2007).

c. **Metabolism of mercury**: Metabolism of mercuric chloride is similar for humans and laboratory mammals. Once absorbed mercuric chloride enters an oxidation reduction cycle (ATSDR, 1999). The oxidation of mercuric chloride may also occur in the brain(WHO, 2005). Liver, lungs, placenta and other tissues(WHO,2005; Berlin *et al*.,2007). The rate of oxidation is dependent on concentration of catalase in the tissue, endogenous production of hydrogen peroxide and availability of mercury at the oxidation site (Clackson, 1989; Berlin *et al*., 2007).

d. **Excretion**: Elimination of mercury is via urine, faeces expired air, sweat and saliva (WHO, 2005). The urine and feaces are the main excretory pathways of mercury compounds inhuman(WHO,2005)with urinary excretion accounting for 13%and 58% of the total body burden after a short and long term exposure respectively(Berlin, 2003)

## 2.2 *Psidium guajava*

The plant *Psidium guajava*popularly known as “guava”is native of South Eastern Asia. It is a small tropical tree that grows up to 35 feet tall widely grown for its fruit in tropics. It is a member of the Myrtaceae family (Table 2.2), with about 133 genera and more than 3800 species (Fagbohun*et al*., 2013). The leaves of this plant are dark green, elliptic, ovate or oblong rounded with an obtuse apex (Plate 2.1). The plant is mostly grown in tropical and subtropical countries. Besides been consumed as fresh fruit, it can be processed into juice, jams and preserved product (Gutierrez *et al*., 2008)

### 2.2.1 Taxonomy of *Psidium guajava*

**Table 2.2** ***Taxonomy of Psidium guajava*****leaves**

| **Domain** | **Eukaryot** |
|---|---|
| **Kingdom** | Plantae |
| **Phylum** | Spermatophyte |
| **Subphylum** | Angiospermae |
| **Class** | Dicotyledonae |
| **Order** | Myrtales |
| **Family** | Myrtaceae |
| **Genus** | Psidium |
| **Species** | *Psidium guajava* |

(Begum *et al*., 2002)



**Plate 2.1 *Psidium guajava*leaves and fruit**

Common names of *Psidium guajava*

English: Guava

Hausa: Gwaiba

Igbo: Gwoba

Yoruba: Guafa(Begum *et al*., 2002)

### 2.2.2 Medicinal uses of *Psidium guajava*

*Psidium guajava*is a well-known traditional medicinal plant and is used in various indigenous systems of medicine. The leavesand bark of *Psidium guajava* tree have a long history of medicinal uses that are still employed today (Fagbohun*et al.,* 2013). The fruits are often included among super fruits, being rich in dietary fiber, vitamins A and C, folic acid and dietary minerals such as potassium, copper and manganese. Having a generally broad, low-calorie profile of essential nutrients, a single common *Psidium guajava* fruit contains about four times the amount of Vitamin C as an orange, these constituents of *Psidium guajava*made it possible to be used traditionally for treatment of various ailments.

More recent ethno- pharmacological studies showed that *Psidium guajava*is used in many parts of the world for the treatment of a number of diseases such as diabetes, hypertension, wounds, inflammations and respiratory disorders (Gutierrez *et al*., 2008). The part of the plant mostly used is the leaves, fruits, bark and the roots. However, the plant as a whole is sometimes used for various medicinal purposes (Geidam*et al*., 2007). The extract of the leaves is used as febrifuge, antispasmodic and for rheumatism in India (Hernandez, 1971). It is also used to treat diarrhea and stomach ache in Columbia, Mexico, Maya, Nahuatl, Zapotec, USA and Mozambique.

The leaves are used in USA as an antibiotic in the form of poultice or decoction for wounds, ulcers and tooth ache (Leonti*et al*., 2001). In South Africa and Caribbean, extract of the leaves is used in management of diabetes and hypertension. Latin America, Central and West Africa and South East Asia use decoction of the leaves as gargle for sore throats, swelling of the mouth, laryngitis, external ulcers on the skin and vaginal irritations (Ojewole, 2005). *Psidium guajava*is

used to expel the placenta after childbirth and in the treatment of infections of the skin, caries, vaginal haemorrhage wounds, fever, dehydration and respiratory disturbances (Gutierrez *et al*., 2008). The root is used in West Africa as a decoction to relieve diarrhoea, coughs, stomach ache, dysentery, toothachesand indigestion. In general, the whole plant or it roots are used in the form of infusion, decoction and paste as skin tonic and as analgesia in painful menstruation, miscarriages, uterine bleeding, premature labour and wounds in Nigeria and many parts of the world today(Gutierrez *et al*., 2008).

**Table 2.2.3** ***Photochemical in ethanolic extract of Psidium guajava leaves***

| PHYTOCHEMICALS | PROPORTION |
|---|---|
| Alkaloids | - |
| Lipids | + |
| Carbohydrates | + |
| Tannins/Polyphenols | ++ |
| Reducing Sugar | + |
| Terpenoids | + |
| Flavonosides | +++ |
| Steroid glycosides | + |
| Anthraquinones | - |
| Saponins | + |

**Key: (+++: Highly present) (++: Moderately present) (+: Faintly present) (-Absent)(Adeyemi*et al*.,2009)**

Pharmacological screening of the ethanolic extract of *Psidium guajava* shows that flavonoid, tannins, alkaloids, saponins and steroid glycosides are present as shown in Table 2.3 (Katteregaet *al*., 2014). Toxicological study in mice and other animal models as well as controlled human studies show both leaves and fruit are safe without any side effect (Kamathet *al.,* 2008).*Psidium guajava*leaves contains a number of major pharmacologically active ingredients responsible for major biological activities such as antidiarrhoeal, antimicrobial, cardioactive, hepatoprotective, anti-allergic, anti-inflammatory, anti-plasmodial, anti-spasmodic, anti-nociceptive, anti-diabetic and anti-oxidative activity(Adeyemiet *al*., 2009). The extracts of*Psidium guajava*leaves were tested for antibacterial potential and found to be effective against *Staphylococcus aureus, Streptococcus mutatis, Pseudomonas aeruginosa, Salmonella enteritidis, Bacillus cereus, Proteus* spp.*Shigella*spp. and *Escherichia coli*; the major causative agents of intestinal infections in humans (Chahet *al*., 2006; Nair and Chanda, 2007).

The methanolic root extract of *Psidium guajava* that consists of quercetin was also found to be fungicidal (Nair and Chanda, 2007). The active flavonoid compound guaijaverin extracted from leaves of same plant was reported to have high potential antiplaque activity (Brotz-Oesterheltet *al*., 2005; Limsonget *al*., 2004). The leave extracts have trypanocidal activity which may be attributed mainly due to the broad antimicrobial property of the flavonoids and the iron chelating property of tannins (Adeyemiet *al*., 2009). In a study carried out with the leave extracts of *Psidium guajava*have shown potent antimicrobial activities against *Propionibacterium acnes* and may be beneficial in treating acne (Qadanet *al*., 2005).

Cellular damage or oxidative injury arising from free radicals or Reactive Oxygen Species (ROS) now appears to be the fundamental mechanism underlying a number of infections, human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive disorders. Free radicals are generated through normal metabolism of drugs, environmental chemicals and other Xenobiotics as well as endogenous chemicals especially stress hormones (Masuda *et al*., 1999). *Psidium guajava*leaves have been used as health tea and contains copious amounts of phenolic phytochemical which inhibit peroxidation reaction in the living body and therefore, can be expected to prevent various chronic diseases such as diabetes, cancer and heart diseases (Kimura *et al*., 1985; Jimenez-Escrig*et al*., 2001).

The decrease of free radicals has antioxidising effect in the body due to the guava leaf polyphenols that prevent arterial sclerosis, thrombosis, cataract and inhibition of senescence changes of the body and skin (Kimura *et al*., 1985). *Psidium guajava* leaves extracts and fruits are a potential source of natural antioxidants (Yan *et al*., 2006). Several studies revealed that guava fruits also exert antioxidant action, collagen formation and radio protective activity(Yan *et al*., 2006). Essential oil leave extracted from *Psidium guajava*leaves was reported to be highly effective in reducing the growth of human mouth epidermal carcinoma and murine leukemia (P388). Another finding suggested that *Psidium guajava*leaves extract have the potential to be developed as new chemotherapeutic agent to inhibit the growth of tumors and cancers (Salib and Michael, 2004).

The alarming increase in diabetes mellitus is becoming a serious problem to human health in all parts of the world particularly Nigeria. With the distinctive ethno medical opinions and natural

medicines mainly originated in herbs, traditional medicine offers good clinical opportunities and shows a brighter future in the therapy of diabetes mellitus and its complications. The decoction of the leaves was screened for hypoglycemic activity in alloxan induced diabetic rats. In both acute and sub acute tests, the extract showed a significant hypoglycaemic activity (Mukhtar*et al*., 2004). Anti-inflammatory and analgesic activities of ethanol extract of leaves were also investigated in rats,*Psidium guajava*leaves showed significant anti-inflammatory activity at a dose of 300 mg/ kgbody weight. A study showed that an ethanol extract of the *Psidium guajava* leaves was investigated for anoxia stress tolerance test and swimming endurance test in Swiss mice and showed significant adaptogenic activity against the stress models (Echemendia and Moron, 2004).

A study to evaluate the antioxidant potential of ethanolic extract of *Psidium guajava* leaves in *invitro* models showed that the extract possesses antioxidant activity in a concentration dependent manner. Based on the result obtained from the study, *Psidium guajava* has ability to minimize oxidative damage in tissues. Superoxide dismutase is an important enzyme in an antioxidant defence system, the study proved that the increasing concentration of the extract have a maximum inhibitory activity against superoxide (Vijayakumar*et al.*, 2015). The ethanolic extract of *Psidium guajava* has proven to be a good source of antioxidant when compared with a standard drug (Vijayakumar*et al*., 2015). A study to evaluatethe antioxidant activities of *Psidium guajava*and*Aloe Vera*showed thatGuava also had significantly higher antioxidant photochemical content and scavenging ability (Chibuike*et al.*,2015). A comparative study of the effect of ethanol extract of *Psidium guajava* leaves with Glibenclamide on experimentally

induced diabetes mellitus in rats showed that *Psidium guajava* significantly reduced blood glucose levels and can be considered a potential anti diabetic agent(Azmary*et al.,* 2017).

## 2.3The Central Nervous System

The central nervous system (CNS) is composed of the brain and the spinal cord. The brain is composed of three parts: the cerebrum, the cerebellum and the brain stem (Moore and Dalley, 2006). The brain is encased in the skull and protected by the cranium (Arthur *et al*., 2010) while the spinal cord is continuous with the brain caudally and is protected by the vertebra (Huijzen*et al*., 2008; Arthur *et al*., 2010).

### 2.3.1 The Cerebral Cortex

The cerebral cortex is the largest part of the brain; it is formed by grey matter and cerebral hemisphere. The cerebral cortex is about 1.5-4mm thick forming convolution with projections called sulci and gyri. The cerebral cortex can be divided into four sectionsknown as lobes. The frontal lobe, parietal lobe, occipital lobe, and temporal lobe have been associated with different functions ranging from reasoning, intelligence, planning to auditory perception. The cerebral cortex contains nerve cells, fibers, neuroglia and blood vessels; anterior, middle and posterior cerebral arteries (Kandel *et al.,* 2000).

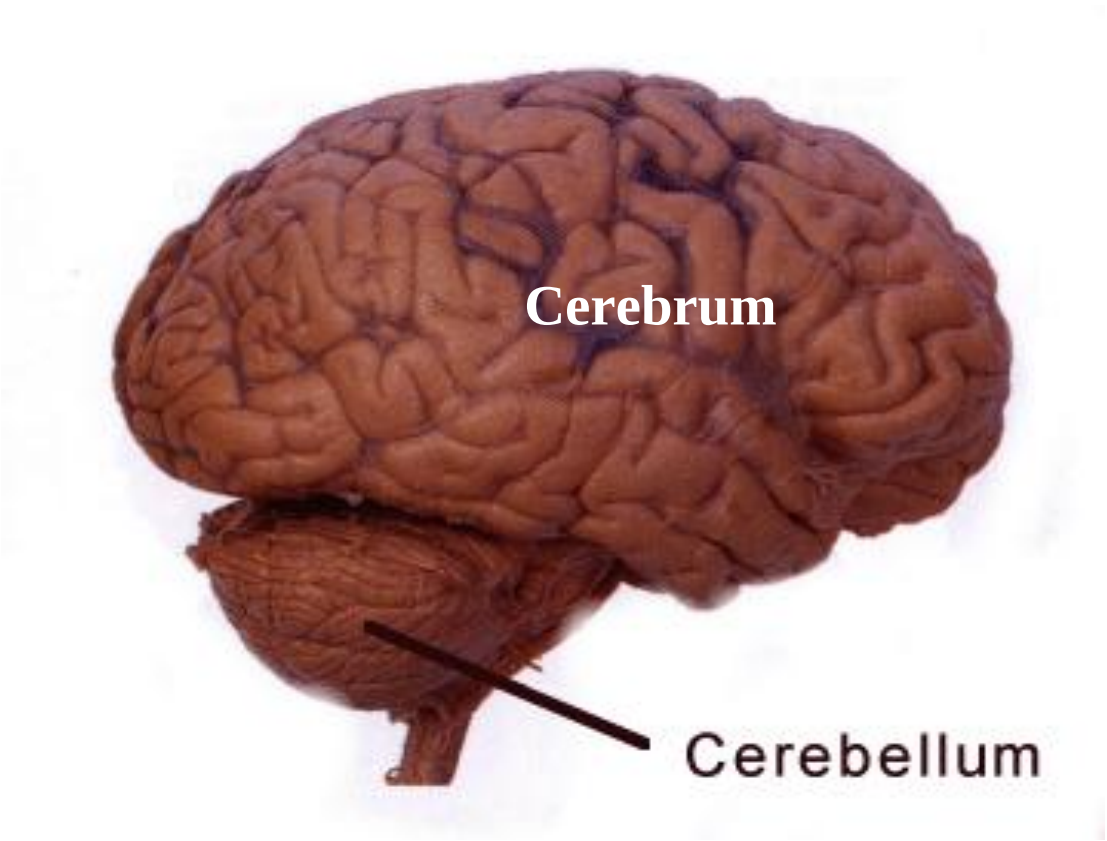



**Plate 2.2**The three major parts of the brain(Moore and Dalley, 2006)

**The cerebral cortex is divided into functional areas namely;**

**Motor area**; the motor area of the classical description is located in the pre central gyrus on the superior lateral surface of the hemisphere, and in the anterior part of the paracentral lobule on the medial surface. It corresponds to area 4 of Brodmann and possibly to the part of area 6 which lies in the pre-central gyrus. Specific region within the area are responsible for specific movement of specific part of the bodyStimulation of the paracentral lobule produces movement of the lower limbs. The trunk and the upper limb are represented in the upper part of the precentralgyrus, while the head and the face are represented in the lower part of the gyrus (Singh, 2008).

**Premotor area**; the premotor area is located just anterior to the motor area. It occupies the posterior part of the superior, middle and inferior gyri. The part represented in the superior and middle frontal gyri correspond to area 6 and area 8 of the brodmann. The part in the inferior precentralgyri corresponds to area 44 and area 45, it constitutes the motor speech area of Broca. Stimulation of the premotorarea results in movement. But these movements are more intricate than those produced by the motor area (Singh, 2008).

**Sensory area**; the central area according to the classical classification is located in the post central gyrus. It corresponds to areas 1,2 and 3 of Brodman. It also extends to the medial side of the hemisphere where it lies in the posterior part of the paracentral lobule. Response can be recorded from the sensory area when the body part are stimulated (Singh, 2008).

**Visual area**; this area is concerned with vision and is located in the occipital lobe, mainly on the medial surface. Area 17 extends into the cuneus and into the lingual gyrus. Posteriorly it extends into thesuperolateral surface where it is limited anteriorly by the lunate sulcus. Area 17 is

continous above and below with the area 18 and beyond this with area 19. Area 18 and 19 are responsible for interpretation of visual impulses reaching area 17. They are often described as psychovisual area.Acoustic area is located at the temporal lobe. It is the area concerned with hearing. It corresponds to areas 41, 42 and 52 (Singh, 2009).

### 2.3.2 Areas of the cerebral cortex

The Frontal Lobe contains areas that Brodmann identified as involved in cognitive functioning and in speech and language.Area 4 corresponds to the precentralgyrus or primary motor area. Area 6is the premotor or supplemental motor area.Area 8 is anterior of the premotor cortex. It facilitates eye movements and is involved in visual reflexes as well as pupil dilation and constriction.Areas 9, 10, and 11 are anterior to area 8. They are involved in cognitive processes like reasoning and judgment which may be collectively called biological intelligence including executive function.Areas 44 and 45 are Broca'sarea(McCaffrey, 2014).

TheParietal Lobeplays a role in somatosensory processes. Areas 3, 2, and 1 are located on the primary sensory strip, with area 3 being above the other two. These are somasthetic areas, meaning that they are the primary sensory areas for touch and proprioception including kinesthesia.Areas5, 7, and 40 are found posterior to the primary sensory strip and are considered presensory association areas where somatosensory processing occurs.Area 39 is the angular gyrus (McCaffrey, 2014).

Areas involved in the processing of auditory information and semantics as well as the appreciation of smell are found in the Temporal Lobe.Area 41 is Heschl'sgyrus, the primary auditory area.Area 42 immediately inferior to area 41 and is also involved in the detection and

recognition of speech. The processing done in this area of the cortex provides a more detailed analysis than that done in area 41. Areas 21 and 22 are the auditory association areas. Both areas are divided into two parts; one half of each area lies on either side of area 42. Collectively they can be called Wernicke's area. Area 37 is found on the posterior-inferior part of the temporal lobe. (McCaffrey, 2014).

The Occipital Lobe contains areas that process visual stimuli. Area 17 is the primary visual area.Areas 18 and 19 are the secondary visual (association) areas where visual processing occurs (McCaffrey, 2014).

### 2.3.3 Layers of the cerebral cortex

The type and size of cell found in the cortex varies at different dept from the surface. Many of the myelinatedfibers are arranged in bands parallel to the pia surface. Brodman (1909) recognized six layer (Fig. 2.3) (Ranson and Clark, 1943);




**Figure 2.3** The cellular layers of the cerebral cortex (Kandel*et al.*, 2000).

The molecular layer: is the most superficial and contains the superficial band of tangential myelinatedfibers and many neuroglia cells. The nerve cells which are not numerous are of two types, the horizontal cells of Cajal, and the granule cells. Within this layer ramifies the terminal branch of the apical dendrite from the pyramidal cell of the deeper layer (Saladin, 2010).

**The external granular layer;** also known as the layer of small pyramidal cells, and contains a large number small nerve cells. Some of these are small pyramid with axons running to the white centre of the hemisphere. Others belong to the short axon group namely Golgi type II or granule cell.

**The pyramidal cell layer;** these may be divided into two sub strata; the more superficial layer contains chiefly the medium sized pyramidal cell while the deeper layer contains the large pyramidal cells. There are also cells of Martinotti and the granular cells.

**The internal granular layer**; also referred to as the layer of small stellate cells is characterized by the presence of large multi polar cells with short axon. Scattered among these cells are the pyramidal cell.

**The ganglionic cell layer;** this layer contains large pyramidal cells which arealthough smaller than those in the deeper strata of the third layer. Axons of some pyramidal cells give off collaterals which ramify in the cortex, and then course to sub cortical region as projection fibers. In the motor area it contains giant pyramidal cells of Betz, which gives origin to some of the fiber of the corticospinal tract. The apical dendrites of these cells are very large and like those of the more superficial pyramidal cells, reach and ramify within the molecular layer. Axons of some

of the inner cells appear to run through the corpus calossum to the opposite cortex. The horizontal fibers of Baillarger's internal line are found in this layer in most of the cortical areas.

**Fusiform cell layer**; this contains irregular fusiform and angular cells, the axon of which enters the subjacent white matter(Ranson and Clark, 1947).

### 2.3.4 Cell of the cerebral cortex

Five different kinds of cells can be enumerated in the cerebral cortex; pyramidal, the granule cells, the fusiform cells, the horizontal cells of Cajal and finally the cells of Martinotti(Ranson and Clark, 1943).

The pyramidal cells are the most numerous and are classified as small, medium, large and giant pyramidal cells. From the base of the pyramidal cell body, an axon extends toward the subjacent white matter, giving off collaterals which ramify in the adjacent cortex. The dendrites are of two kinds: the large apical dendrite and numerous smaller ones attached to the base and sides of the pyramid.The apical dendrite appearsas an extension of the cell body and is directed toward the surface of the cortex, near which it ends in spreading branches. Its length varies with the depth of the cell body from the surface(Ranson and Clark, 1943).

The granule cells also known as the pyramidal cells, are for the most part of small size, and their short axons branch repeatedly and terminates at the neighbourhood of the cell origin. Although they occur in most layers of the cortex, they are especially numerous in the second and third layers which are accordingly designated as internal and external granular cell layer(Ranson and Clark, 1947).

The cells of Martonotti, are found in most of the cortical strata, and have as their distinguishingcharacteristics, axons which are directed toward the surface of the cortex and ramify in the superficial layer. The horizontal cells of cajal, which are present only in the superficial layer, and are fusiform, with long branching dendrite directed horizontally. Their axon forms targentialfibers in the superficial layer.Fusiform cells or polymorphous cells are found in the deepest stratum of the cortex. Their axons enter the subjacent white matter(Ranson and Clark, 1947).

**Functions associated with the cerebral cortex**

The cerebral cortex is the most highly developed part of the human brain and is responsible for determining intelligence, motor function, planning and organisation, touch sensation, thinking, perceiving, producing and understanding language. The cerebral cortex is divided into lobes; frontal lobe, parietal lobe, temporal lobe(Kandel*et al*., 2000:Shipps, 2007)

### 2.3.5 The hippocampus

**Morphology and formation of the hippocampus**

The hippocampus is a brain structure which lies under the medial temporal lobe of the cerebral cortex, one on each side of the brain. The hippocampus together with the dentate gyrus,sibiculum

is termed “the hippocampal function”. It belongs to the limbic system and plays importantroles in the consolidation of information from short term memory to long term memory and spatial navigation (Chris and Neil, 2008)

**Cells and layers of the hippocampus**

The hippocampus is composed of multiple subfields. Though, terminologies vary among authors, the term most frequently used are dentate gyrus and the cornusammonis (CA). The dentate gyrus contains the fasia dentate and the hilus while the cornusammonis is differentiated into fields CA1, CA2 and CA3 (Chris, 2008). The CA regions are structures depth-wise in clearly defined strata(Phillippe, 2007; Annabelle, 2009).

**Stratum Alveus**: is the deepest layer and contains the axons from pyramidal neurons passing on towardsthe fornix which is 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;theoriens,pyramidal and radiatum are located in this stratum(Annabelle,2009)

**Stratum pyramidale** contains the cell bodies of the pyramidal neurons, which are the principal excitory neurons of the hippocampus. This stratum tends to be the one of the more visible strata of the naked eye. This stratum contains synapses in CA3 region of the hippocampus(Chris and Niel, 2008).

**Stratum lucidum**isthe thinnest in the hippocampus and itis only found in CA3 region. The mossy fibres from the dentate gyrus granule cells course through this stratum in CA3, through synapses can be found in stratum pyramidale.

**Stratum radiatum** like stratum oriens contains schaffer and commissural fibres and interneuron such as basket cells.

**Stratum lacunosum** is a thin layer stratum that contains Schaffer collateralfibres but due to its small size it is often grouped together with stratum molecular into a single stratum called stratum lacunosum-moleculare(Chris and Niel,2008) while stratum Molecular is the most superficial stratum in the hippocampus.

**The dentate gyrus**

The dentate gyrus is composed of unidirectional projections dispersed towardsCA3 pyramidal cells of the hippocampus(Treves*et al*., 2008) the three layers of the dentate gyrus includes; molecular, granular and polymorphic layers. The middle layer is most prominent and contains granule cells that project to the CA3 subfield of the hippocampus (Nolte, 2002).

**Functions of hippocampus**

The hippocampus is believed to play several important functions ranging from memory formation, organisation and storage(Nakashiba*et al*., 2012). Hippocampus is essential in the formation of new memories, and connecting emotions and senses such as smell and sound to memories (Bliss, 2010). 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.

## Memory

Memory is the process of storing information from learning, which acquire information about experiences overtime (Goelet*et al*., 1986;Mark*et al*., 2007)

## Types of memory

### Sensory memory

Sensory memoryisthe earliest stage of memory. During this stage, sensoryinformation from the environment isstored for a very brief period of time, gradually for nolonger than half-second for visual information and 3or 4 for auditory information(Mandler,1967;Bruce *et al*.,2006).

### Short term memory

Most of the information kept in short term memory can be stored forapproximately 20-30 seconds but are quickly forgottenbecause of the limited capacity (Cowan, 2001) which means that information is not retained indefinitely. However, memory capacity can be increased through chuckingprocess (Miller, 1956).

### Long term memory

Long term memory involves the posterior cinglate and can store information in larger quantities for potentially unlimited duration, sometimes a wholelife spans(Alberini, 2001). Its capacity is immeasurably large. Hence, we can remember telephonenumbers for many years through repetition; this information is said to be stored in long term memory(Baddeley, 1996). The hippocampus is essential for learning new information and the consolidation of informationfrom

short term to long term memory, although it does not seem to store information of its own self (Gross, 1993).

### 2.3.6The cerebellum

The Cerebellum is often referred to as the little brain or as a neuronal machine. It coordinates many function of the brain (Albus,1971). The cerebellum influences mainly motor behaviour, eye movement and coordination(Cooper *et al*., 1999). Cerebellar projections are also thought to influence respiration cognition and mediate detection of sensory discrepancy and production of sensory events(Nixon and Possimgham, 2001). The mammalian cerebellum is complicated and interrupted by many transverse fissures. The deep primary and posteriolateral fissures divides the mammalian cerebellum into an anterior, a posterior and a flocculonodular lobe, respectively.

The shallower fissures subdivide the cerebellum into ten (10) lobes (Voogd and Ruigrok, 1997). Longitudinally, two furrows create a thin strip in the midline called the vermis with a hemisphere on each side. Although the distinction between the vermis and the hemisphere is not obvious in the anterior cerebellum, a division by a shallow groove which contains the paravermal vein in each hemisphere is futher subdivided into an intermediate and a lateral zone. The cerebellum is supplied by superior cerebellar artery, anterior inferior cerebellar artery and posterior inferior cerebellar artery (Voogd and Glickstein, 1998).

### 2.3.7 Histology of the cerebellum

The cerebellar cortex forms a series of deeply convoluted folds or folia supported by a branching central medulla of white, the cortex is seen to consist of three layers:

a. The outer molecular layer

b. The inner granular layer

c. Purkinje cell layer

Molecular layer: The outer molecular layer is made up of few neurons and large numbers of unmyelinated fibres, basket cells, Stellate cells and as well parallel fibres, which are the axons of the granule cells. The extensive dendritic tree of the Purkinje cell extends into the molecular layer (Young *et al.,* 2007).

Purkinje layer: Purkinje layer of cerebellar cortex is the middle and most important layer of the cerebellar cortex. Purkinje cells have very large cell bodies, a relatively fine axon extending down through the granular cell layer, and extensively branching dendritic system which arborizes into the outer molecular layer. All of the inputs to the cerebellum are directed toward influencing the firing Purkinje cells, and only axons of Purkinje cells leave the cerebellar cortex (Young *et al*., 2007).

The inner granular cell layer; it is the innermost layer of the cerebellar cortex and it is deep and contains Golgi cells, granule cells, and glomeruli cells. The non-myelinated axons of the numerous small neurons present in the granular layer ascends outwards to the molecular layer where they bifurcate to run parallel to the surface to synapse with the dendrites of the Purkinje cells. Each glomerulus is surrounded by a glial capsule and contains a

granule cell and axons of Golgi cells, which synapse with granule cells. The granule is the only excitatory neuron within the cerebellar cortex (Young *et al*., 2007).

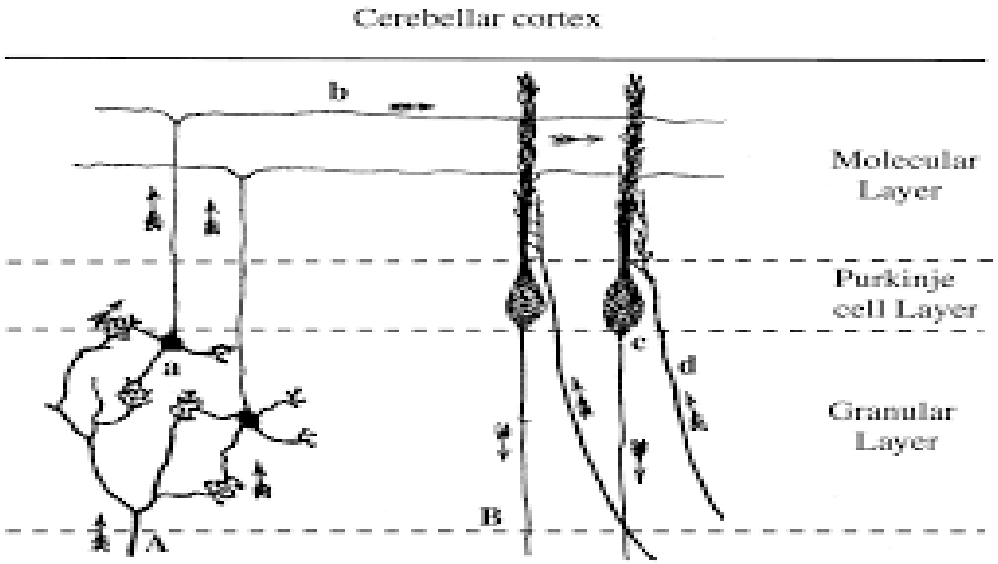



**Figure 2.5:Layers of the Cerebellar cortex (Ito, 2001).**

# CHAPTER THREE

# MATERIALS AND METHOD

## 3. 1Materials

Materials used in the present study include the following; Adult Wistar rats(150-200g), Mercuric chloride, fresh leaves of *Psidium guajava,* Ascorbic Acid, Light Microscope, Amscope, Computer, Tissue Processing Reagents, Cages, Animal feed, Distilled water, Weighing Balance (Kitchen and Digital weighing scale), 1ml needle and syringe, Sacrificing chamber, Bouin's fluid, Gloves, Dissecting tray, Dissecting kit, Gloves, Sacrificing chamber, Centrifuge Machine, Ketamin Injection, Pipette, Glass slide, Cover slip, Beam Walking Maze setup, Elevated Plus Maze set up and Morris Water Maze setup.

## 3.2Experimental Animal

Thirty-Five (35) adult Wistar rats were purchased from the Animal house facility, Department of pharmacology and therapy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria-Nigeria. The animals were acclimatized for a period of two weeks and fed with standard diet. The pelletized growers feed contained crude protein (14.5%), fat (7.0%), crude fiber (7.2%), calcium (0.8%) and available phosphorus (0.4%). Drinking of water was allowed *ad libitum.* Theanimals were weighed and randomly divided into seven (7) groups of five (5) animals per group.

### 3.3Chemical Substances

Mercuric chloride manufactured by May and Bakers Chemical Laboratory Limited Dagenham England wit-N202 was purchased from Steve Moore Laboratory store,Samaru- Zaria, Kaduna State was used for this study.

Ascorbic acid manufactured by Sam Pharmaceuticals Limited, Ilorin, kwara State, Nigeria with batch number S42238 purchased from Rukkayah PharmacySamaru- Zaria, Kaduna State was used for this study.

### 3.4 Preparation of *Psidium guajava*

Fresh leavesof*Psidium guajava* was gotten from a farm within Samaru, Zaria, Kaduna State. The leaves were identified and authenticatedby comparing withthe existing specimen with a voucher number 3253in the Herbarium of Botany, Biological Sciences Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria. The leaves were dried at room temperature, extraction of the guava leaves was done in the Department of Pharmacognosy and drug development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.The method of maceration as reported by Kolawole *et al.* (2011) for the preparation of ethanol extracts was employed. Ethanol extraction of the guava leaves was prepared by placing a sample of 1000g of guava leaves in 1.5 L pure ethanol (purity 94.0%) for 4 days at room temperature. After 4 days, the solution was filtered using filter and funnel.The filtrate was then poured into the evaporating dishes and evaporated on the water bath (Digital thermostatic water bath Mc Donald Scientific international – 220volt, 50 Hz and 10A) at a temperature of $80^0$C.With reference to the powdered sample, a yield of 14.2 % of the extracts was obtained.

### 3.5Acute toxicity study

An acute toxicity study of the plant extract was carried out according to Lorkes (1983) to determine the oral median lethal dose ($LD_{50}$).The study was conducted in two (2) phases. In phase one (1) nine (9) animals were used for the experiment. These animals were divided into three (3) Groups with three (3) animals per Group. Animals were given ethanolic extract of *Psidium guajava* leaves at doses of 10, 100 and 1000mg/kg body weight respectively and were placed under observation for 24hours to monitor behavior as well as if mortality will occur. After twenty-four (24) hours of no mortality, the second phase of the experiment was conducted. Three (3) animals were used for the second phase with one (1) animal in each Group. The animals were administered higher doses (1600, 2900 and 5000mg/kg body weight) of ethanolic extract of *Psidium guajava* leaves, they were also observed for 24hours for behavioral changes as well as mortality. The $LD_{50}$ was calculated by the formula;

$LD_{50}= \sqrt{(D_o \times D_{100})}$

$D_0$ = highest dose that gave no mortality

$D_{100}$ = lowest dose that produced mortality

### 3.6Experimental Protocol

Based on the $LD_{50}$of ethanol extract of *Psidium guajava* leaves for Wistar rats which is greater than 5000mg/kg body weight doses of 10% (500mg bwt) and 20% (1000mg bwt) were used in this study.

Based on the reported $LD_{50}$ of Mercuric Chloride whichis 166 mg/kg body weight (Berlin *et al.*, 2007; Sadeeq*et al.*, 2013). 25% of the $LD_{50}$ (41.5mg/kg bwt) was administered in this study.

Based on the reported $LD_{50}$ of ascorbic acid which is 11,900mg/kg body weight (MSDS, 2008), 10 %( 1190mg/kg) of this $LD_{50}$ was used in this study.

**3.7Experimental Procedure**

The rats were divided into Seven (7) groups of five (5) rats each. The animals in Group one (1) was administered with distilled water from 1st to 42ndday; Group two (2) was administered with 41.5mg/kg body weight of mercuric chloride from 1st to 21st day, Group three (3) was administered with 1000mg/kg body weight of ethanolic extract of *Psidium guajava* leaves from 1st to 21st day; Group four (4) was administered with 41.5mg/kg body weight of mercuric chloride from 1st to 21st day and distilled water from 22nd to 42nd day; Group five (5) was administered with 41.5mg/kg body weight of mercuric chloride from 1st to 21st day and 500mg/kg body weight of ethanolic extract of *Psidium guajava* leaves from 22nd to 42nd day while Group six (6) was administered with 41.5mg/kg body weight of mercuric chloride from 1st to 21st day and 1000mg/kg body weight of ethanolic extract of *Psidium guajava* leaves from 22nd to 42nd day. Group seven (7) was administered with 41.5mg/kg body weight of mercuric chloride from 1st to 21st day and 1190mg/kg body weight of ascorbic acid from 22nd to 42nd day. All administration was carried out orally.

**Table 3.1Treatment Schedule**

| **Groups (n = 5)** | **Treatment (dosage/kg body weight)** | **Duration** |
|---|---|---|
| 1 | Distilled water (control) | $1^{st}$ – $42^{nd}$ day |
| 2 | 41.5mg/kg body weight mercuric chloride | $1^{st}$ – $21^{st}$day |
| 3 | 1,984.4mg/kg body weight of ethanol extract of *Psidium guajava* leaves | $1^{st}$ – $21^{st}$ day |
| 4 | 41.5mg/kg of mercuric chloride + Distilled water | $1^{st}$ – $21^{st}$day<br>$22^{nd}$ – $42^{nd}$ day |
| 5 | 41.5mg/kg body weight mercuric chloride + 500mg of ethanol extract of *Psidium guajava* leaves | $1^{st}$ – $21^{st}$day<br>$22^{nd}$ – $42^{nd}$ day |
| 6 | 41.5mg/kg body weight mercuric chloride + 1000mg of ethanol extract of *Psidium guajava* leaves | $1^{st}$ – $21^{st}$day<br>$22^{nd}$ – $42^{nd}$ day |
| 7 | 41.5mg/kg body weight mercuric chloride + 1190 mg/kg body weight *vitamin C* | $1^{st}$ – $21^{st}$day<br>$22^{nd}$ – $42^{nd}$ day |

n= number of animals per group

**Route of administration:** Mercuric chloride, *Psidium guajava* and Vitamin C were administered orally with the aid of orogastric tube

### 3.8Neuro Behavioural Studies

All experimental procedures were conducted in the Animal House Centre, Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.The following neurobehavioral tests were conducted: Morris water Maze test, Beam walking test and Elevated Plus maze. Throughout the training and experimental period, the beam walking test was conducted early hours of the morning(6am), the Morris Water Maze test was conducted at noon Six(6) hours after the beam waking test) while the Elevated Plus Maze test was conducted in the evenings(6pm). This was done to reduce the impact of stress on the animals for each task, stress is known to impact learning and cognition negatively, the order of the test was from the least stressful to the most stressful.

### 3.8.1 Spatial learning and memory test using Morris water maze (MWM)

The Morris Water Maze (MWM) which was first established by neuroscientist Richard G. Morris in 1981 was used in order to test hippocampal-dependent learning, including acquisition of spatial memory and long-term spatial memory(Morris, 1981). The MWM is a relatively simple procedure typically consisting of seven day trials before treatment, the main advantage being the differentiation between the spatial (hidden-platform) and non-spatial (visible Platform) conditions(Eichenbaum *et al*., 1990).

**Apparatus preparation**

A circular poolmade ofaluminium with a diameter of 150 cm and a depth of 50 cm was obtained with gray coated interior. A 10 cm diameter gray coated platform was placed in the pool. The pool was filled with water until the platform was 1cm above the water surface; the water equilibrated to about room temperature (25 °C). The bottom of the pool was divided into four equal parts with a vertical and horizontal marking, using a black paint.

**Testing Procedure**

The rats were transferred from their housing facility to the point of the behavioural test. They were kept in an area where they cannot see the pool or spatial cues to adjust to the new environment for some time before testing. To begin testing, the rats were lifted from the home cage at the tail, supported around the neck,and gently place into the water, facing the edge of the pool. As soon as the rat was placed in the pool of water, it finds the platform before the 60 sec cut off, the rat was allowed to stay on the platform for 5 seconds then return to its home cage (Plate 3.1). In situations where the rat could not find the platform, it was placed on the platform and allowed to stay there for 20 seconds before returning it to its home cage. The process was repeated for all rats in the trail. Each subsequent trial was started with a different platform location and starting direction of north, east and west. When testing was completed, the rats were returned to their housing facility. Rats were dried off and normothermia was assured prior to returning to animal facility. For each rat, the direction of the platform was changed as well as the starting point for the trial. The time it took the rat to locate the platform was recorded for each of the three(3) trials and the average taken to give single escape time latency for each test object.

The rats were trained for 7 days before the experiment and tested weekly throughout the experimental period.



**Plate 3.1 showing: Morris Water Maze Experiment**

### 3.8.2 Elevated plus maze test

The elevated plus maze test was performed according to the methods of Shrestha and Singh (2013). The test was employed to study anxiety related behaviours in Wistar rats.

**Equipment preparation**

The apparatus consisted of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm) which were connected through central platform (10 cm × 10 cm). The arms were arranged in a cross shape with the two open arms facing each other and two closed arms facing each other. The maze was elevated 45 cm above the floor.

**Testing Procedure**

To begin testing, the rats were lifted from the home cage at the tail, supported around the neck, and placed gently at the centre of the plus maze with their faces directed towards one of the closed armsand then the activity of the rat within the maze was carefully observed for a period of five(5)minutes . The number of entries into closed arm (Plate 3.2a) and open arm (Plate 3.b)as well as period of permanence in those areas was observed and recorded in seconds. The number of rearing and head deep was also recorded. The floor and the walls of the open and closed arms werecleaned with 70% alcohol before each trial.After each trial, the rats were returned to their cages.




**Plate 3.2 Shows the elevated plus maze setup (a) shows the test rat in the closed arm and experimental animal in the open arm (b)**

### 3.8.3 Beam walking test

Motor coordination and balance was assessed by measuring the ability of the animals to traverse a narrow beam to reach an enclosed safety platform (Carter *et al*., 1999).

**Equipment preparation**

The test consisted of elevated platforms connected by a 100 cm long wooden beam with a width of 3 cm. The beam was placed horizontally, with one end mounted on a narrow support and the other end attached to an enclosed hamster box (20 x 20 x 20 cm). Beam-walking ability was measured using a 6-point scale described by (Puurunen, 2001).

**Procedure**

The rats were trained for seven (7) days on the beam walking apparatus before the onset of administration**.** To begin testing, the rats were lifted from the home cage at the tail, supported around the neck, and gently at the start point of the beam with their faces directed towards the goal box.Beam walking ability was recorded in seconds; the time the animal spent on the platform, the time spent to traverse the beam, the time taken (latency) until the rat entered the goal box (up to 60 s) was recorded. The apparatus was cleaned with 70% alcohol after each trial and the rats were returned to their cages.

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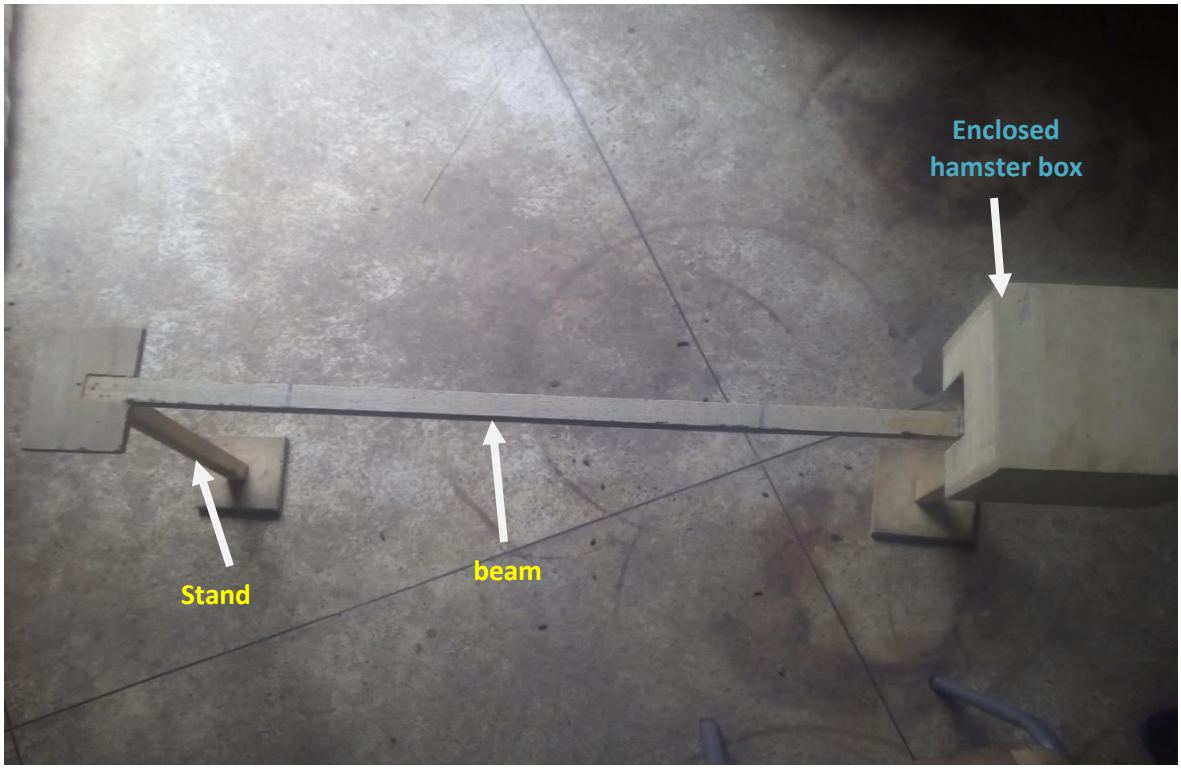



**Plate 3.3 Showing the beam walking test setup, with stand, the beam and the enclosed hamster box.**

### 3.9Morphological Assessment

#### 3.9.1 **Change in weight**

All experimental animals' body weights were taken; at the onset of treatment, weekly throughout the study and at the end of the study before sacrifice. The weight change of the animals in each Group was calculated by subtracting the initial weight of animals from the final weight after experiment.

### 3.10Animal Sacrifice

After the administration, the animals wereeuthanised by 75mg/kg body weight of Ketamin injectionintraperitoniallyin the sacrificing chamber. The skull was opened through a midline to view the tissues. The tissues namely the Cerebrum, Cerebellum and Hippocampus were dissected out, freed from adherent tissues and fixed in Bouins fluid. The fixed tissues were taken to the histology laboratory of Human Anatomy Department, Ahmadu Bello University, Zaria for tissue processing.

### 3.11Estimation of Oxidative Parameter

#### 3.11.1Preparation of tissue homogenates

The brain tissues; Cerebellum, Cerebral cortex and Hippocampus were collected and then 1 g of tissues were immediately homogenized. The tissue homogenates were centrifuged at 3000 rpm for 10 min and the supernatant were utilized for different estimations. Protein amount in brain homogenates were measured according to the method of Bradford (1976).

### 3.11.2 Malondialdehyde assay

MDA levels in brain samples were measured by the method of Uchiyama and Mihara (1978). This method depends on the formation of MDA as an indicator of lipid peroxidation, which reacts with thiobarbituric acid producing thiobarbituric acid reactive substances (TBARS), measured spectrophotometrically at 532 nm.

### 3.11.3 Superoxide dismutase activity assay

Activity of SOD enzyme was evaluated by the method of Sun *et al*. (1988). The analysis of SOD was based upon the principle in which xanthine reacts with xanthine oxidase to produce superoxide radicals. The SOD activity was measured by the level of suppression of this reaction. Results were expressed as U/mg protein.

### 3.11.4 Catalase activity assay

Catalase enzyme activity was evaluated using a spectrophotometric test based on the yellow complex with molybdate and hydrogen peroxide, which was described in detail by Goth (1991) in plasma was expressed with 1, 1, 2, 2,-tetramethoxypropane as the standard (nmol/ml).

### 3.12Tissue Processing

This was done by passing the tissue through ascending grades of alcohol of 70%, 90% and 95% sand 100% for dehydration for two changes of (50 mins each changes respectively). It was then passed into xylene for 40 minutes to clear the alcohol and make it mixable with molten paraffin wax. After this, tissue was transferred into molten paraffin wax for infiltration (Drury and Wallington, 1973). Embedding commenced with the use of molten paraffin as the embedding

media at a temperature of 600C for 40 minutes. After the tissue were embedded in paraffin wax, they were mounted on wooden blocks and then sectioned as follows, the blocked tissue were affixed to a rotating microtome and trimmed to get an even surface prior to sectioning. Thin section 8 - 10 microns each were then cut and the sections were floated on warm water bath to spread the tissue properly prior to mounting on a glass slide. The tissue were then passed through changes of xylene for dewaxing and then taken to water by passing the slide with the tissue intact through descending grades of alcohol-absolute, 100%, 95%, 90%, and 70%, two changes each for each and finally water. The tissues were stained with Crysel fast violet stain and haematoxylin for twenty-five minutes, the excess of which was rinsed off under running tap water. Counter staining for Haematoxylin was then done using eosin, the excess of which was also rinsed off under tap water. After proper staining, the sections were dehydrated through ascending grades of alcohol, followed by clearing with xylene. DPX was used as mountant and the slide were covered with cover slip. Slides were viewed under light microscope and photomicrograph obtained using amscope digital camera.

**Staining:** Two types of staining were done, which includes Haematoxylin and Eosin (H and E) for general tissue structure and Crysl violet fast for demonstration of Nisil substance.

### 3.12.1 Haematoxylin and Eosin (H and E) staining method

The method of H and E staining technique involved deparafinization with Xylene, hydrating the tissue sections in descending grades of alcohol from 100%, 95%, 90% and 70%. Each of these steps were for two changes and lasted for thirty (30) minutes and the tissues were further washed in running tap water. The tissues were stained with haematoxylin for twenty-five (25) minutes, washed with water and then differentiated in acid alcohol. The tissues were counter-stained with

eosin and blued in Scott water, hydrated in ascending grades of alcohol and cleared in xylene for three (3) changes for twenty (20) minutes each. The tissues were then mounted with cover slips using a mounting media. Tissue sections were viewed under light microscope and the photomicrographs were taken using digital amscope.

### 3.12.2 Special Stain using cresyl violet method

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

### 3.13Data Analysis

All the results were analyzed using the Statistical package for Social Scientist (SPSS *version 18)* and the results were expressed as Mean ± SEM. A one-way analysis of variance was done to assess if there were differences in body weight, neurobehavioral assay (Morris water maze and beam walking) and antioxidant activities in experimental animals. A p-value $\leq 0.05$ was considered significant and turkey's LSD post hoc test was also done to test for the least significant difference within the experimental groups. The results were presented in graphs and tables as appropriate.

# CHAPTER FOUR

# RESULTS

## 4.1 Acute Toxicity Study

No lethality was observed when ethanolic extract of *Psidium guajava*leaves was administered orally in Wistar rats even as doses as high as 5000mg/kg bwt. Aside from weakness (noticed in animals that took 5000mg/kg), there were no signs of toxicity after 48 hours and 14 daysobservation in the experimental group. The absence of death at 5000mg/kg bodyweight showed that the $LD_{50}$ of the extract is greater than 5000mg/kg body weight.

## 4.2Physical Observation of Animals During Administration

The result of physical observation showed that animals in Group I (Control)and Group 3 (1000mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) were very active with no visible physical changes observed while animals in Group 2(41.5mg/kg bwt of $HgCl_2$), Group 4(41.5mg/kg bwt of $HgCl_2$+ Distilled $H_2O$), Group 5(41.5mg/kg bwt of $HgCl_2$ + 500mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) Group 6 (41.5mg/kg bwt of $HgCl_2$+ 1000mg/kg bwt of ethanolic extract of *Psidium guajava* leaves)**,** and Group 7(41.5mg/kg bwt of $HgCl_2$ +1190mg/kg bwt of ascorbic acid)showed decreased physical activity, gnawing and restlessness. grooming, frequent urination and watery faeces were also noticed in these Groups within the first 21 days (3weeks) of mercury administration. However,the experimental animals in Group 5 as well as those of Groups 6 and 7 showed increased physical activity progressively during the last 21 days (3weeks) of *Psidium guajava* and Vitamin C administration.

### 4.3Body Weight Before and During the Experiment

The result of body weight assessment showed that there was increase in the body weights of the animals in Groups I (Distilled water)and Group 3 (1000mg/kg bwt ethanolic extract of *Psidium guajava* leaves)during the period of administration whereas the degree of weight gains by animals in Group 2 (41.5mg/kg bwt of Mercuric chloride) and 4 (41.5mg/kg bwt of Mercuric chloride)was significantly lower when compared to Group 1 and other treatment group. Animals in Group 5 (41.5mg/kg bwt of Mercuric chloride + 500mg/kg bwt ethanolic extract of *Psidium guajava* leaves) as well as those of Groups 6 (41.5mg/kg bwt of Mercuric chloride + 1000mg/kg bwt ethanolic extract of *Psidium guajava* leaves) and 7(41.5mg/kg bwt of Mercuric chloride + 1190mg/kg bwt of Vitamin C) showed progressive increase in weight when compared to Group 2 and 4 exposed to mercuric chloride only as shown in Table 4.1

**Table 4.1: Effect of Mercuricchloride exposure, *Psidium guajava* leaves and ascorbic acid onweight of Wistar rats**

| | Initial Weight (g) | Final Weight (g) | Weight Change (g) |
|---|---|---|---|
| Group 1 | 132.33±18.37 | 206.00±12.53 | $73.67\pm5.84^{ab}$ |
| Group 2 | 152.33±24.36 | 179.67±18.94 | $26.67\pm1.45^{acde}$ |
| Group 3 | 153.33±27.64 | 216.67±18.94 | 62.0±1.45 |
| Group 4 | 167.00±17.21 | 200.00±17.56 | $33.00\pm3.61^{bde}$ |
| Group 5 | 163.33±26.71 | 204.67±19.78 | $40.67\pm6.96^{c}$ |
| Group 6 | 172.67±16.75 | 225.67±15.21 | $53.00\pm4.04^{d}$ |
| Group 7 | 152.67±21.11 | 208.00±15.50 | $56.67\pm5.61^{e}$ |

**Group 1 = (**Distilled $H_2O$), **Group 2 = (**41.5mg/kg $HgCl_2$ only**), Group 3 = (**1000mg/kg bwt EEPGL**), Group 4 = (**41.5mg/kg$HgCl_2$+ Distilled $H_2O$**), Group 5 = (**41.5mg/kg $HgCl_2$ + 500mg/kg bwt EEPGL**), Group 6 = (**41.5mg/kg$HgCl_2$+ 1000mg/kg bwt EEPGL**), Group 7 = (**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C**).**

### 4.3 Effect of *Psidium guajava* leaves on mercuric chloride induced cognitive defect In Wistar rats using Morris water maze test

At the end of the training, all the animals learnt how to locate the hidden platform, this was reflected in their ability to locate the platform within a short period of time as shown in table 4.1 The results of spatial learning and memory showed significant increase ($p<0.05$) in the mean time taken to complete Morris water maze task in animals treated with 41.5 mg/kg bwt of mercuric chlorideonly when compared to the control animals and animals treated with *Psidium guajava*leaves extract following mercury exposure. However, there was no significant difference in the mean time taken to complete Morris water maze task in animals treated with *Psidium guajava*leaves extract and Vitamin C following mercury exposure when compared with the control animals as shown in Table 4.2

**Table 4.2: Effect of mercuricchloride exposure, *Psidium guajava* leaves and ascorbic acid on spatial learning and memory**

| | Training | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|
| | (*P* =0.289) | (*P* =0.044) | (*P* =0.002) | (*P* =<0.001) | (*P* =0.002) | (*P* =0.003) | (*P* =0.041) |
| Group 1 | 31.33±3.38 | 15.0±2.65 | $9.22\pm1.20^{ab}$ | $5.67\pm0.67^{abcde}$ | $5.33\pm1.45^{abcd}$ | $4.67\pm1.20^{abc}$ | $3.33\pm0.33^{ab}$ |
| Group 2 | 32.83±4.49 | 15.0±3.61 | 27.0±1.53 | $40.0\pm5.20^{a}$ | - | - | - |
| Group 3 | 21.0±2.75 | $10.0\pm1.16^{a}$ | $6.67\pm1.67^{cdef}$ | 5.67±0.67 | - | - | - |
| Group 4 | 25.83±6.06 | 19.67±3.71 | $26.33\pm3.71^{c}$ | $31.0\pm5.57^{b}$ | $34.33\pm6.36^{a}$ | $43.0\pm6.43^{b}$ | $47.33\pm5.61^{acd}$ |
| Group 5 | 34.33±1.26 | $25.67\pm5.24^{ab}$ | $31.67\pm6.74^{d}$ | $37.33\pm5.49^{c}$ | $39.0\pm5.03^{b}$ | $30.67\pm6.06^{c}$ | $27.67\pm11.20^{b}$ |
| Group 6 | 27.83±6.65 | 14.33±3.18 | $31.67\pm5.24^{ae}$ | $40.67\pm2.91^{d}$ | $37.33\pm4.63^{c}$ | $25.67\pm4.10^{db}$ | 14.0±2.89c |
| Group 7 | 20.33±6.06 | 12.67±0.88 | $22.0\pm4.36^{\mathbf{bf}}$ | $34.0\pm5.51^{e}$ | $29.07\pm3.69^{d}$ | $24.33\pm3.84^{eb}$ | 15.33±3.18d |

**Group 1 = (**Distilled $H_2O$), **Group 2 = (**41.5mg/kg $HgCl_2$ only**), Group 3 = (**1000mg/kg bwt EEPGL**), Group 4 = (**41.5mg/kg$HgCl_2$+ Distilled $H_2O$**), Group 5 = (**41.5mg/kg $HgCl_2$ + 500mg/kg bwt EEPGL**), Group 6 = (**41.5mg/kg$HgCl_2$+ 1000mg/kg bwt EEPGL**), Group 7 = (**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C**).**

**Means in a column sharing superscripts are significantly different from one another**

### 4.4Effect of *Psidium guajava* leaves on mercuric chloride induced anxiety related behaviours in Wistar rats using Elevated plus Maze test

The result of the elevated plus maze test performed by experimental rats showed that animals treated with 41.5mg/kg bwt of mercuric chloride(Groups 2, 4,5,6, and 7) had the least number ofentriesinto open and closed arm, least duration in open arm, highest duration in closed arm and least number of grooming, rearing and head deep when compared to animals in the Group 1(Control) which were active throughout the experiment, having the highest number entry/ duration in closed and open arms, highest number of rearing, grooming and head deep.Following treatment with *Psidium guajava* leaves extract, animals in Group 5, 6 and 7 showed increased activity as shown on (Table 4.3 and 4.4) although the difference observed within the groups was not statistically significant.

**Table 4.3Effect of mercuric chloride exposure, *Psidium guajava* leaves and ascorbic acid on anxiety related behaviour using Elevated plus maze test**

| | NEOA | NECA | REARING | HEAD DIP | DOA | DCA |
|---|---|---|---|---|---|---|
| Group 1 | 19.83 | 13.83 | 15.67 | 20.00 | 3.60±0.29 | 59.80±17.51 |
| Group 2 | 3.83 | 6.00 | 5.17 | 8.50 | 1.11±0.06 | 209.20±2.91 |
| Group 3 | 11.67 | 14.17 | 16.17 | 17.00 | 3.85±0.35 | 62.80±20.99 |
| Group 4 | 5.67 | 5.33 | 7.17 | 3.50 | 0.64±0.20 | 237.80±11.81 |
| Group 5 | 11.67 | 13.83 | 5.83 | 7.00 | 2.11±0.09 | 157.60±11.95 |
| Group 6 | 10.17 | 11.00 | 14.00 | 15.50 | 2.40±0.06 | 162.00±3.52 |
| Group 7 | 14.17 | 13.83 | 13.00 | 15.50 | 2.30±0.64 | 157.80±38.30 |
| P | 0.064 | 0.053 | 0.091 | 0.144 | 0.068 | 0.099 |

**Group 1 = (**Distilled $H_2O$), **Group 2 = (**41.5mg/kg $HgCl_2$ only**), Group 3 = (**1000mg/kg bwt EEPGL**), Group 4 = (**41.5mg/kg$HgCl_2$+ Distilled $H_2O$**), Group 5 = (**41.5mg/kg $HgCl_2$ + 500mg/kg bwt EEPGL**), Group 6 = (**41.5mg/kg$HgCl_2$+ 1000mg/kg bwt EEPGL**), Group 7 = (**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C**).**

**NEOA: Number of entries into open arm, DOA: Duration or time spent in the open arm, NECA: Number of entries into closed arm, DCA: Duration or time spent in the closed arm**

**Table 4.4Effect of mercuric chloride exposure, *Psidium guajava* leaves and ascorbic acid onanxiety related behaviours using Elevated plus Maze test**

| | NEOA | NECA | REARING | HEAD DIP | DOA | DCA |
|---|---|---|---|---|---|---|
| Group 1 | 17 | 11.83 | 16.33 | 16.67 | 3.08±0.38 | 91.00±22.86 |
| Group 4 | 2 | 8 | 2.33 | 2 | 0.80±0.25 | 227.80±14.84 |
| Group 5 | 7 | 11.83 | 6.33 | 5.67 | 2.64±0.28 | 115.60±15.27 |
| Group 6 | 10.67 | 9.33 | 11.83 | 10.17 | 2.99±0.26 | 96.40±15.57 |
| Group 7 | 11.5 | 8 | 10.83 | 11 | 2.89±0.16 | 102.60±9.70 |
| P | 0.075 | 0.839 | 0.127 | 0.084 | 0.086 | 0.061 |

**Group 1 = (**Distilled $H_2O$), **Group 2 = (**41.5mg/kg $HgCl_2$ only**), Group 3 = (**1000mg/kg bwt EEPGL**), Group 4 = (**41.5mg/kg$HgCl_2$+ Distilled $H_2O$**), Group 5 = (**41.5mg/kg $HgCl_2$ + 500mg/kg bwt EEPGL**), Group 6 = (**41.5mg/kg$HgCl_2$+ 1000mg/kg bwt EEPGL**), Group 7 = (**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C**).**

**Means in a column sharing superscripts are significantly different from one another**
**NEOA: Number of entries into open arm, DOA: Duration or time spent in the open arm, NECA: Number of entries into closed arm, DCA: Duration or time spent in the closed arm. Differences were not significant (p< 0.05).**

### 4.5Effect of *Psidium guajava* leaves on motor deficit in Wistar rats using beam walking test

From the result of the beam walking performed by the test rats by week 3, the highest time taken to cross the beam (TTCB) was observed in mercuric chloride treated Groups; Group 2(41.5mg/kg $HgCl_2$**),**Group 4 **(**41.5mg/kg$HgCl_2$**+** Distilled $H_2O$**),** Groups 5**(**41.5mg/kg $HgCl_2$ **+** 500mg/kg bwt EEPGL**),** Group6(41.5mg/kg$HgCl_2$**+** 1000mg/kg bwt EEPGL**)**andGroup 7**(**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C). By week 6, the highest time taken to cross the beam was only observed in Group 4 when compared to Group 1(Distilled $H_20$ and other treatment Groups as values obtained were statistically significant. However decrease in time taken to cross the beam was observed in Group 5, 6 and 7 at the end of 6 weeks as values obtained were not statistically significant when compared to Group 1(control) as shown in Table 4.4

**Table 4.5:Effect of mercuric chloride exposure, *Psidium guajava* leaves and ascorbic acid on motor coordination using beam walking test**

| | Training | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|
| | (*P* =0.279) | (*P* =0.008) | (*P* =<0.001) | (*P* =<0.001) | (*P* =0.028) | (*P* =0.031) | (*P* =0.024) |
| Group 1 | 15.02±3.20 | 4.90±1.33 | $4.89\pm0.89^{abcd}$ | $3.58\pm0.57^{abcdef}$ | $3.66\pm0.69^{abcde}$ | $3.81\pm0.73^{abc}$ | $3.15\pm0.54^{abc}$ |
| Group 2 | 18.18±4.73 | 10.04±1.09 | $16.27\pm0.74^{a}$ | $25.75\pm2.05^{b}$ | - | - | - |
| Group 3 | 25.71±2.73 | 13.22±2.20 | $10.99\pm2.13^{ef}$ | 6.25±1.64 | - | - | - |
| Group 4 | 18.86±1.94 | 9.57±2.82 | 14.0±3.51 | $25.04\pm3.80^{c}$ | $29.87\pm6.45^{b}$ | $26.80\pm6.58^{b}$ | $25.55\pm6.52^{b}$ |
| Group 5 | 28.34±4.04 | 13.94±0.87 | $19.60\pm0.34^{b}$ | $28.43\pm0.91^{d}$ | $29.11\pm0.98^{c}$ | $21.28\pm2.78^{c}$ | $16.76\pm2.04^{c}$ |
| Group 6 | 21.44±5.98 | 16.21±2.83 | $22.62\pm6.91^{ce}$ | $27.80\pm1.14^{e}$ | $20.93\pm1.90^{d}$ | 17.31±1.75 | 7.08±1.03 |
| Group 7 | 18.67±3.44 | 18.73±2.67 | $25.27\pm2.79^{df}$ | $35.45\pm0.57^{f}$ | $30.49\pm2.31^{e}$ | 16.53±1.97 | 9.84±0.70 |

**Group 1 = (**Distilled $H_2O$), **Group 2 = (**41.5mg/kg $HgCl_2$ only**), Group 3 = (**1000mg/kg bwt EEPGL**), Group 4 = (**41.5mg/kg$HgCl_2$+ Distilled $H_2O$**), Group 5 = (**41.5mg/kg $HgCl_2$ + 500mg/kg bwt EEPGL**), Group 6 = (**41.5mg/kg$HgCl_2$+ 1000mg/kg bwt EEPGL**), Group 7 = (**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C**).**

**Means in a column sharing superscripts are significantly different from one another.**

## 4.6 BIOCHEMICAL STUDIES

The result revealed that the level of Catalase (CAT) were lower in groups exposed to mercuric chloride (Groups 2 and 4) when compared to the Group 1(Control) and other treatment groups as values obtained were statistically significant ($p<0.05$) as shown in Fig. 4.1. There was significant increase in Malondialdehyde (MDA) concentration in Groups 2 and 4 compared to Group 1 and other Groups as shown in Fig 4.2. The level of glutathione (GSH) in Groups 2 and 4 was significantly lower when compared to Group 1 and other treatment Groups ($p<0.05$) as shown in Fig 4.3. In addition, the study showed a significant decrease ($p<0.05$) in SOD biomarkers in Group 2and Group 4 whencompared with Group 1 and other treatment groups and a significant increase in SOD concentration in Group3 when compared to all the groups as shown in Fig 4.4

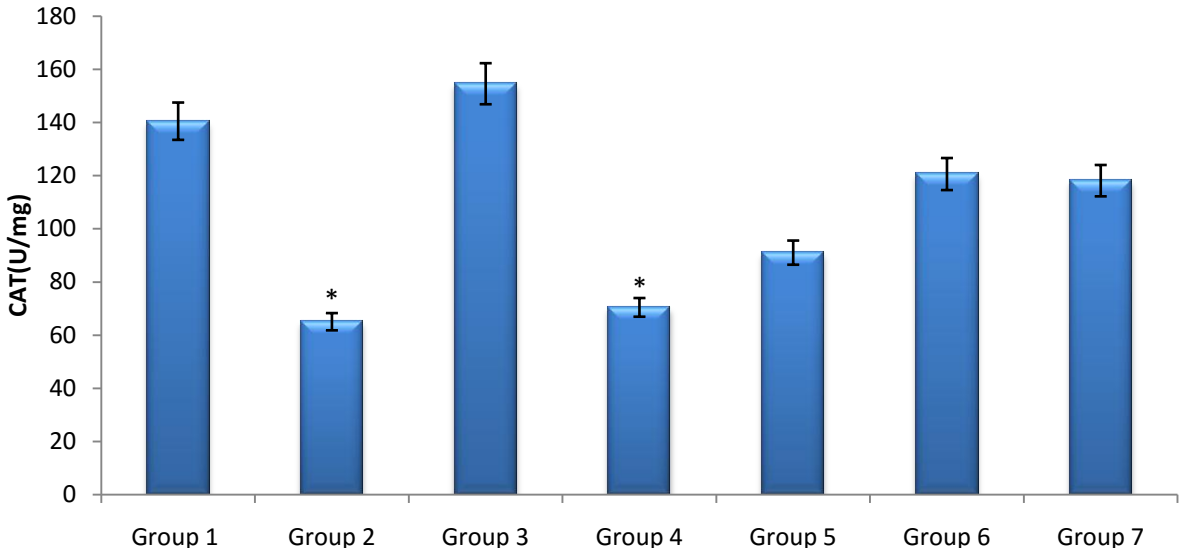



**Figure 4.1: CAT concentration in the brain tissues of animals exposed to mercuric chloride**

**Group 1** = **(**Distilled $H_2O$), **Group 2** = **(**41.5mg/kg $HgCl_2$ only**), Group 3** = **(**1000mg/kg bwt EEPGL**), Group 4** = **(**41.5mg/kg$HgCl_2$+ Distilled $H_2O$**), Group 5** = **(**41.5mg/kg $HgCl_2$ + 500mg/kg bwt EEPGL**), Group 6** = **(**41.5mg/kg$HgCl_2$+ 1000mg/kg bwt EEPGL**), Group 7** = **(**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C**).**

***= Statistically significant CAT = Catalase**

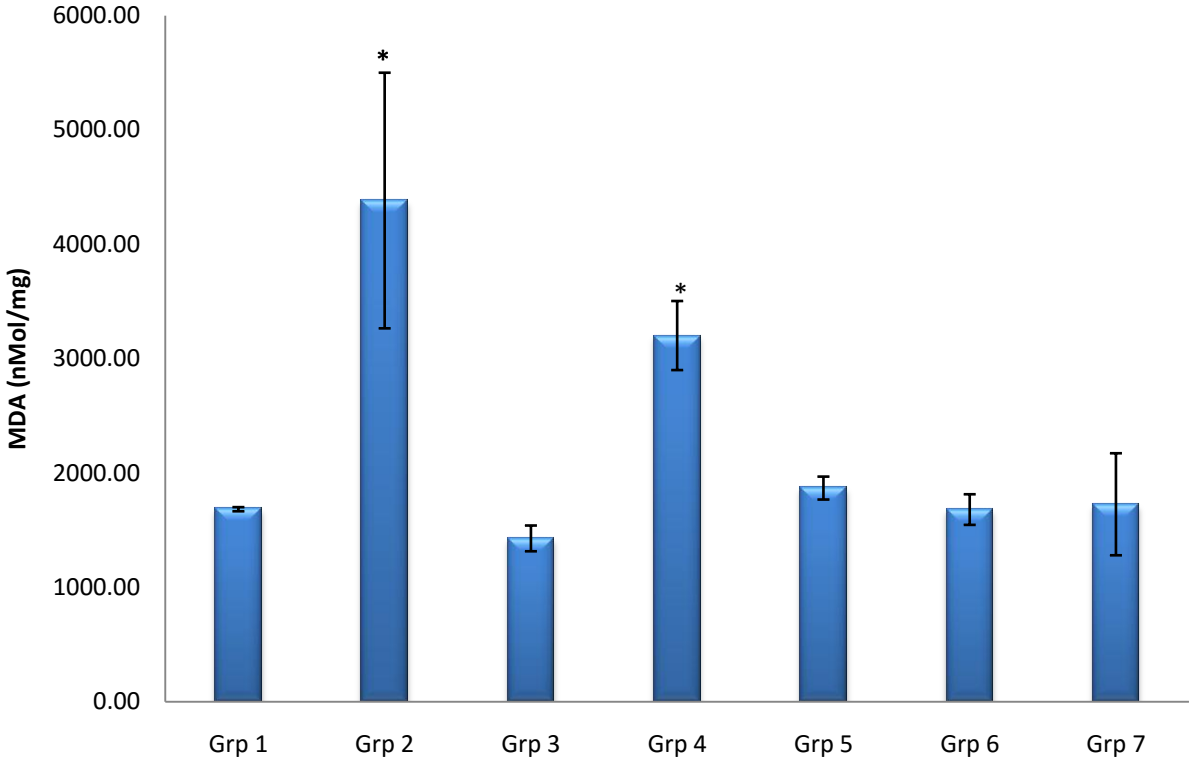



**Fig 4.2: MDA concentration in the brain tissues of animals exposed to mercuric chloride**

**Group 1 = (**Distilled $H_2O$), **Group 2 = (**41.5mg/kg $HgCl_2$ only**), Group 3 = (**1000mg/kg bwt EEPGL**), Group 4 = (**41.5mg/kg$HgCl_2$+ Distilled $H_2O$**), Group 5 = (**41.5mg/kg $HgCl_2$ + 500mg/kg bwt EEPGL**), Group 6 = (**41.5mg/kg$HgCl_2$+ 1000mg/kg bwt EEPGL**), Group 7 = (**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C**).**
***=Statistically significant MDA = Malondialdehyde.**

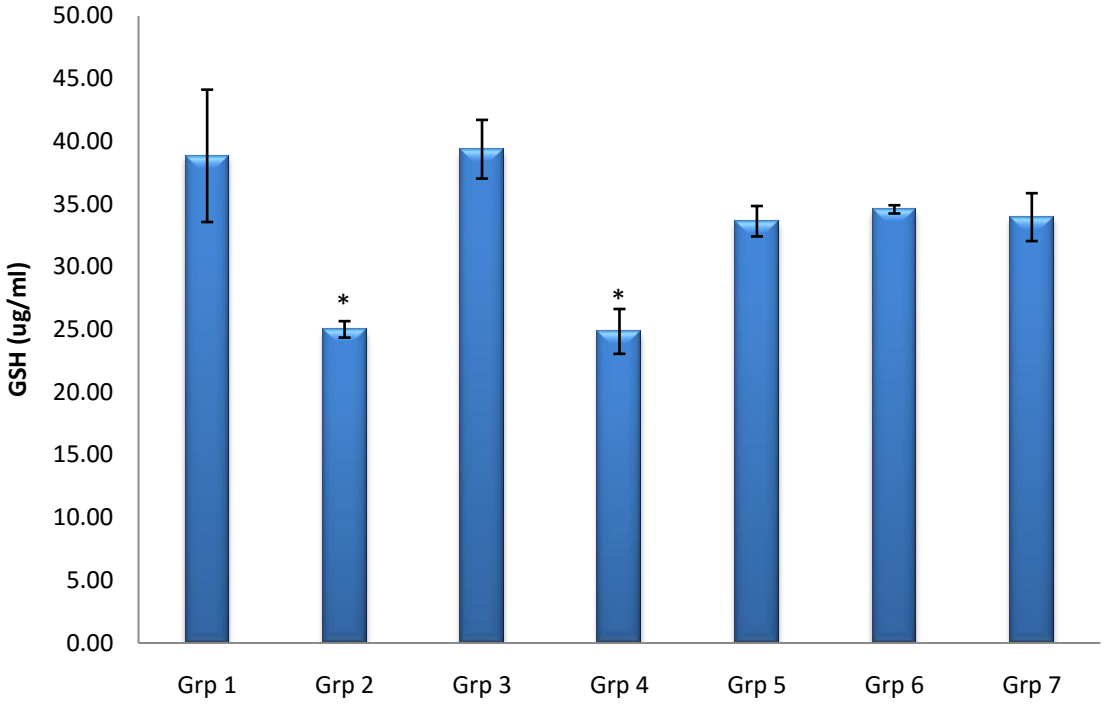



**Fig 4.3: GSH concentration in the brain tissues of animals exposed to mercuric chloride**

**Group 1** = **(**Distilled $H_2O$), **Group 2** = **(**41.5mg/kg $HgCl_2$ only**), Group 3** = **(**1000mg/kg bwt EEPGL**), Group 4** = **(**41.5mg/kg$HgCl_2$+ Distilled $H_2O$**), Group 5** = **(**41.5mg/kg $HgCl_2$ + 500mg/kg bwt EEPGL**), Group 6** = **(**41.5mg/kg$HgCl_2$+ 1000mg/kg bwt EEPGL**), Group 7** = **(**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C**).**
***= Statistically Significant; GSH= Gluthathione**

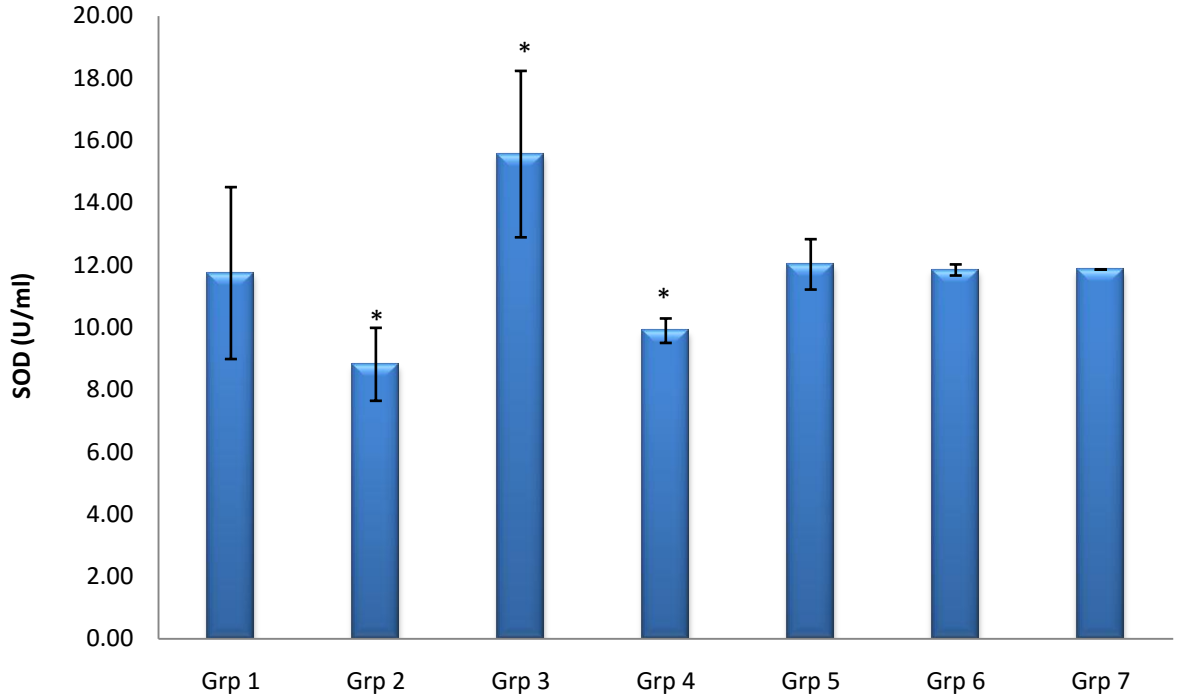



**Fig 4.4: SOD concentration in the brain tissues of animals exposed to mercuric chloride**

**Group 1 = (**Distilled $H_2O$), **Group 2 = (**41.5mg/kg $HgCl_2$ only**), Group 3 = (**1000mg/kg bwt EEPGL**), Group 4 = (**41.5mg/kg$HgCl_2$+ Distilled $H_2O$**), Group 5 = (**41.5mg/kg $HgCl_2$ + 500mg/kg bwt EEPGL**), Group 6 = (**41.5mg/kg$HgCl_2$+ 1000mg/kg bwt EEPGL**), Group 7 = (**41.5mg/kg $HgCl_2$ +1190mg/kg bwt of Vitamin C**).**

***= Significant difference ($p \leq 0.05$). SOD= Superoxide Dismutase**

## 4.7 HISTOLOGICAL STUDIES

The results from histological observations showed histological changes in the tissues studied. The histological analysis showed that animals in Group I had normal cytoachitecture of the Cerebellar cortex with the Purkinje cells of the Cerebellar cortex appearing normal as shown in Plates I and XXII respectively. The animals in Group 2 presented with disorientation of the Pukinje cell layer and degenerating Pukinje cells as shown in Plates II and XXIII. The animals in Group 3**,** showed normal Purkinje cells with normal orientation of the layers in the Cerebellar cortex as shown in Plate III and XXIV. The animals in Group 4showed some normal Purkinje Cells and degenerating purkinje cells as shown in plate IV and XXV. While animals in Group 5, Group 6 and Group 7 presented some normal Pyramidal cells and some evidence of degenerated cells as shown in Plates V,VI, VII and XXVI, XXVII, and XXVIII respectively.

The cerebral cortical sections of Wistar rats in the Control Group showed normal histoarchitecture of the cerebral cortex; the characteristic appearance of neurones arranged into six layers, the layer differing in neuron morphology, size and population density. Particularly the internal pyramidal layer (Layer V) revealed large pyramidal cells called ganglion or Betz cells of the motor cortex and stallate cells as shown in PlatesVIII, X and XXIX, XXX while the result of the histological studies of the cerebral cortex of the treated Wistar rats showed neuronal degenerative changes and vacuolated cell bodies as shown in Plates IX, XI, XII, XIII, XIVand XXXI, XXXII, XXXIII, XXXIV and XXXV respectively

The hippocampus of Group I animals showed normal cytoarchitecture of CA3 region of the hippocampus with Pyramidal cells appearing normal with intact nuclei as shown in Plate XVand XXXVI while Group 2 animals presented with degenerated Pyramidal cells of the CA3 region of

the hippocampus as shown in plate XVI and XXXVII. The hippocampus of animals in Group 3 showed normal cytoachitecture of CA3 region of the hippocampus with pyramidal cells appearing normal with intact nuclei as shown in Plate XVII and XXXVIII. Group 4 animals showed degenerated Pyramidal cells, and some usual Pyramidal cells in Plate XVIII and XXXIX. Group 5, 6, 7 showed normal cytoachitecture of some Pyramidal cells with little evidence of degeneration as shown in Plates XIX, XX, XXI and XXXX, XXXXI and XXXXII respectively.

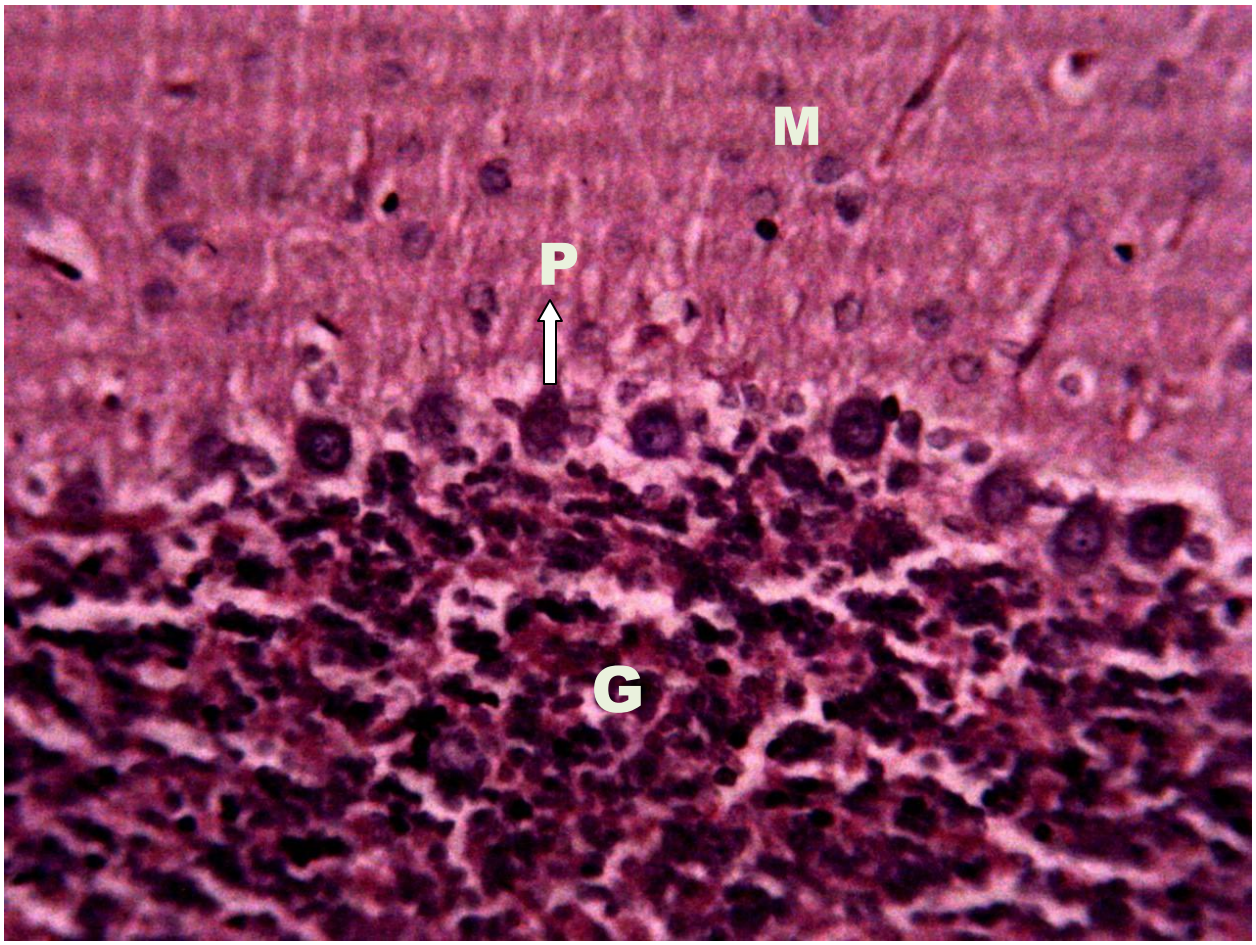



**Plate I Section of the cerebellar cortex of Wistar rats in Group 1 (Control) showing normal orientation of Pukinje cell layer and normal Pukinje cells P: Purkinje cell layer, M: Molecular cell layer and G: Granular cell layer (H and E×250).**

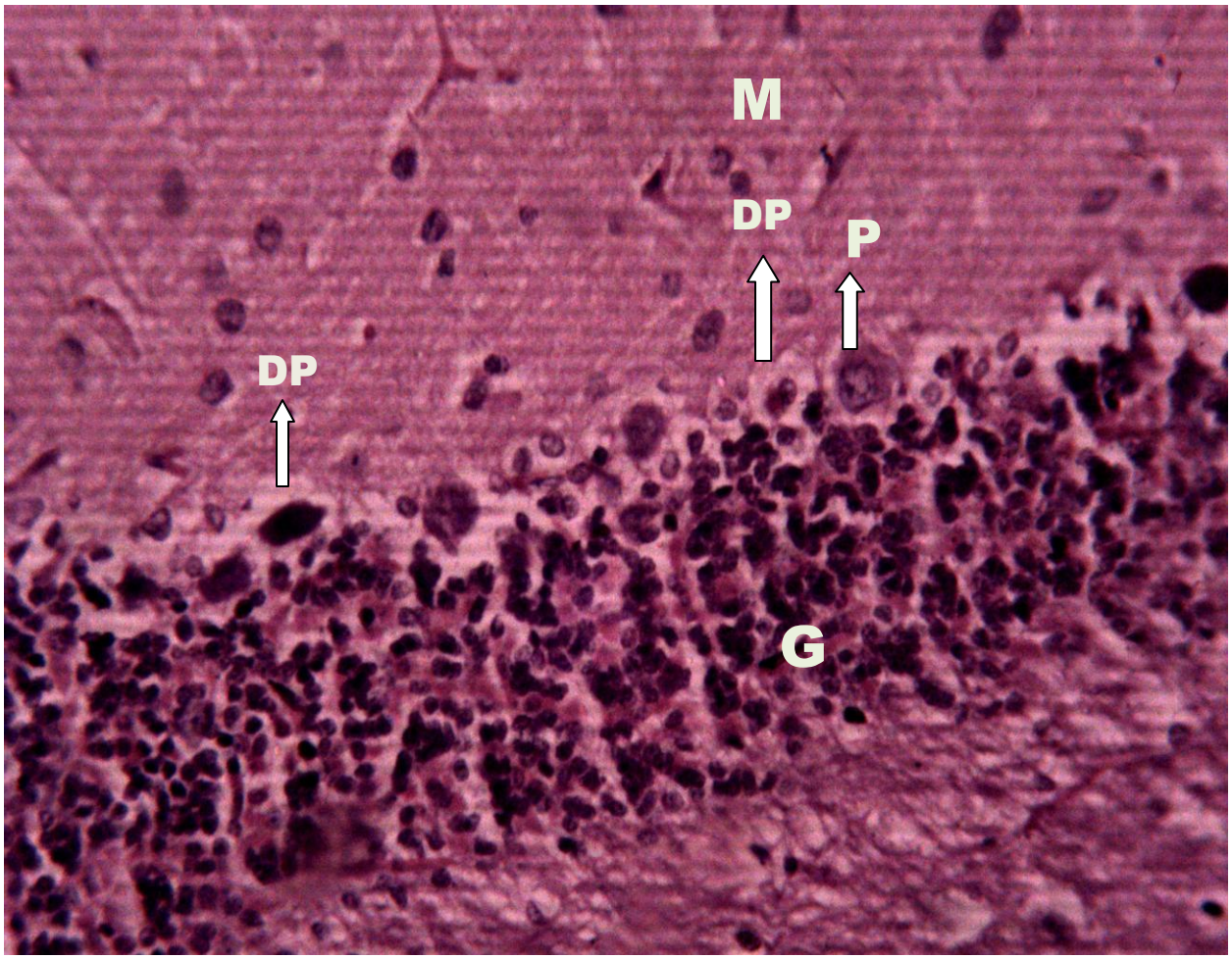



**Plate II Section of the cerebellar cortex of Wistar rats in Group 2 (41.5mg/kg bwt $HgCl_2$) showing disorientation of Pukinje cell layer , DP: degenerated Purkinje cell ; P: Pukinje cell M: Molecular cell layer and G: Granular cell layer (H and E×250).**

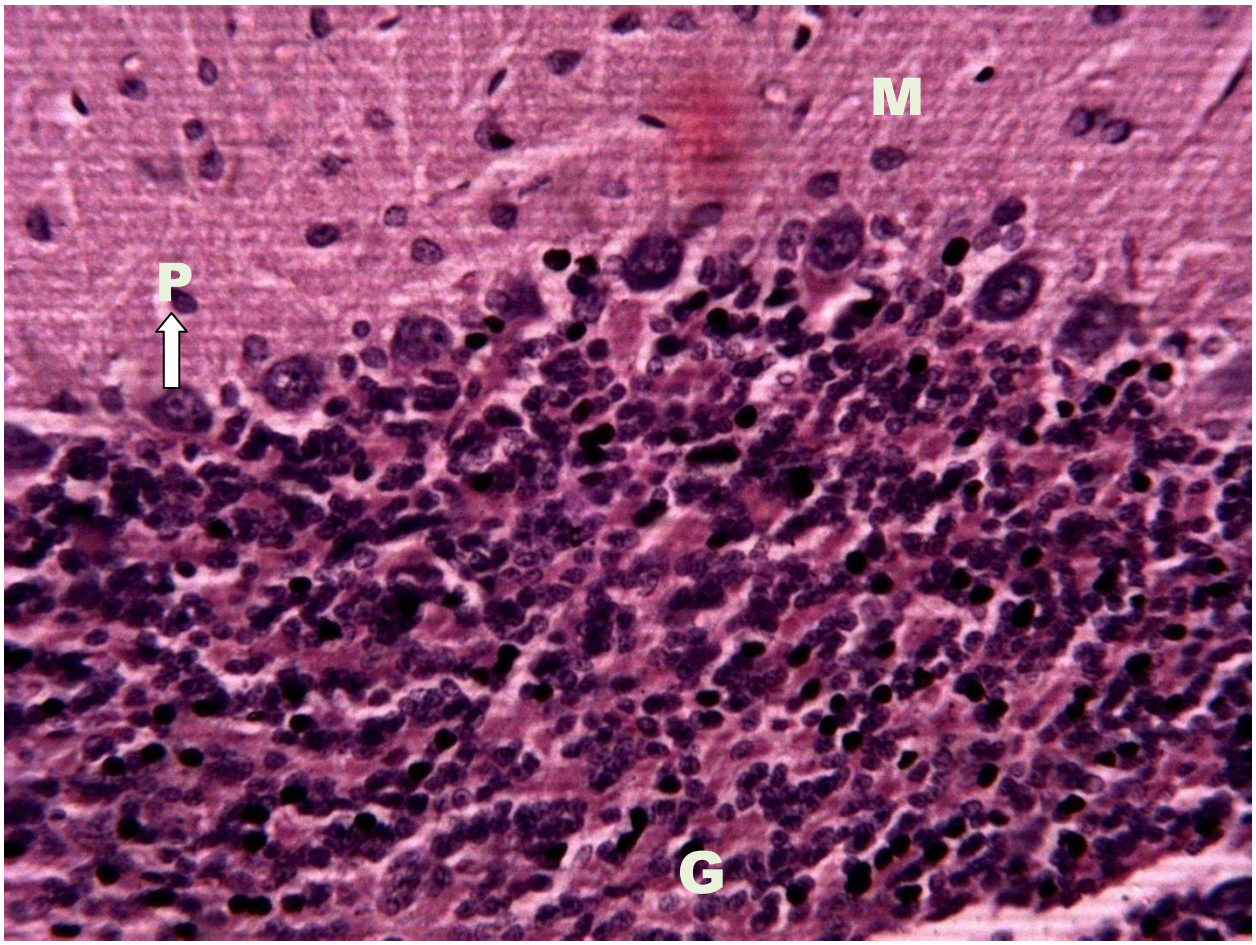



**Plate IIISection of the cerebellar cortex of Wistar rats in Group 3(1000mg/kgbwt ethanolic extract of *Psidium guajava* leaves) showing normal orientation of P: Purkinje cell layer, M: Molecular cell layer and G: Granular cell layer (H and E×250).**

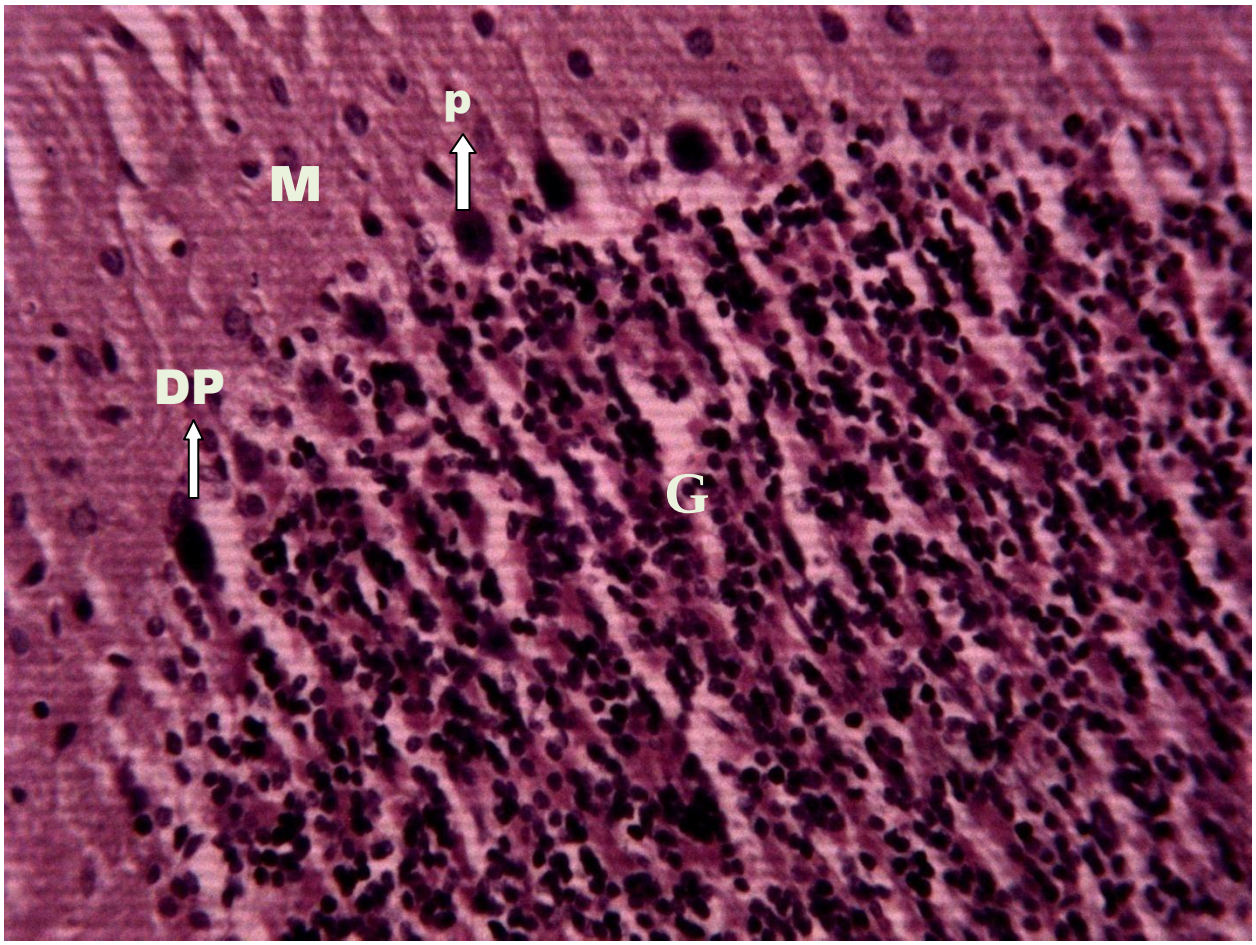



**Plate IV Section of the cerebellar cortex of Wistar rats in Group 4 (41.5mg/kg bwt$HgCl_2$ + distilled water) showing DP: degenerated Pukinje cells P;purkinje cell layer; M: Molecular cell layer and G: Granular cell layer (H and E×250).**

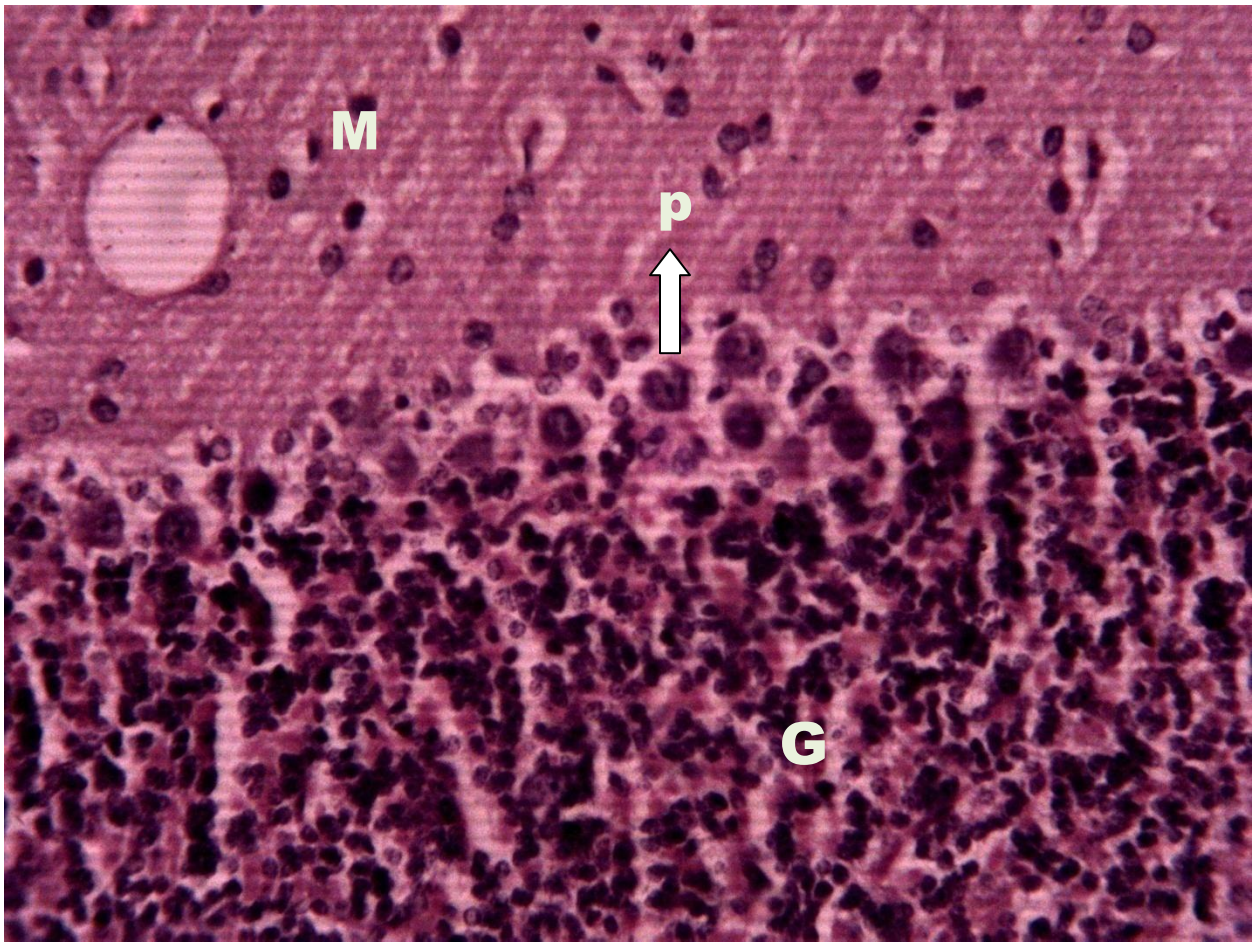



**Plate V Section of the cerebellar cortex of Wistar rats in Group 5(41.5mg/kg bwt $HgCl_2$ + 500mg/kgbwt of ethanolic extract of *Psidium guajava* leaves) showing some normal Purkinje Cells P: Purkinje cell, M: Molecular cell layer and G: Granular cell layer (H and E×250).**

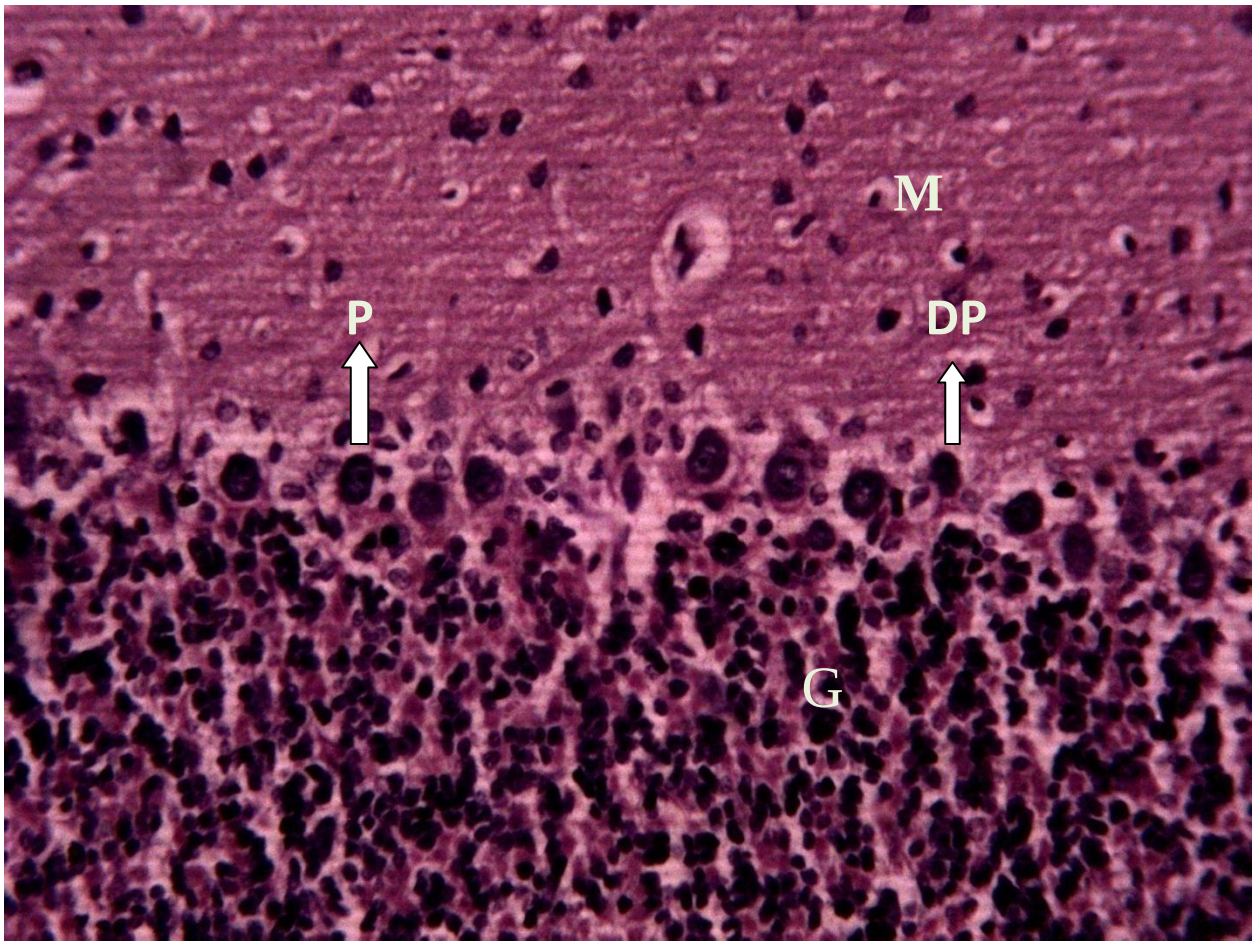



**Plate VI Section of the cerebellar cortex of Wistar rats in Group 6(41.5mg/kgbwt $HgCl_2$ + 10 00mg/kgbwt of ethanolic extract of *Psidium guajava* leaves) showing some normal purkinje cell and DP: degenerating purkinje cells; P: Purkinje cell, M: Molecular cell layer and G: Granular cell layer (H and E×250).**

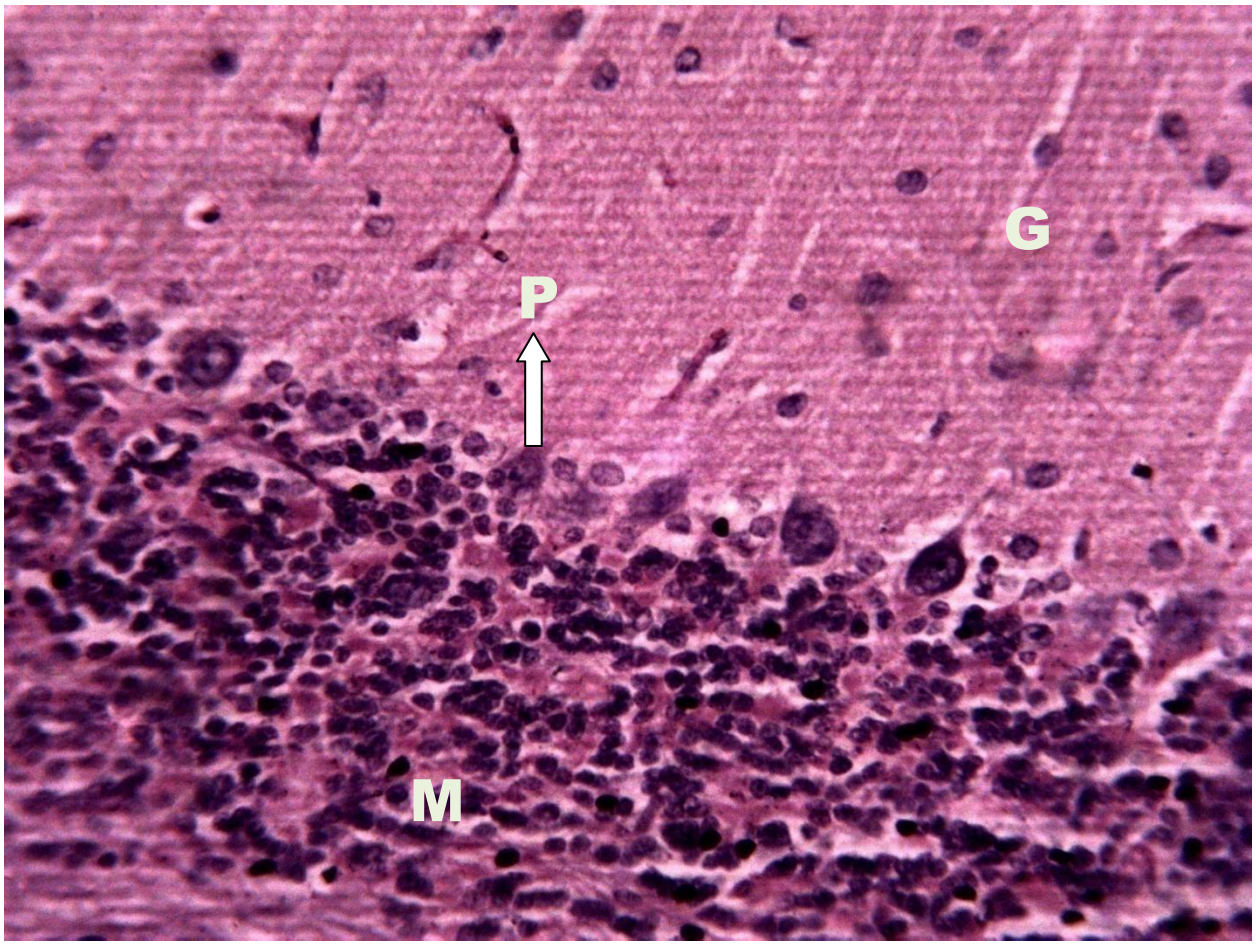



**Plate VII Section of the cerebellar cortex of Wistar rats in Group 7(41.5mg/kgbwt $HgCl_2$ + 1000mg/kgbwt ascorbic acid) showing some normal Purkinje cells P: Purkinje cell, M: Molecular cell layer and G: Granular cell layer (H and E×250).**

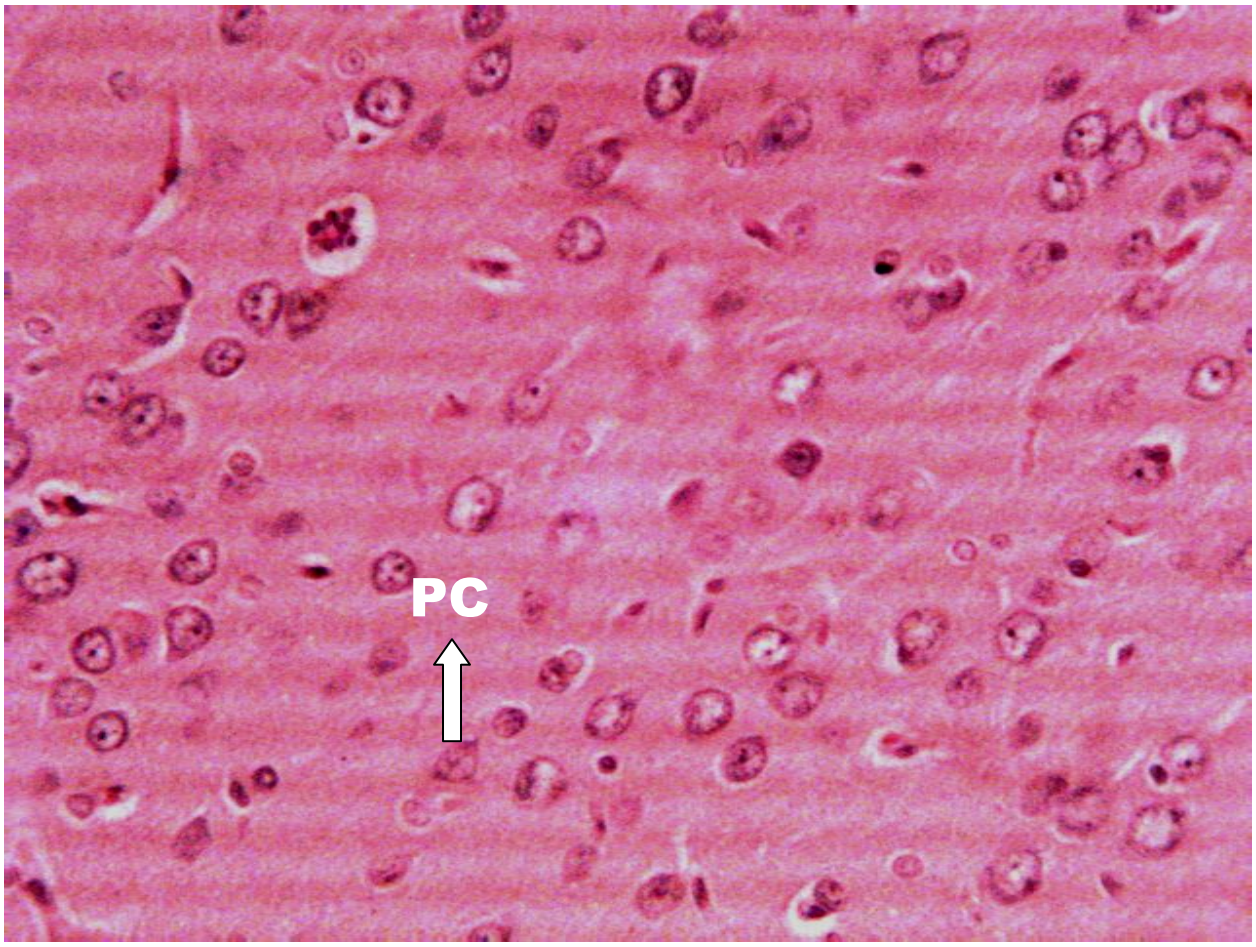



**Plate VIII Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 1(control) showing normal cytoarchtecture of pyramidal cell layer PC: Pyramidal cell (H and E×250).**

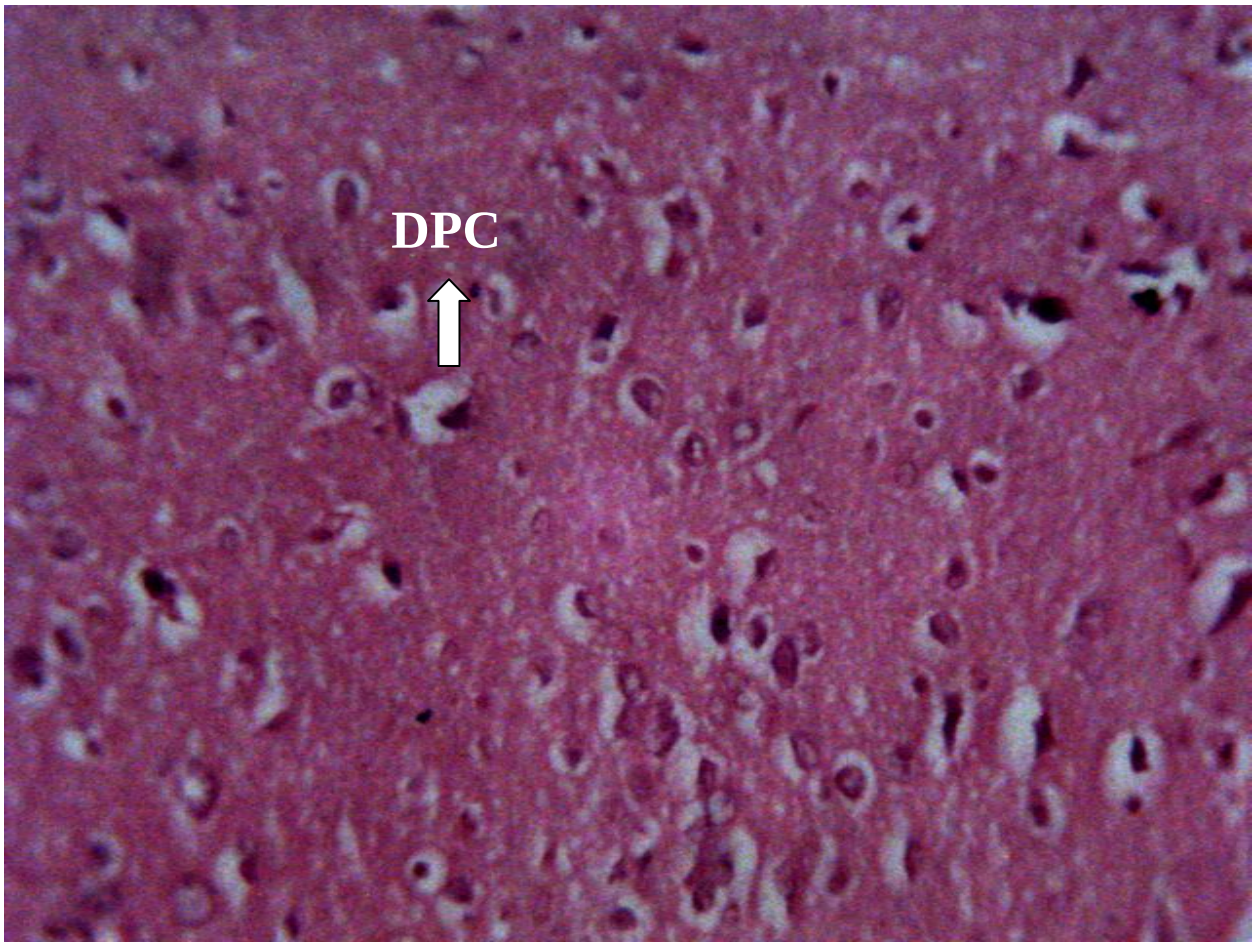



**Plate IX Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 2(41.5mg/kg bwt $HgCl_2$) showing distortion in the cytoarchtecture of pyramidal cells and evidence of degeneration.DPC: degenerating pyramidal cells; S: Stallet cell (H and E×250).**

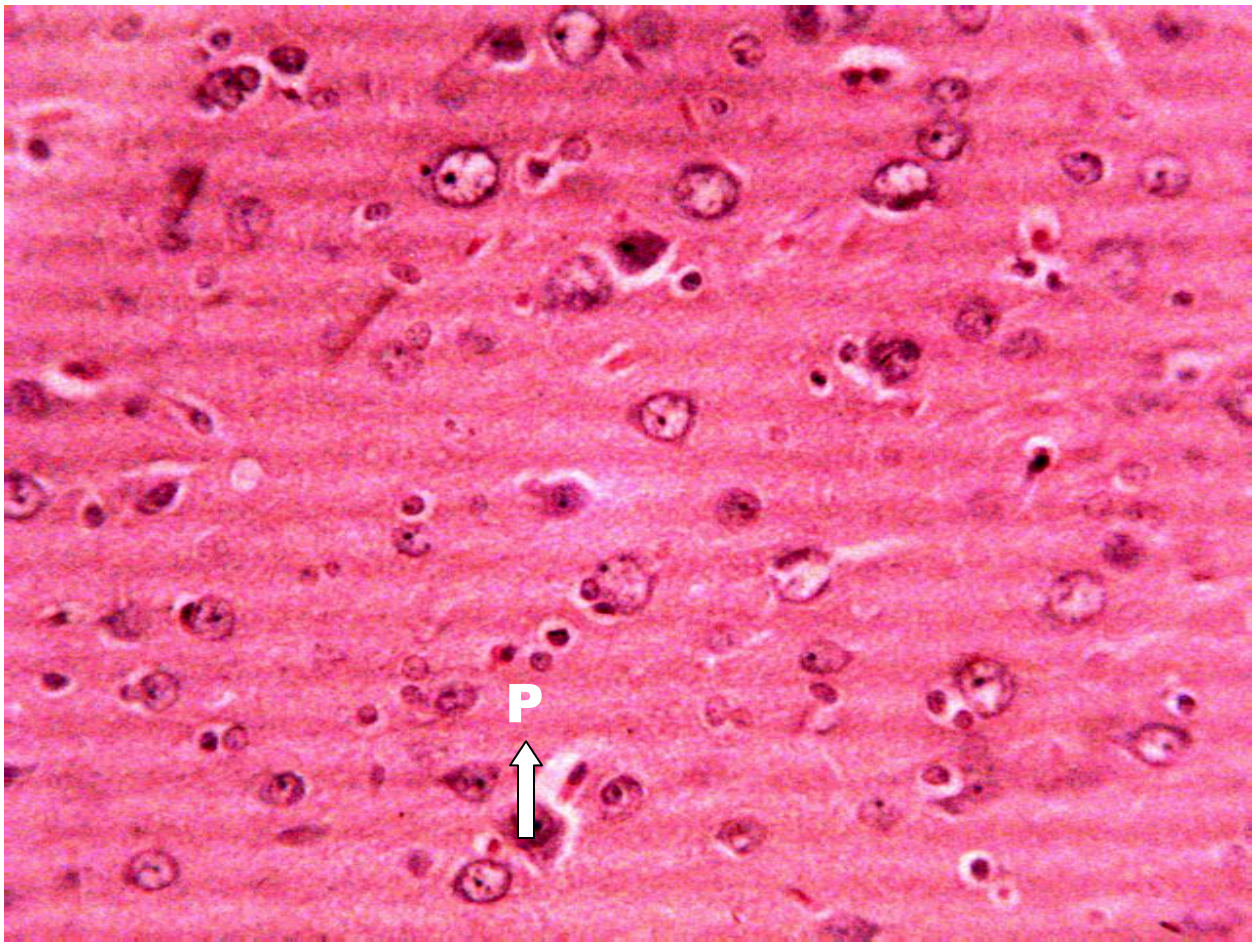



**Plate XSection of the cerebral cortex(internal pyramidal layer) of Wistar rats in Group 3(1000mg/kgbwt ethanolic extract of *Psidium guajava* leaves) showing normal cytoarchtecture of the pyramidal cell. PC: Pyramidal cell (H and E×250).**

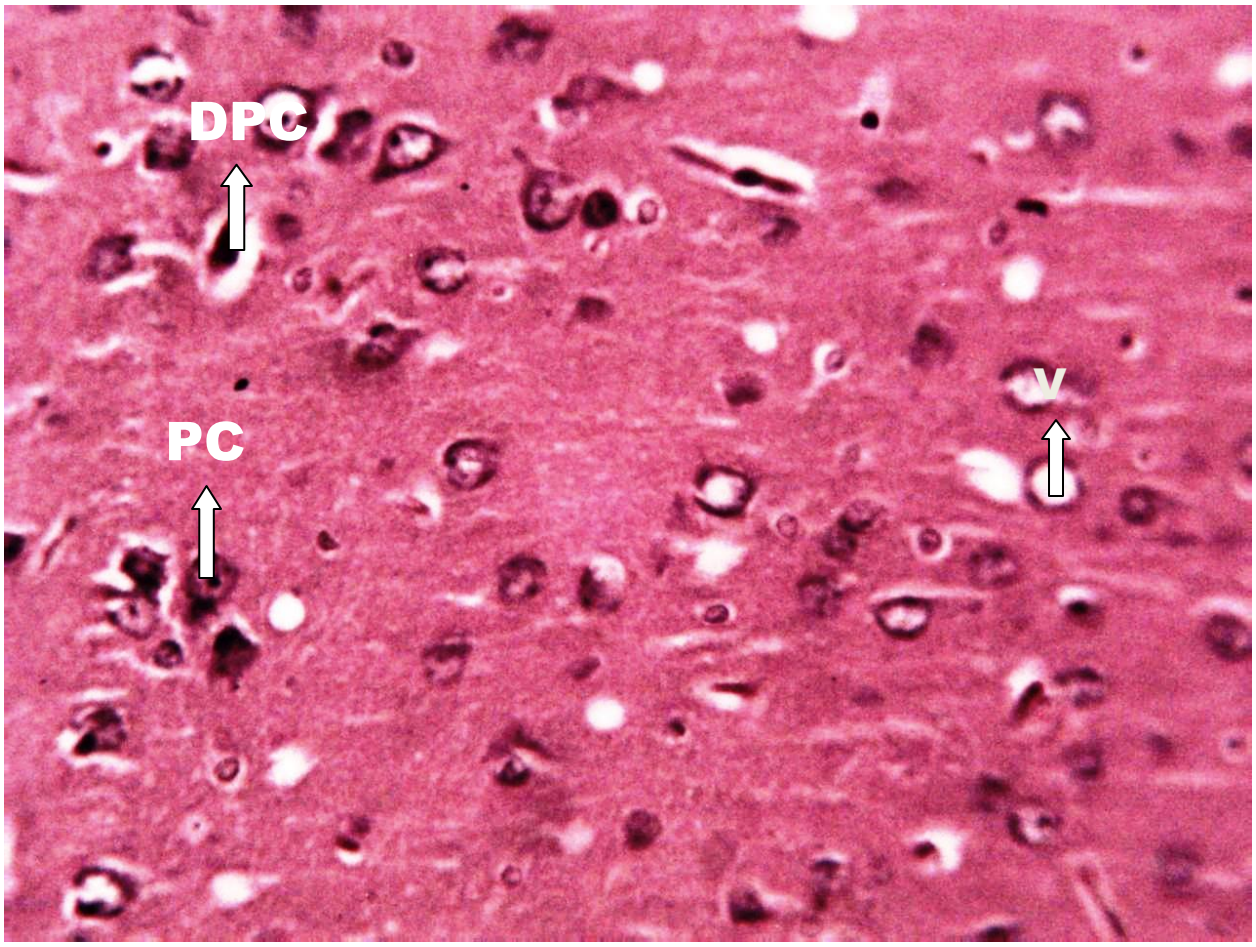



**Plate XI Section of the cerebral cortex(internal pyramidal layer) of Wistar rats in Group 4(41.5mg/kgbwt $Hgcl_2$ + Distilled $H_2O$) showing distortionin the cytoarchtecture of some pyramidal cells. PC: Pyramidal cells, Vacuolation and Degenerating pyramidalcells(DPC) (H and E×250).**

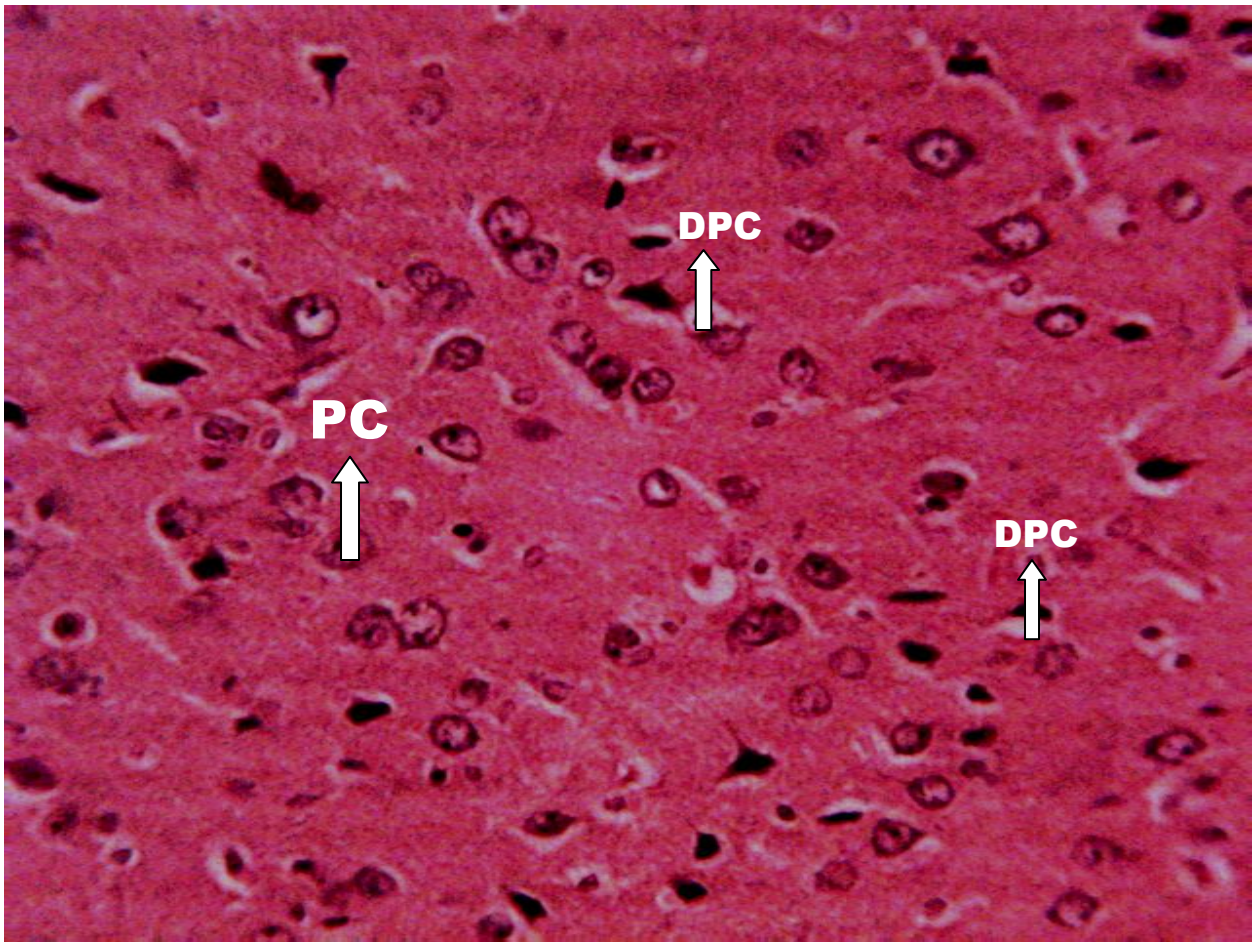



**Plate XII Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 5 (41.5mg/kgbwt $Hgcl_2$ + 500mg/kgbwt ethanolic extract of *Psidium guajava*) showing some normal pyramidal cell PC: pyramidal cells and evidence of degenerated cell bodies (DCB) and DPC: degenerated pyramidal cells. (H and E×250)**

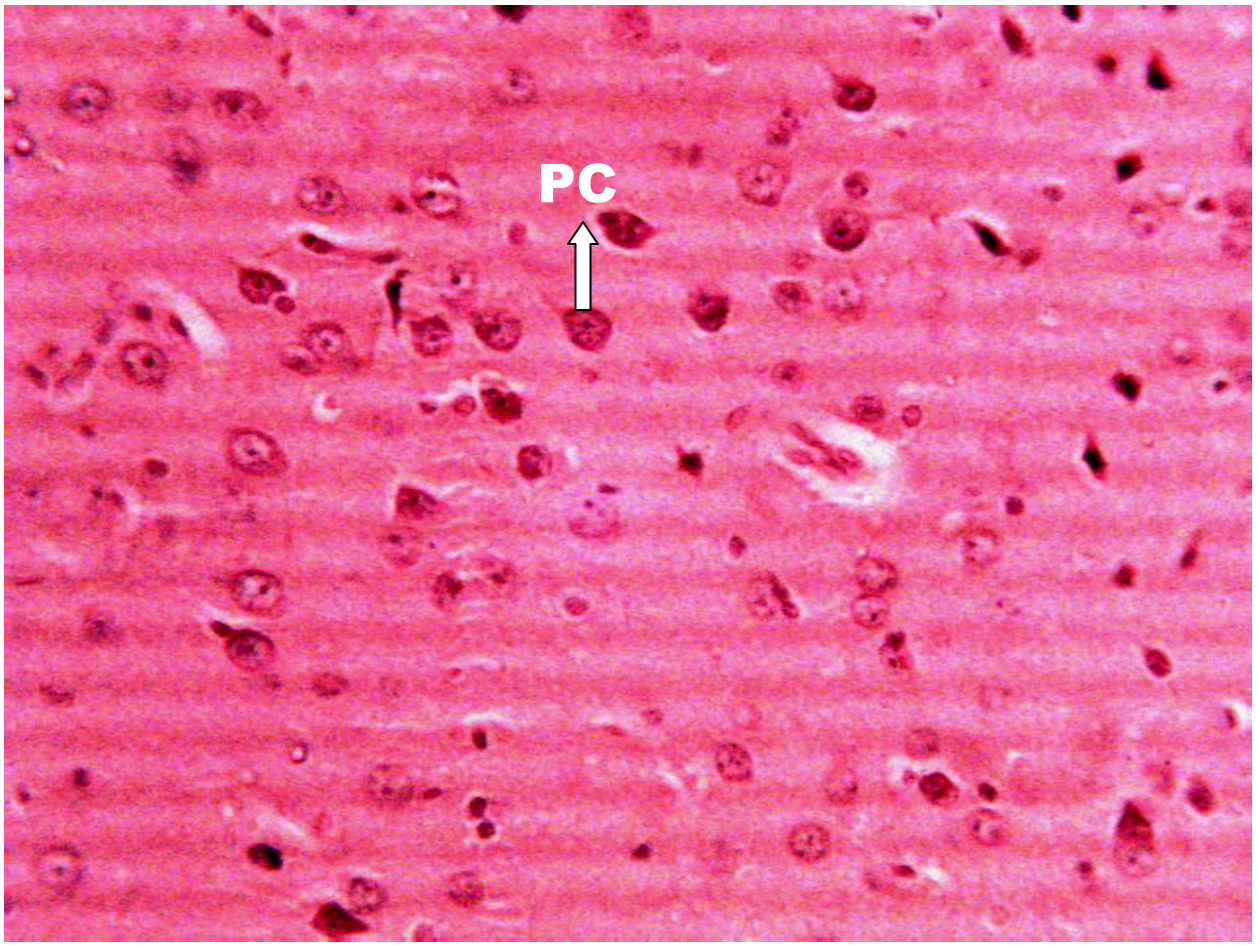



**Plate XIII Section of the cerebral cortex (internal pyramidal layer) of Wistar rats in Group 6(41.5mg/kgbwt $Hgcl_2$ + 1000mg/kgbwt ethanolic extract of *Psidium guajava*) showing normal cytoarchitecture of pyramidal cells. PC=pyramidal cell (H and E×250).**

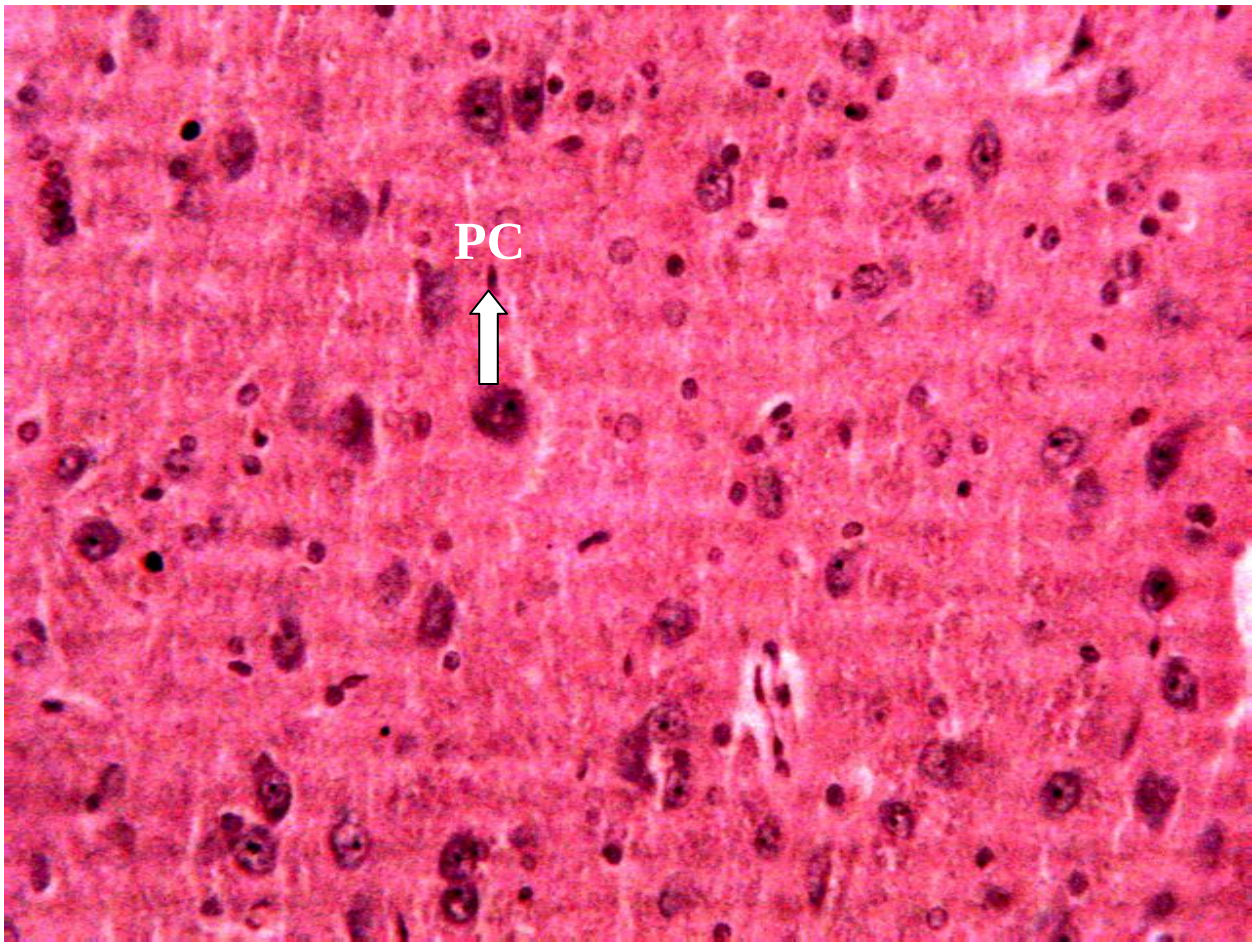



**Plate XIV: Section of the cerebral cortex(internal pyramidal layer) of Wistar rats in Group 7(41.5mg/kgbwt $Hgcl_2$ + 1000mg/kgbwt ascorbic acid) showing normal cytoarchitecture of pyramidal cells PC: Pyramidal cell (H and E×250).**

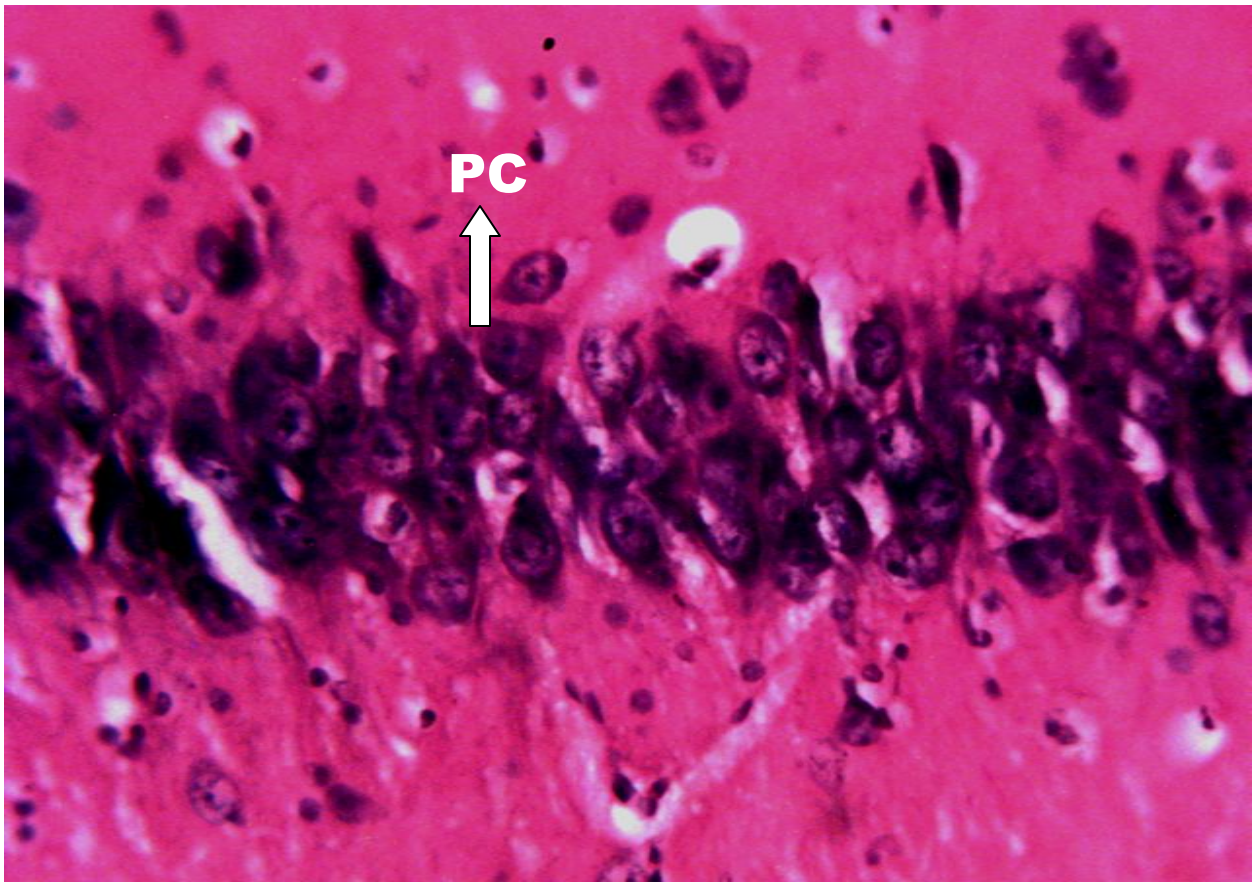



**Plate XV: A section of the hippocampus (CA3) of Group I Wistar rats(Control) showing normal orientation of CA3region and normal Pyramidal cells (PC) (H&E Stain × 250).**

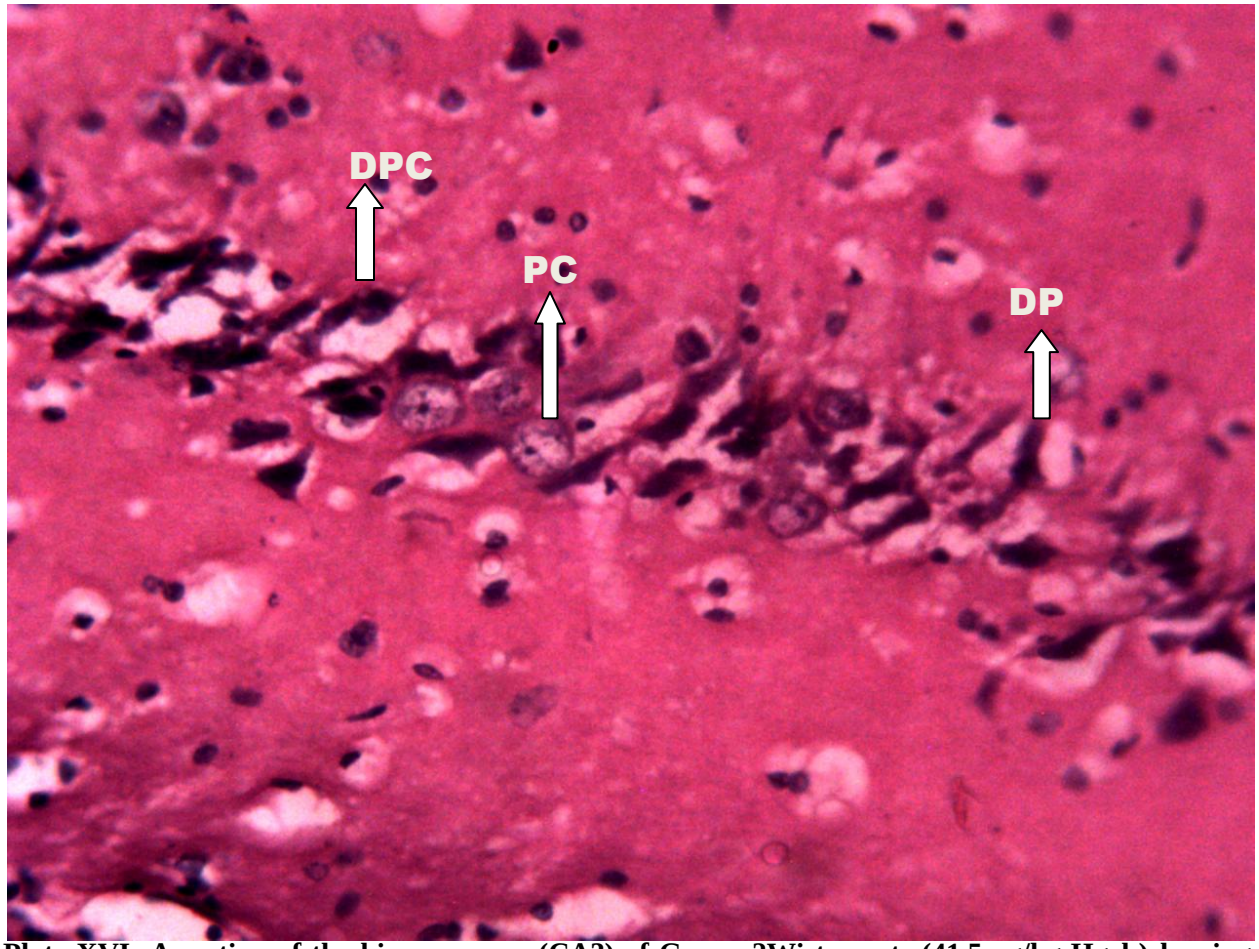



**Plate XVI: A section of the hippocampus (CA3) of Group 2Wistar rats (41.5mg/kg $Hgcl_2$)showing some evidence of cell degeneration (DPC) and some Pyramidal cells (PC) (H&E Stain × 250).**

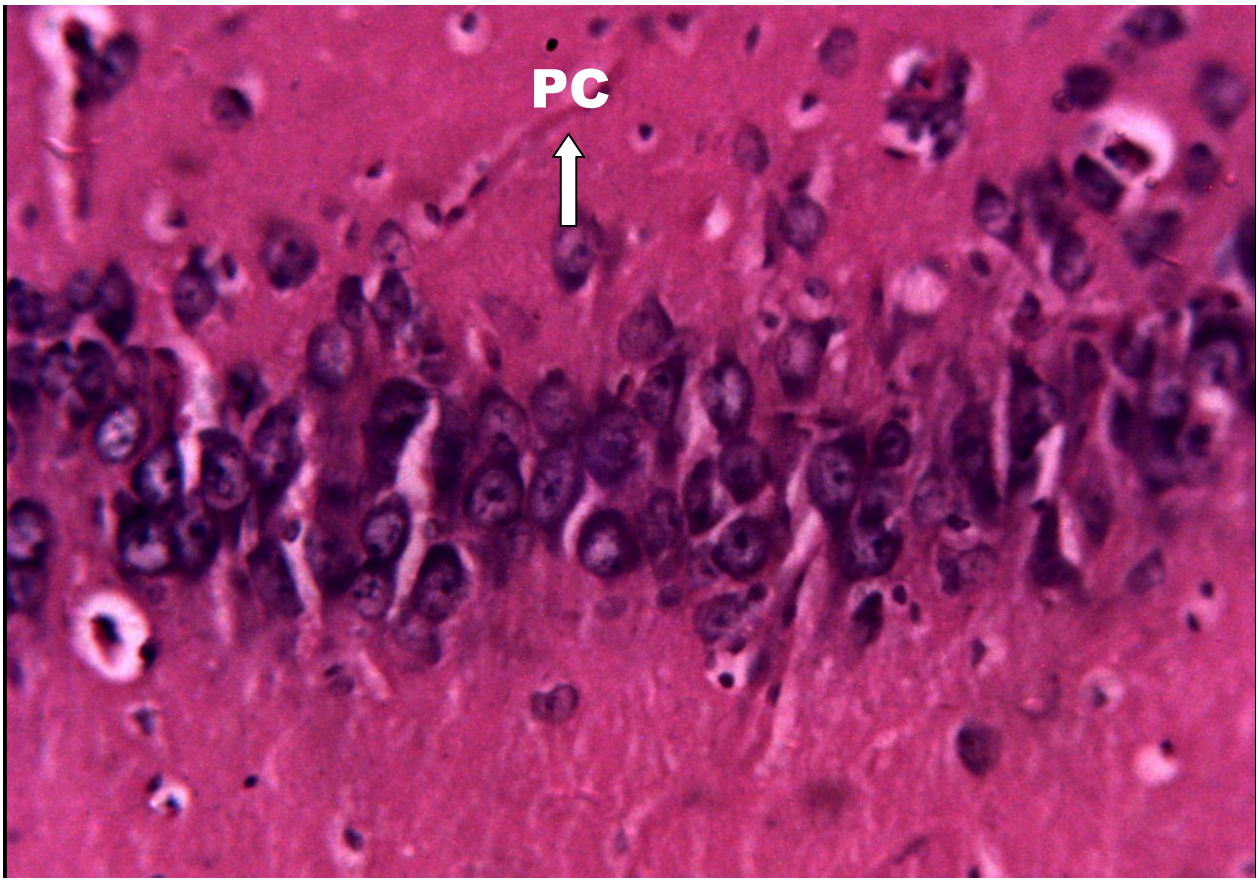



**Plate XVII: A section of the hippocampus (CA3) of Group 3 Wistar rats (1000mg/kgbwt ethanolic extract of *Psidium guajava*leaves) showing the CA3 region area and some Pyramidal cells (PC) (H&E Stain × 250).**

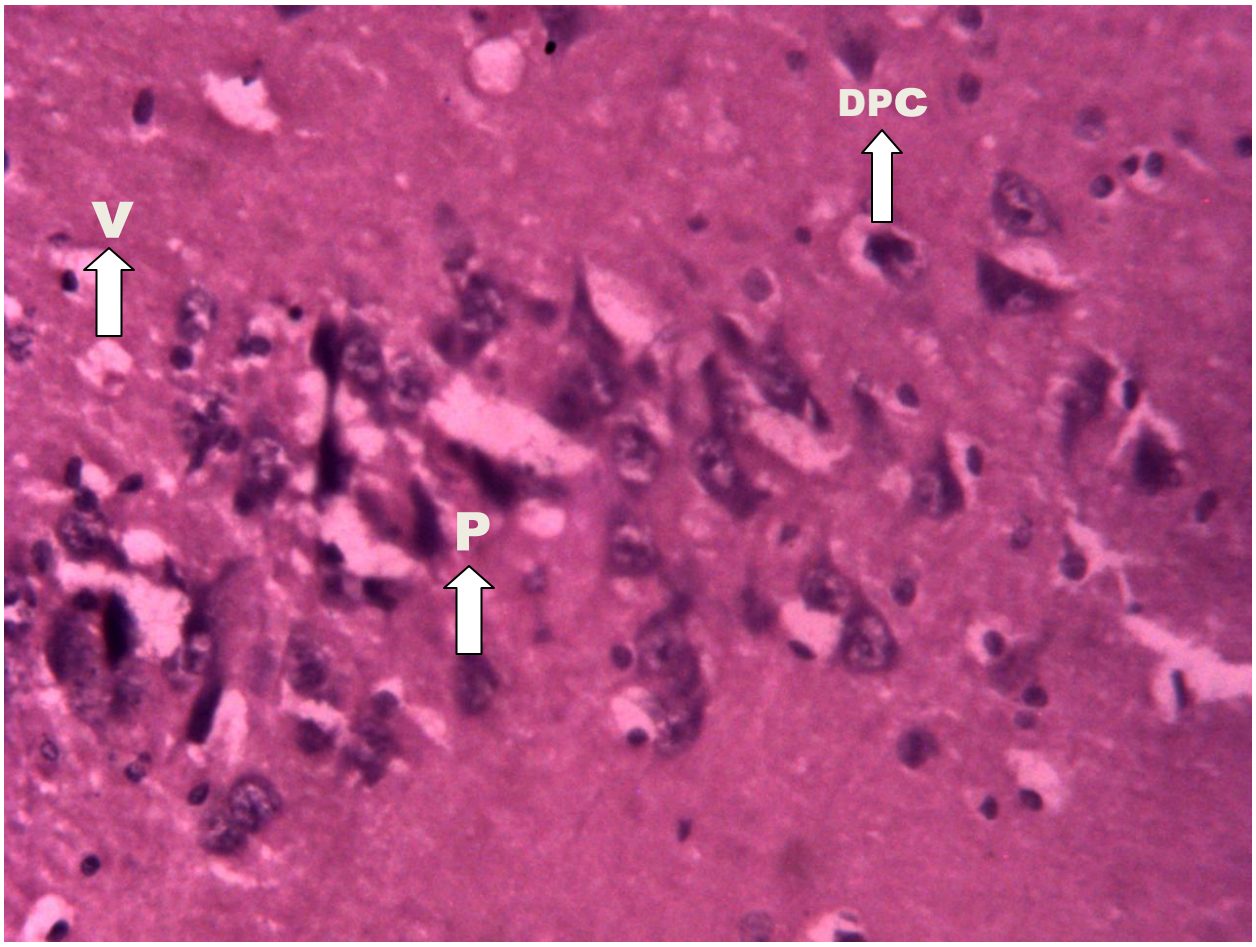
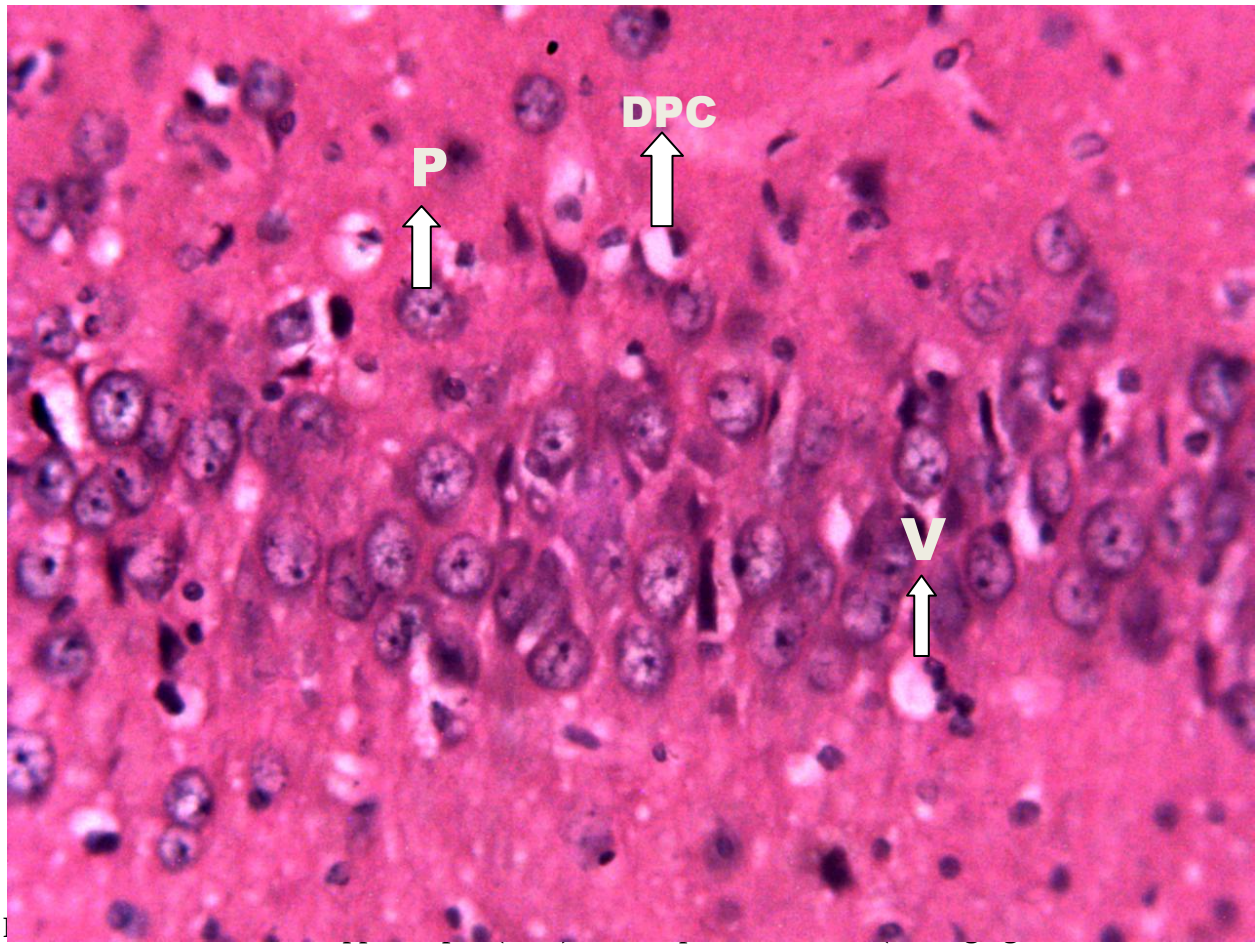



**Plate XVIII:Section of the hippocampus (CA3) of Group 4 Wistar rats (41.5mg/kg $Hgcl_2$ + Distilled $H_2O$) showing disorientation of CA3 region, some evidence of Vacuolation (V) and degenerated pyramidal cell (DPC); PC= Pyramidal cell (H&E Stain × 250).**




**+ 500mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) showing the CA3 region and some normal Pyramidal cells (PC),Evidence of degeneration (DPC) and Vacuolation(V) (H&E Stain × 250)**

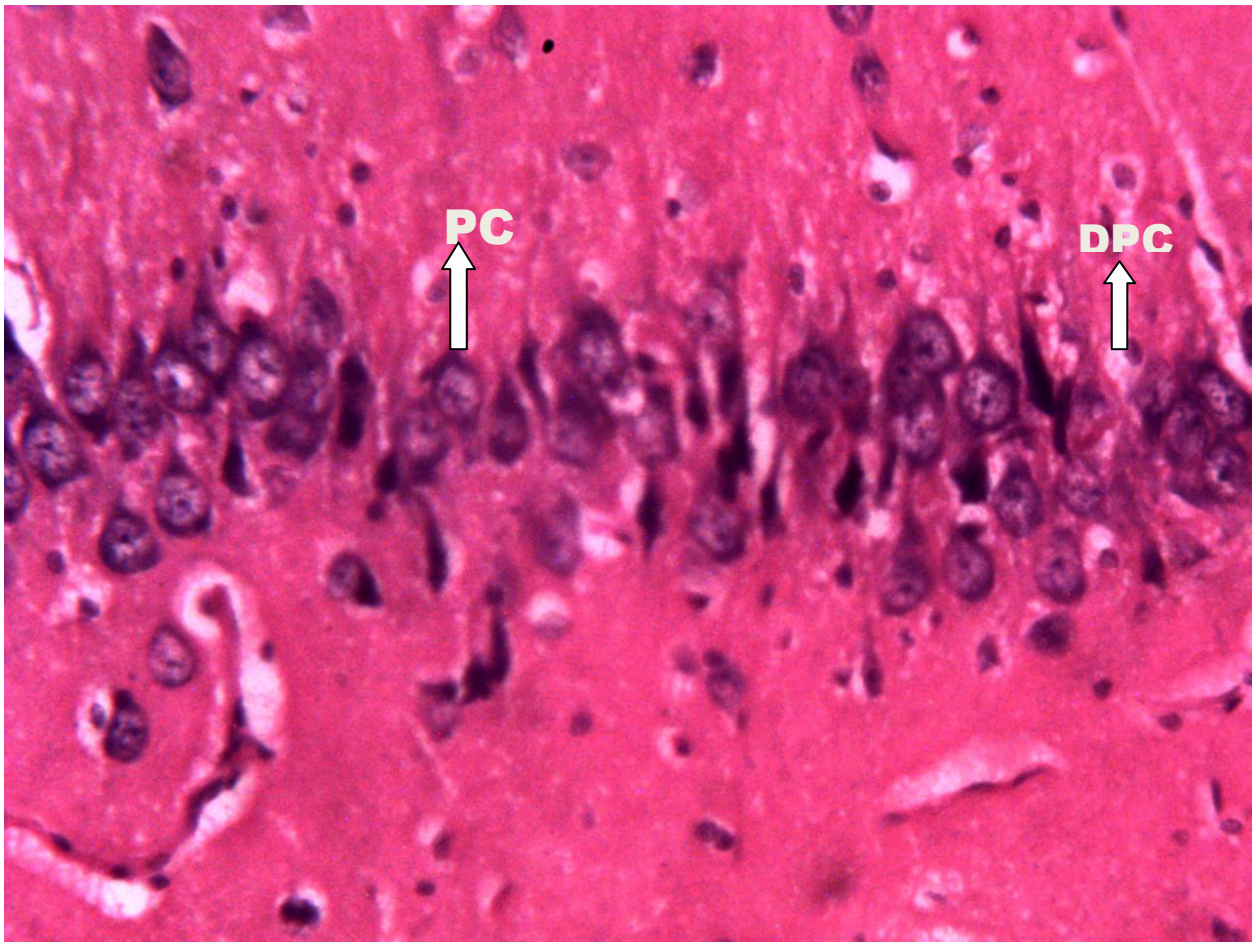



**Plate XX:Section of the hippocampus (CA3) of Group 6 Wistar rats (41.5mg/kg $HgCl_2$ + 1000mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) showing the CA3 region, some normal Pyramidal cells (PC) and evidence of few degenerating pyramidal cells(DPC) (H&E Stain × 250).**

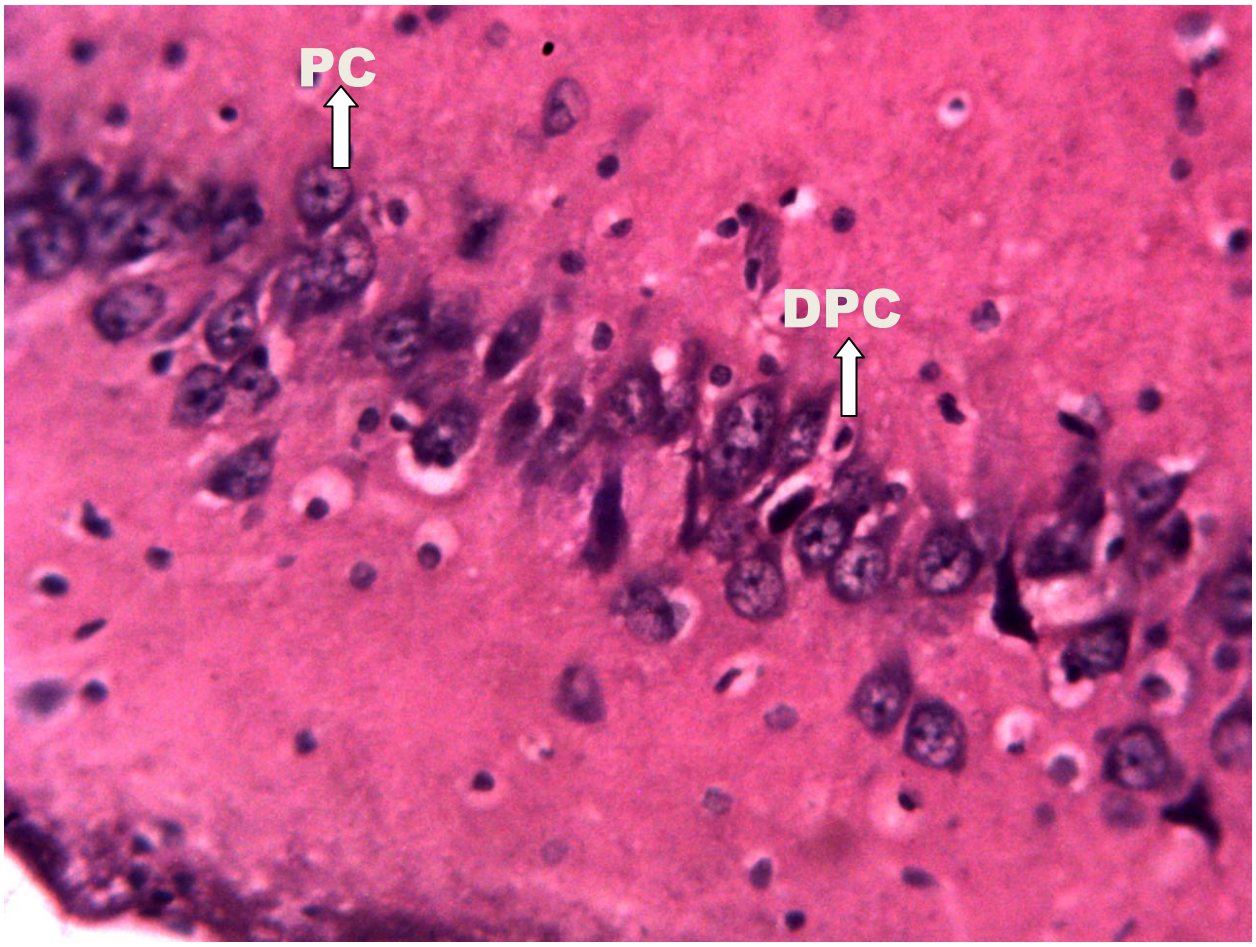



**Plate XXI: A section of the hippocampus (CA3) of Group 7 Wistar rats (41.5mg/kg $HgCl_2$ + 1000mg ascorbic acid) showing disorientation of CA3 region, some degenerating Pyramidal cell (DPC) and some Pyramidal cells (PC) (H&E Stain; X250).**

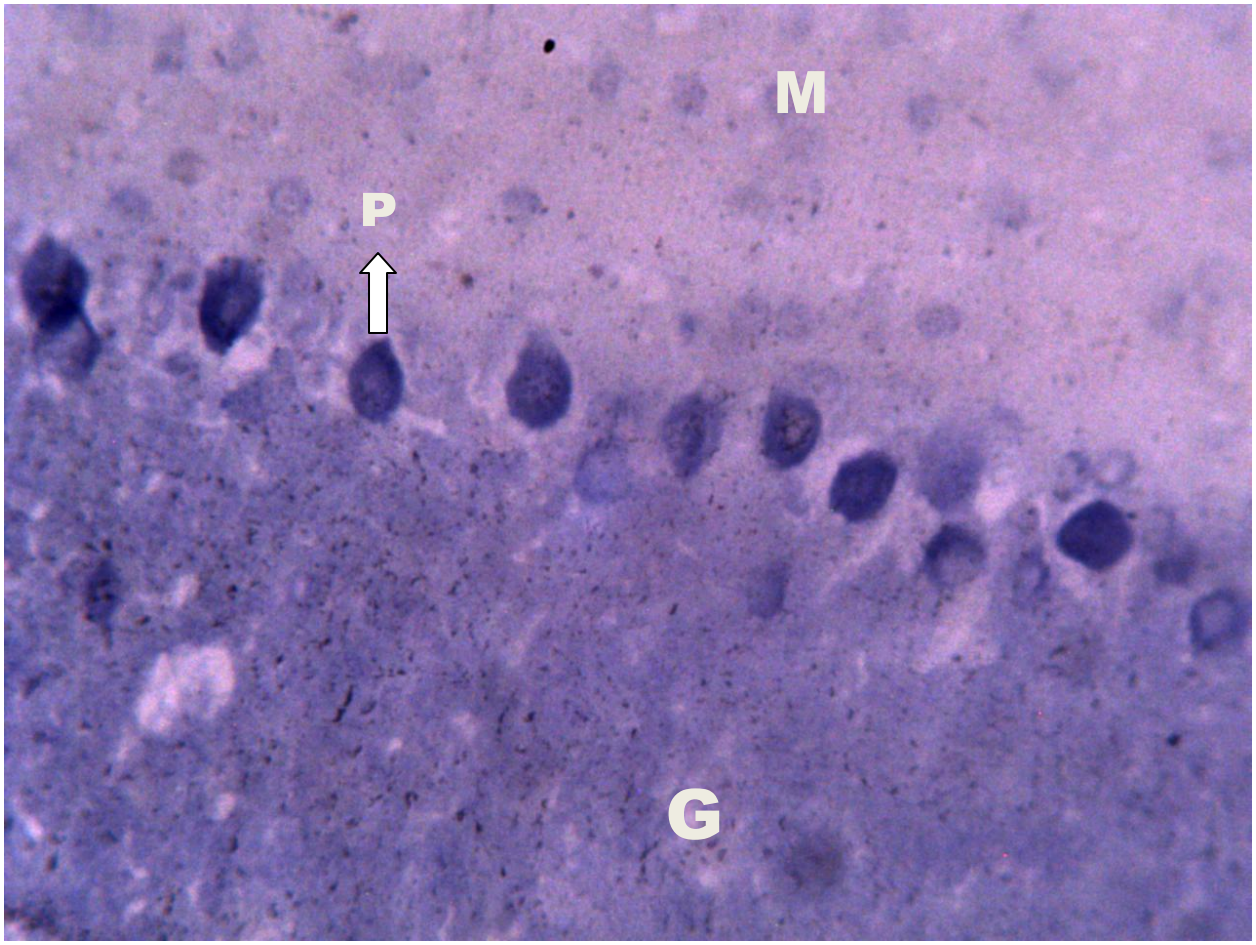



**Plate XXII: Section of cerebellar cortexofGroup 1 Wistar rats (control) showing characteristic three lamina G (Granular layer)P (Purkinje layer/cells) and M (Molecular layer with normal Nissl substance (X250).**

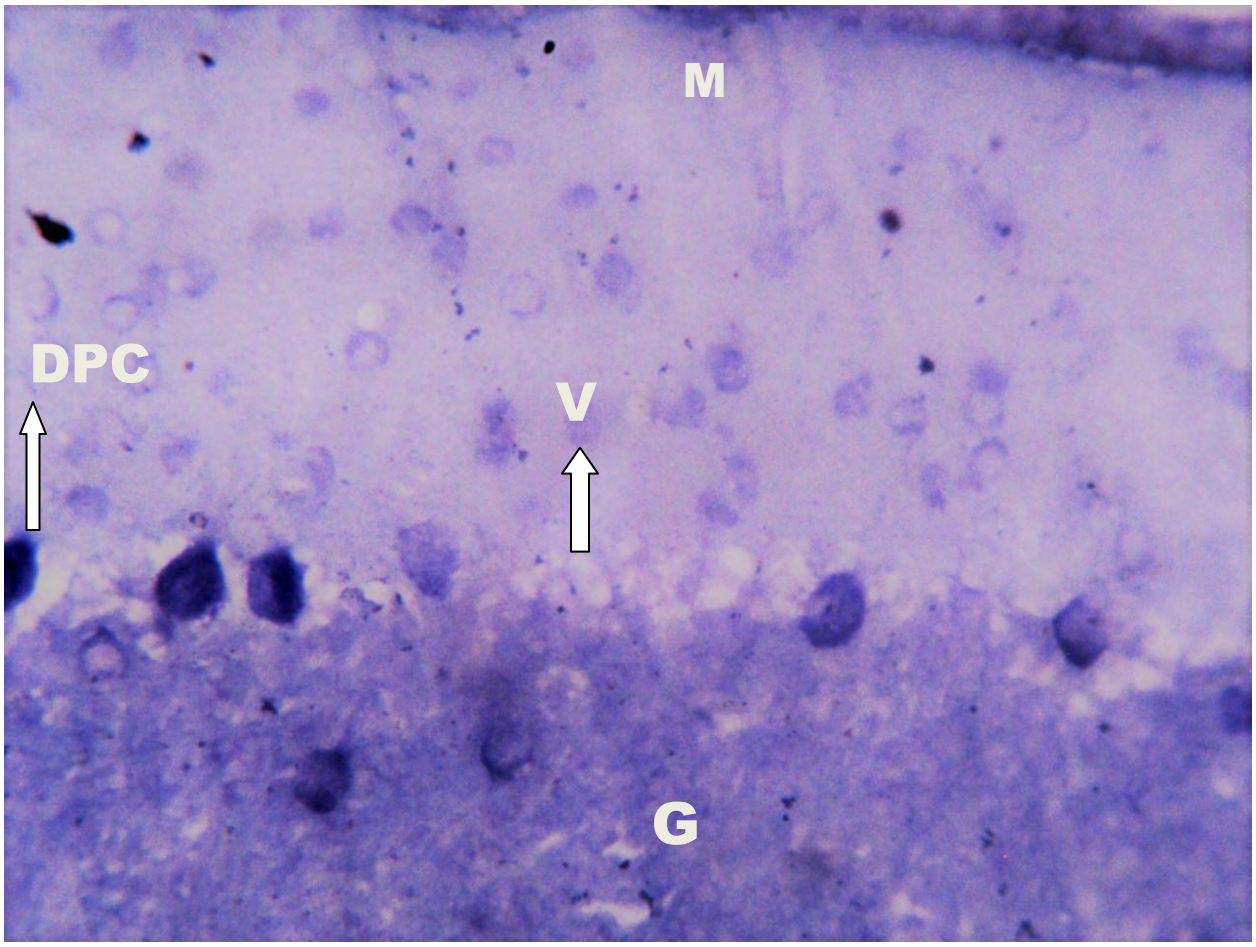



**Plate XXIII: Section of the Cerebellar Cortex of in Group 2Wistar rats (41.5mg/kgbwt $Hgcl_2$) showing Purkinje cell (P) with Vacuolation(V)Granular layer (G) Molecular layer (M); CFV stain (Mag x 250).**

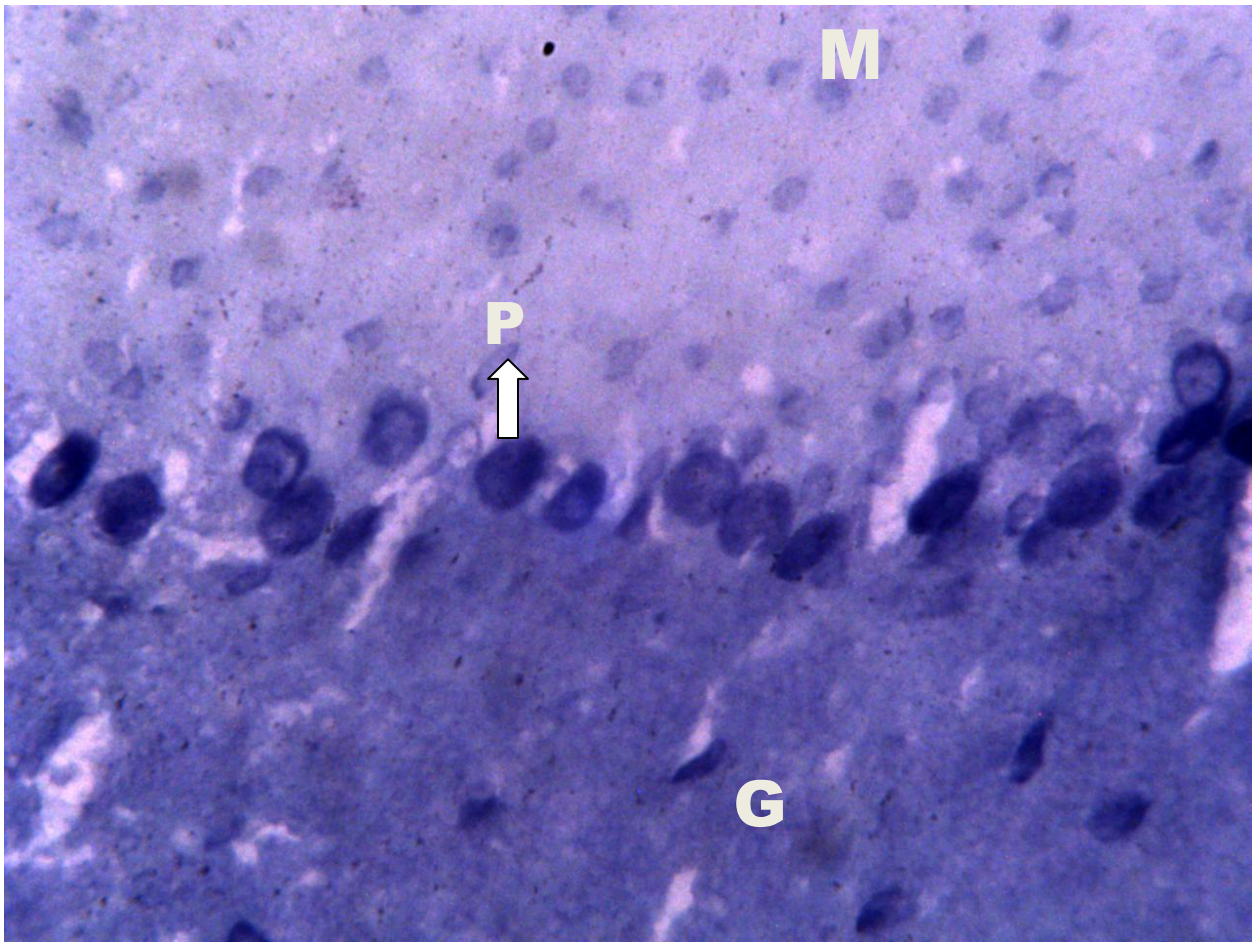



**Plate XXIV: Section of cerebellum Group 3 Wistar rats (1000 mg/kgbwt of ethanolic extract of *Psidium guajava* leaves) showing normal orientation of cells in the three layers G (Granular layer)P (Purkinje layer/cells) and M (Molecular layer with normal Nissl substance (X250).**

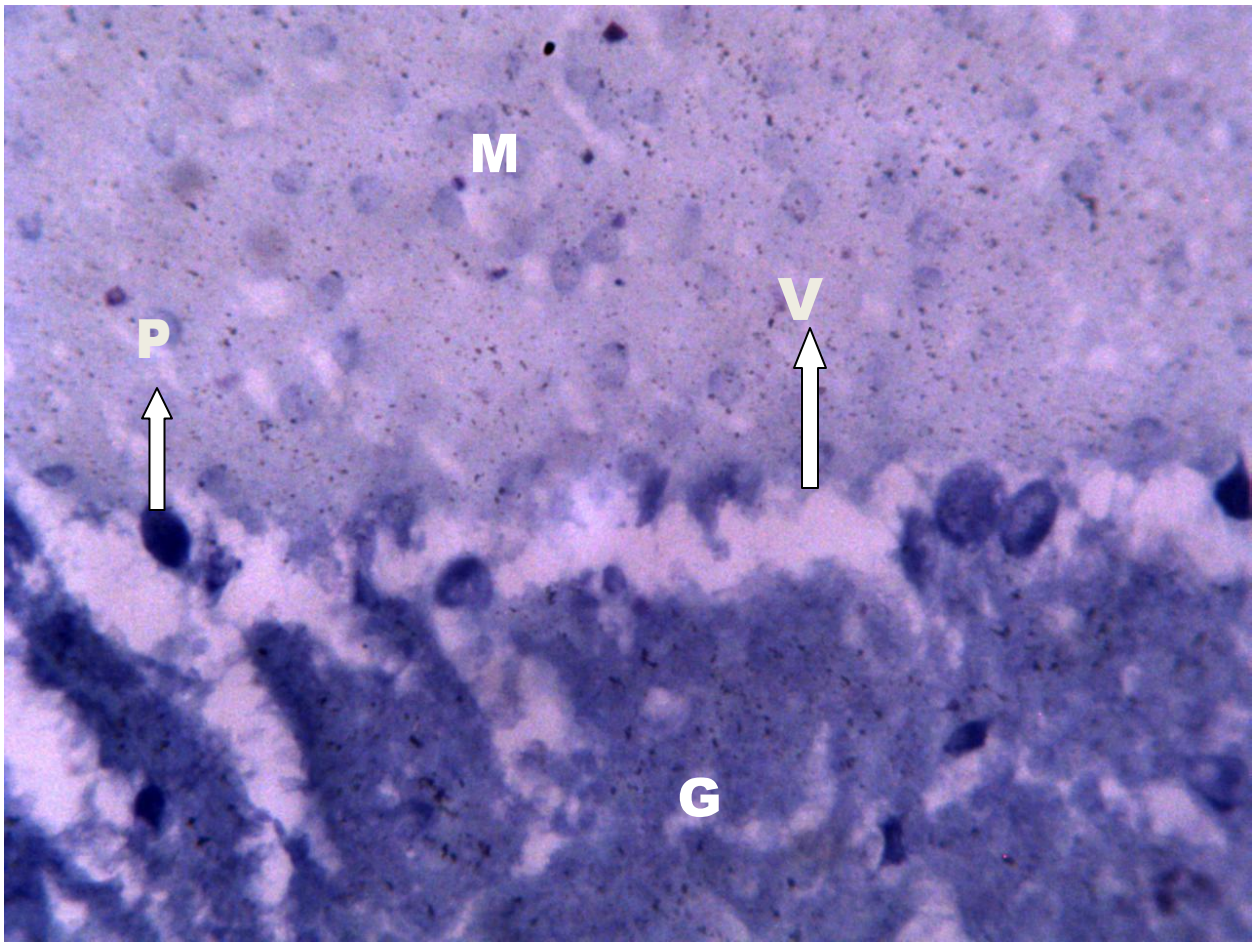



**Plate XXV: Section of the Cerebellar Cortex of Wistar rats in Group 4 (41.5mg/kgbwt $Hgcl_2$+ Distilled) showing Purkinje cell (P) with Vacuolation (V)Granular layer (G) Molecular layer (M); CFV stain (Mag X250).**

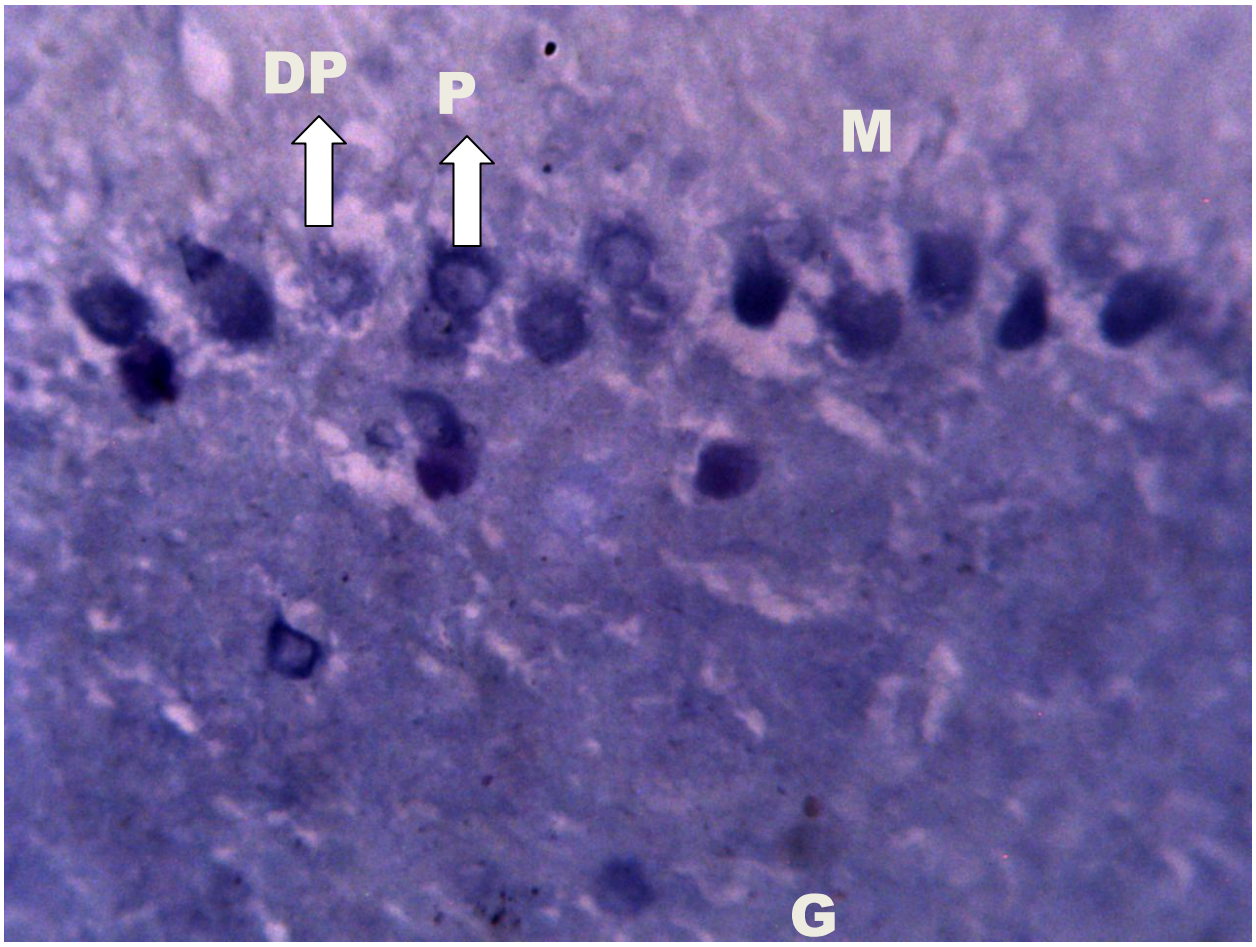



**Plate XXVI: Section of the Cerebellar Cortex of Wistar rats in Group 5 (41.5mg/kgbwt $Hgcl_2$+ 500mg/kgbwt of *Psidium guajava*leaves extract) showing Purkinje cell (P) with evidence of degeneration (DP) Granular layer (G) Molecular layer (M); CFV stain (Mag x 250).**

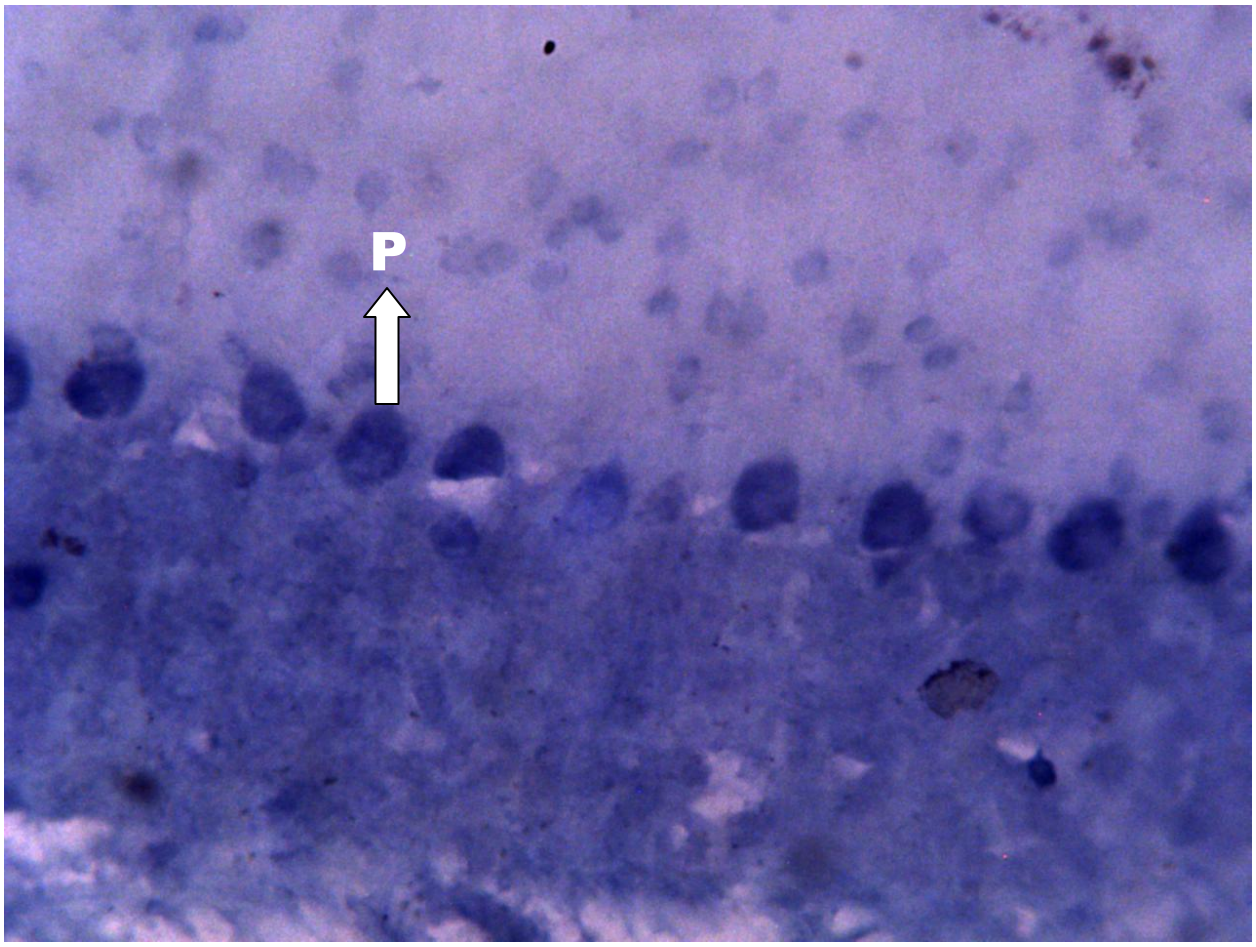



**Plate XXVII: Section of the Cerebellar Cortex of Wistar rats in Group 6 (41.5mg/kgbwt $Hgcl_2$+ 1000mg/kgbwt of *Psidium guajava*leaves extract) showing Purkinje cell (P) with evidence of degeneration (DP) Granular layer (G) Molecular layer (M); CFV stain (Mag x 250).**

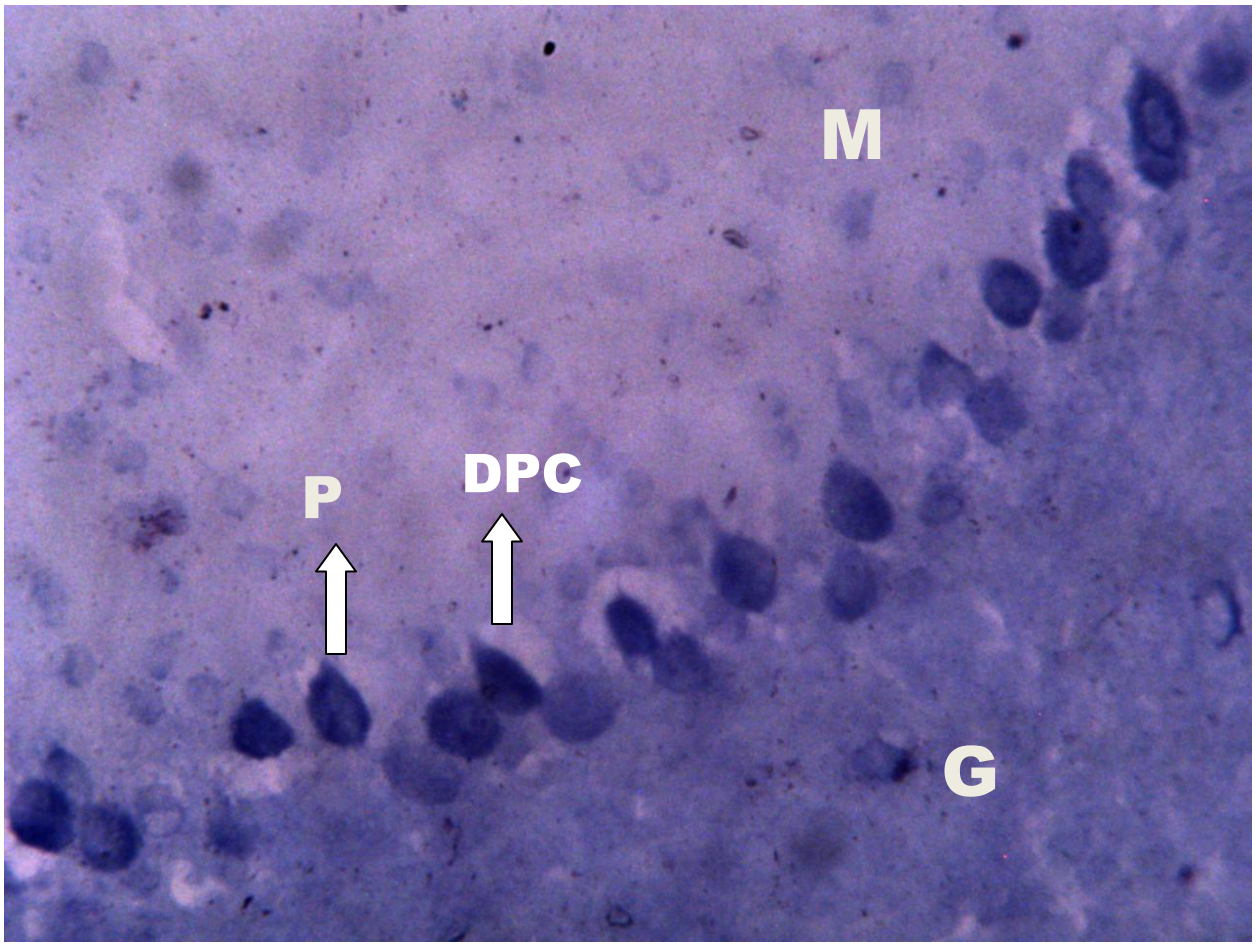



**Plate XXVIII: Section of the Cerebellar Cortex of Wistar rats in Group 7 (41.5mg/kgbwt $Hgcl_2$+ 1190mg/kgbwt of Ascorbic Acid) showing Purkinje cell (P) Granular layer (G) Molecular layer (M); CFV stain (Mag x 250).**

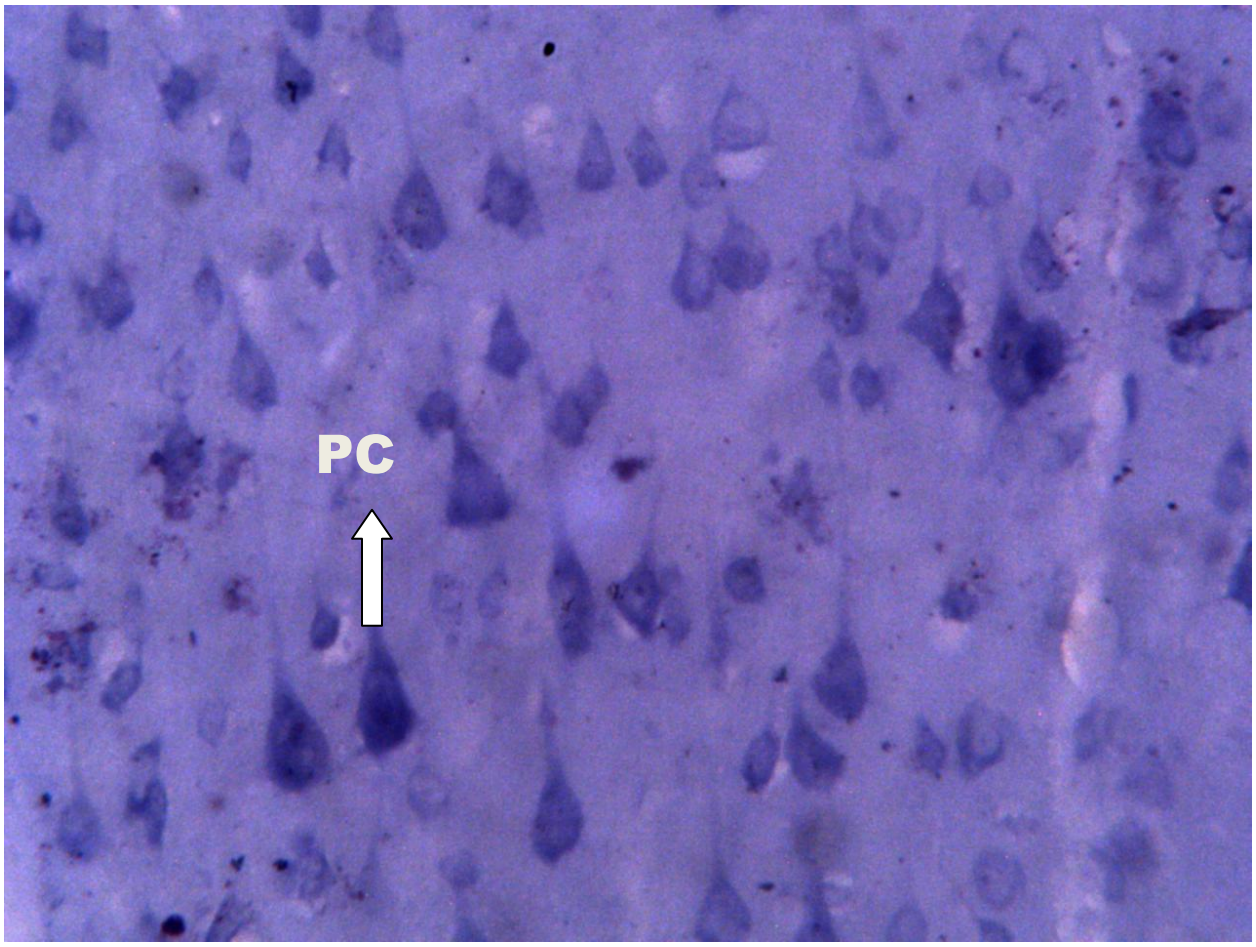



**Plate XXIX: Section of cerebral cortex (Internal pyramidal layer) of Group I (Control) showing normal architecture of Pyramidal cells (PC) with intact nuclei (Cresyl violet Stain × 250).**

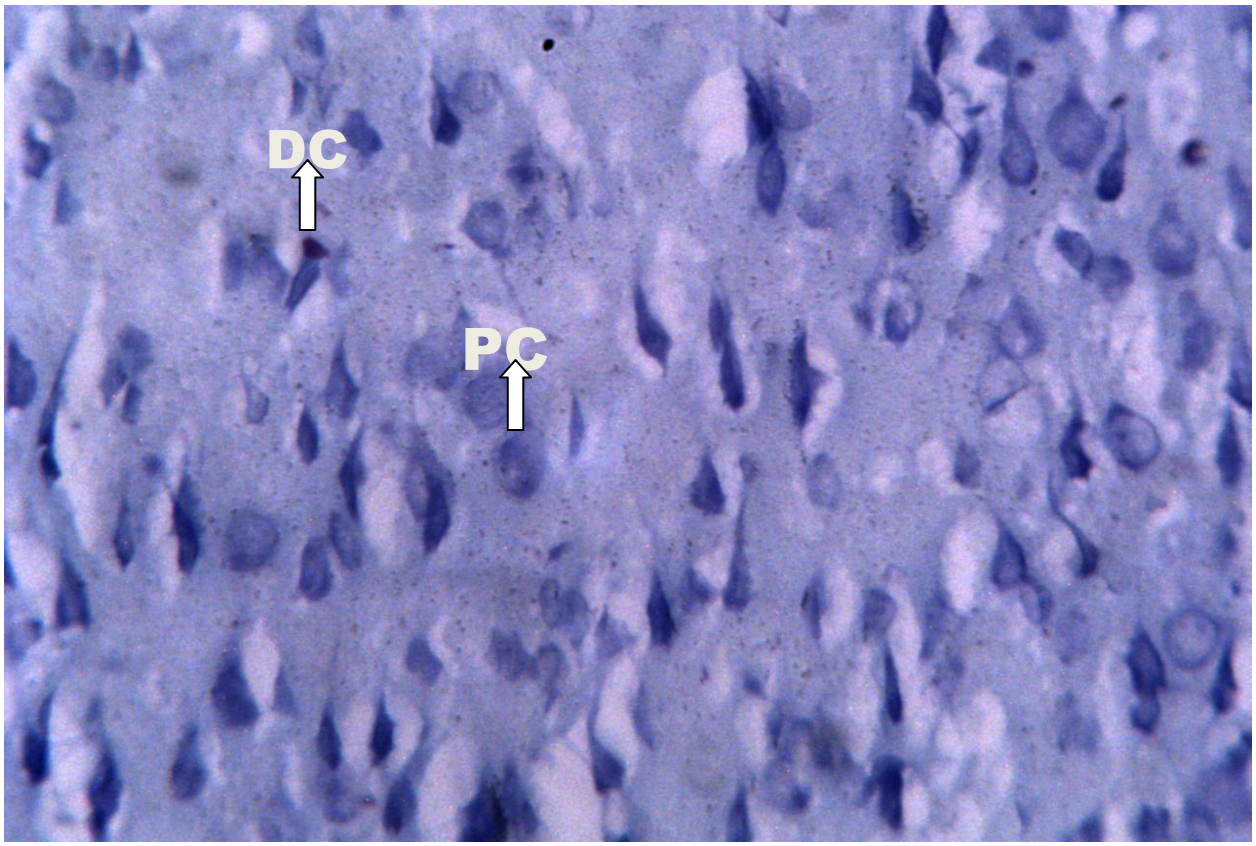



**Plate XXX: Section of cerebral cortex (Internal pyramidal layer) of Group 2 Wistar rats (41.5mg/kg $Hgcl_2$) showing normal Pyramidal cells (PC) Vacuolation and evidence of degenerating cells (DC) (Cresyl violet Stain X250).**

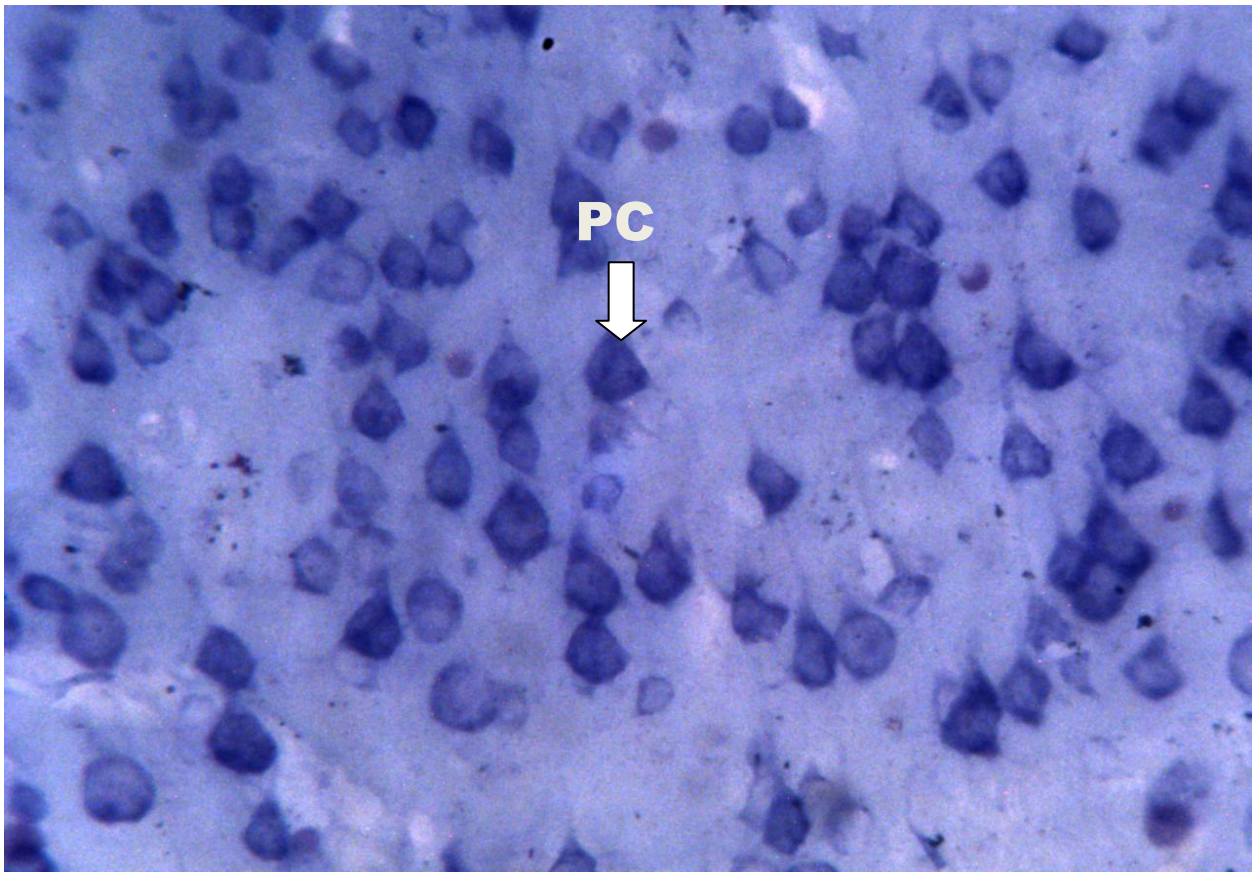



**Plate XXXI: Section of cerebral cortex (Internal pyramidal layer) of Group 3 Wistar rat (1000mg/kg bwt of ethanolic extract of *Psidium guajava*leaves) showing some normal Pyramidal cells (PC) (Cresyl violet Stain × 250).**

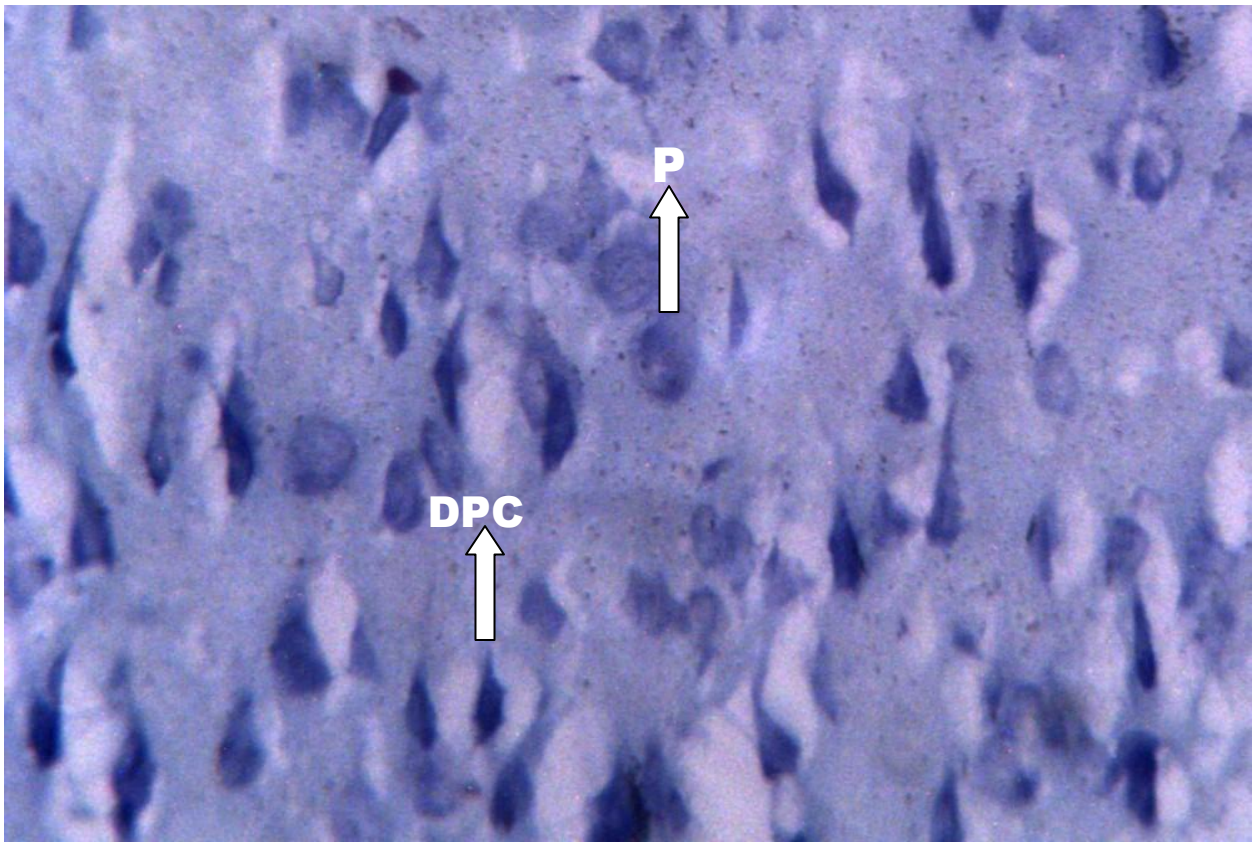



**Plate XXXII: Section of cerebral cortex(Internal pyramidal layer) of Group 4 Wistar rat (41.5mg/kg $HgCl_2$+ distilled $H_2O$) showing some normal Pyramidal cells (PC) and degenerated Pyramidal cells (DPC) (Cresyl violet Stain × 250).**

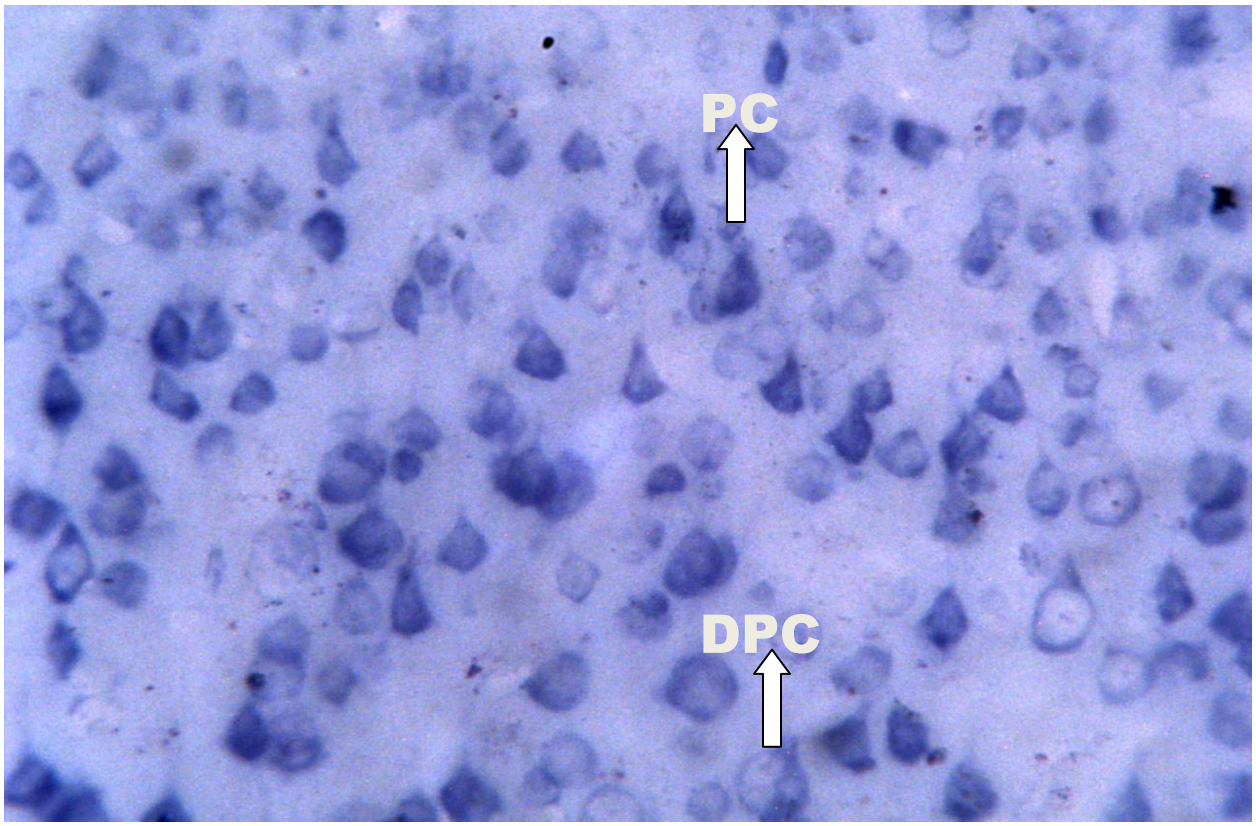



**Plate XXXIII: Section of cerebral cortex(Internal pyramidal layer) of Group 5 Wistar rats(41.5mg/kg $HgCl_2$+ 500mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) showing some degenerated Pyramidal cells (DPC), and some normal pyramidal cell (PC) (Cresyl violet Stain × 250).**

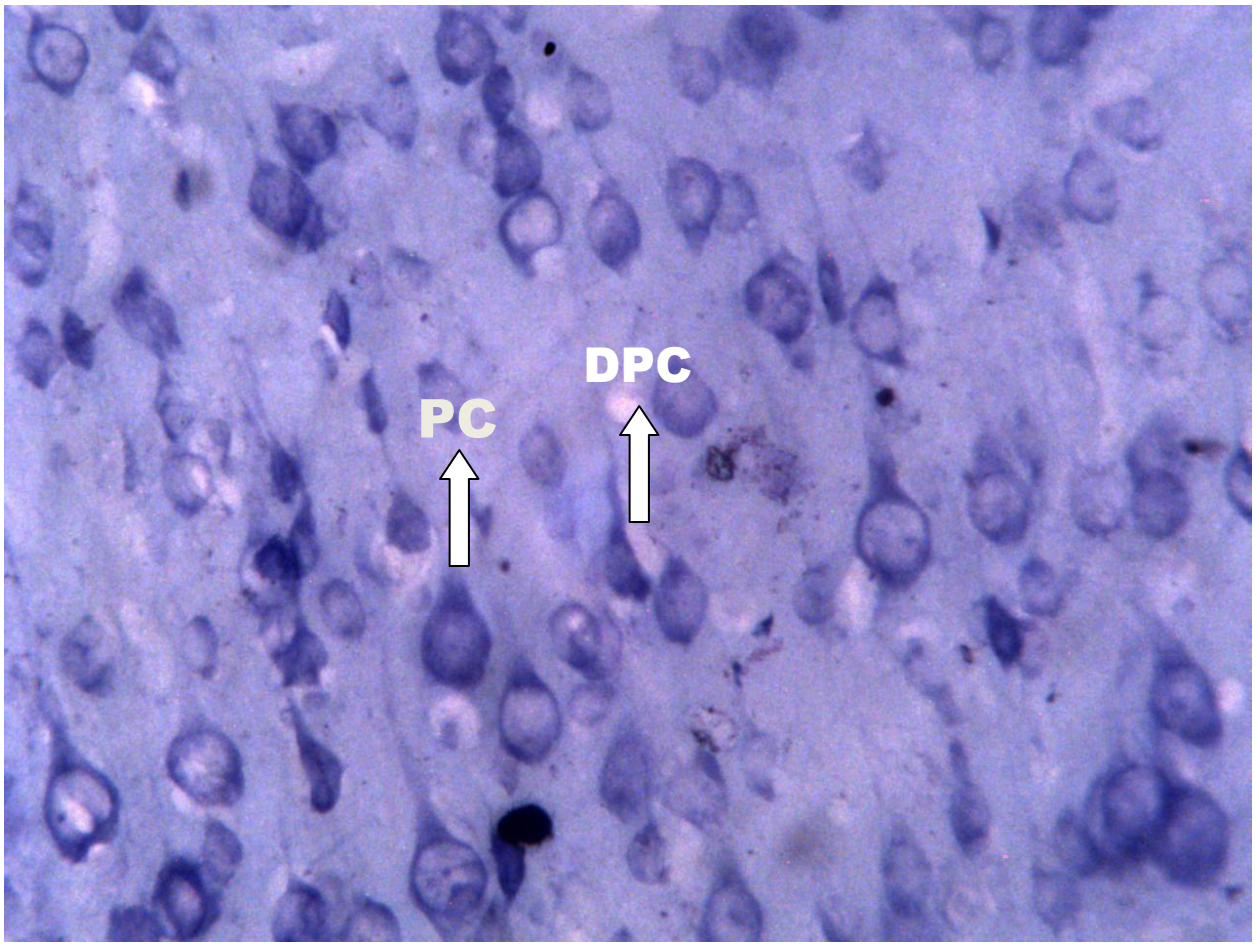



**Plate XXXIV: Section of cerebral cortex(Internal pyramidal layer) of Group 6Wistar Rat (41.5mg/kg $HgCl_2$+ 1000mg/kgbwt of ethanolic extract of *Psidium guajava* leaves) showingPyramidal cells (PC) and degenerating pyramidal cells (DPC) (Cresyl violet Stain × 250).**

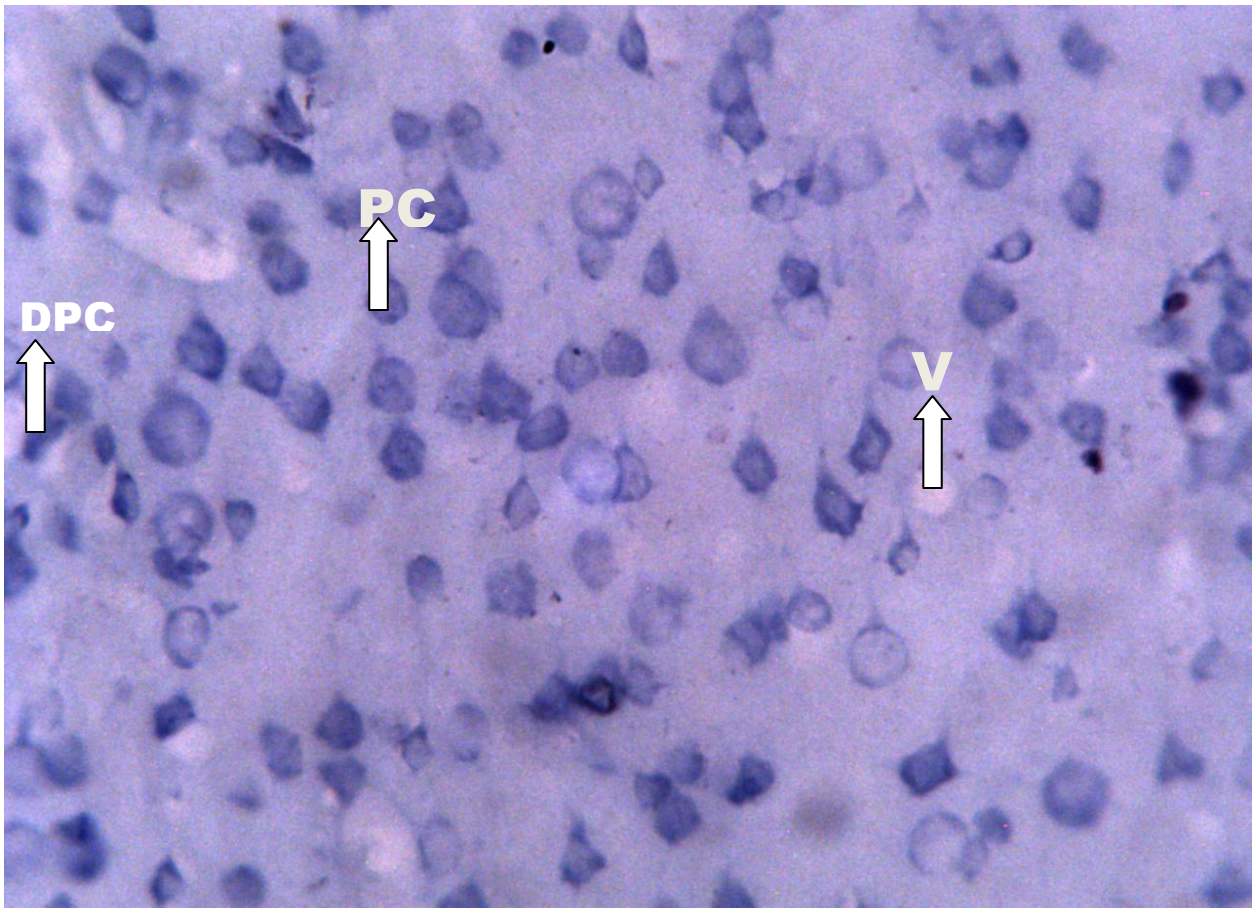



**Plate XXXV: Section of cerebral cortex(Internal pyramidal layer)of Group7Wistar rat (41.5mg/kg $HgCl_2$+1000mg/kgbwt of ascorbic acid) showing Pyramidal cells (PC) and vacuolated cells (V) (Cresyl violet Stain × 250).**

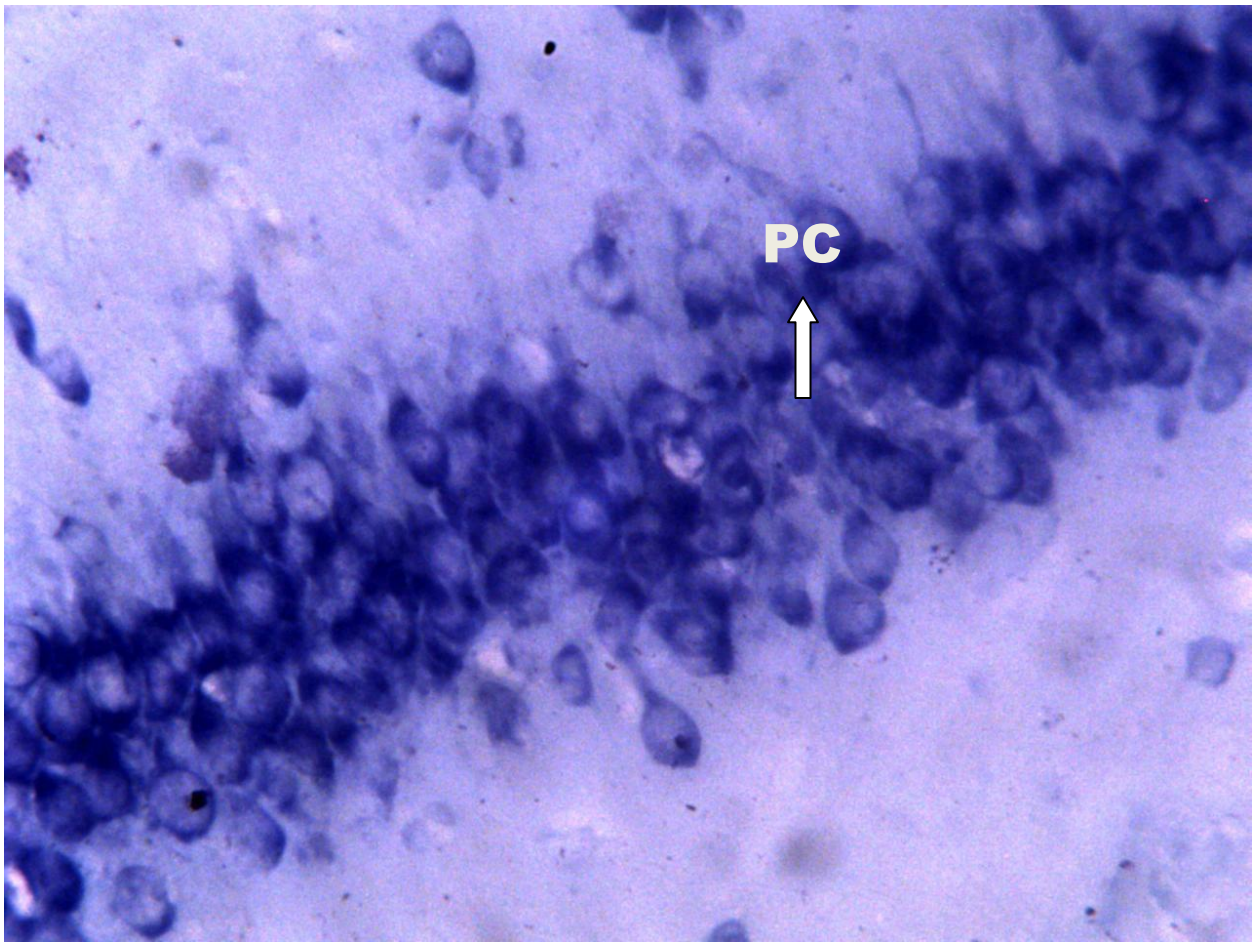



**Plate XXXVI: Section of the hippocampus (CA3) of Group I Wistar rats (Control) showing normal orientation of CA3 region, Pyramidal cell (PC) with intact nuclei (Cresyl violet Stain; X250).**

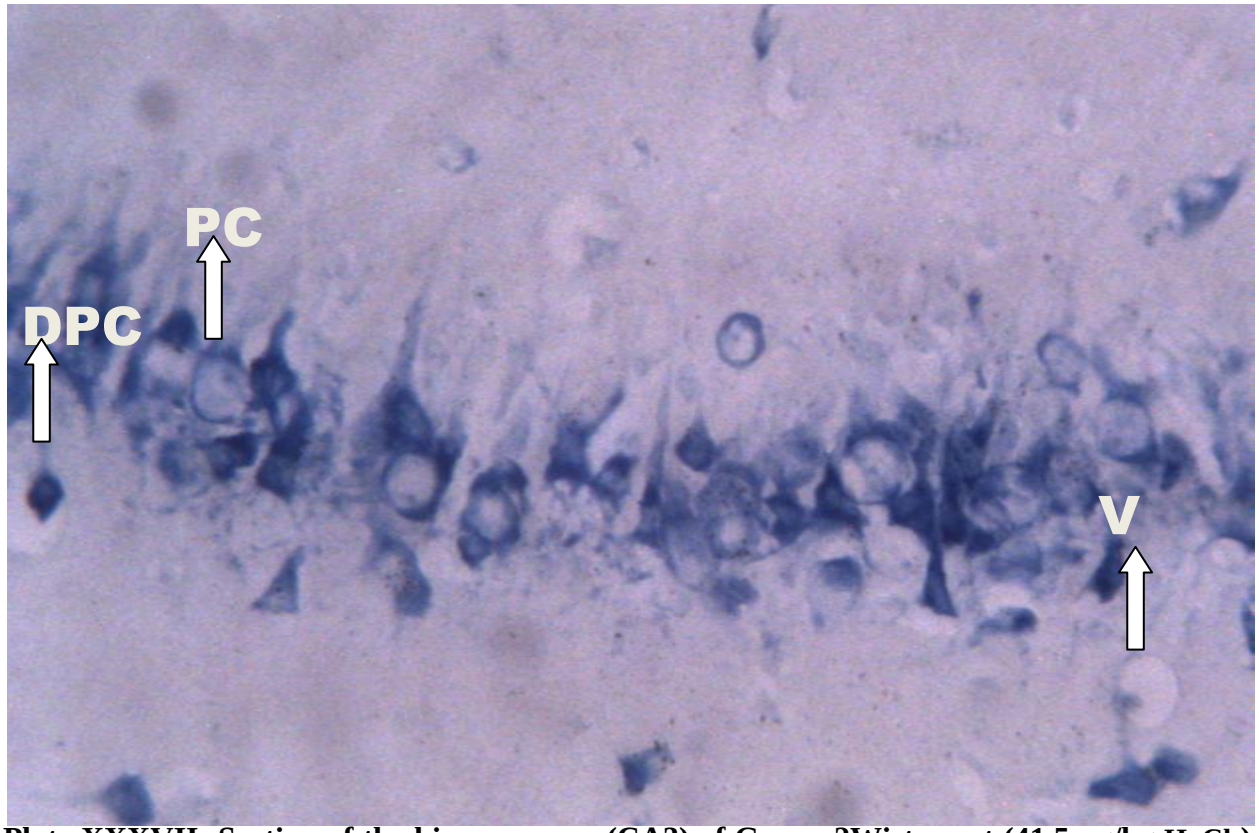



**Plate XXXVII: Section of the hippocampus (CA3) of Group 2Wistar rat (41.5mg/kg $HgCl_2$) showing pyramidal cell (PC), Vacuolation (V) and degenerated Pyramidal cells (DPC) with loss of nuclei (Cresyl violet Stain × 250).**

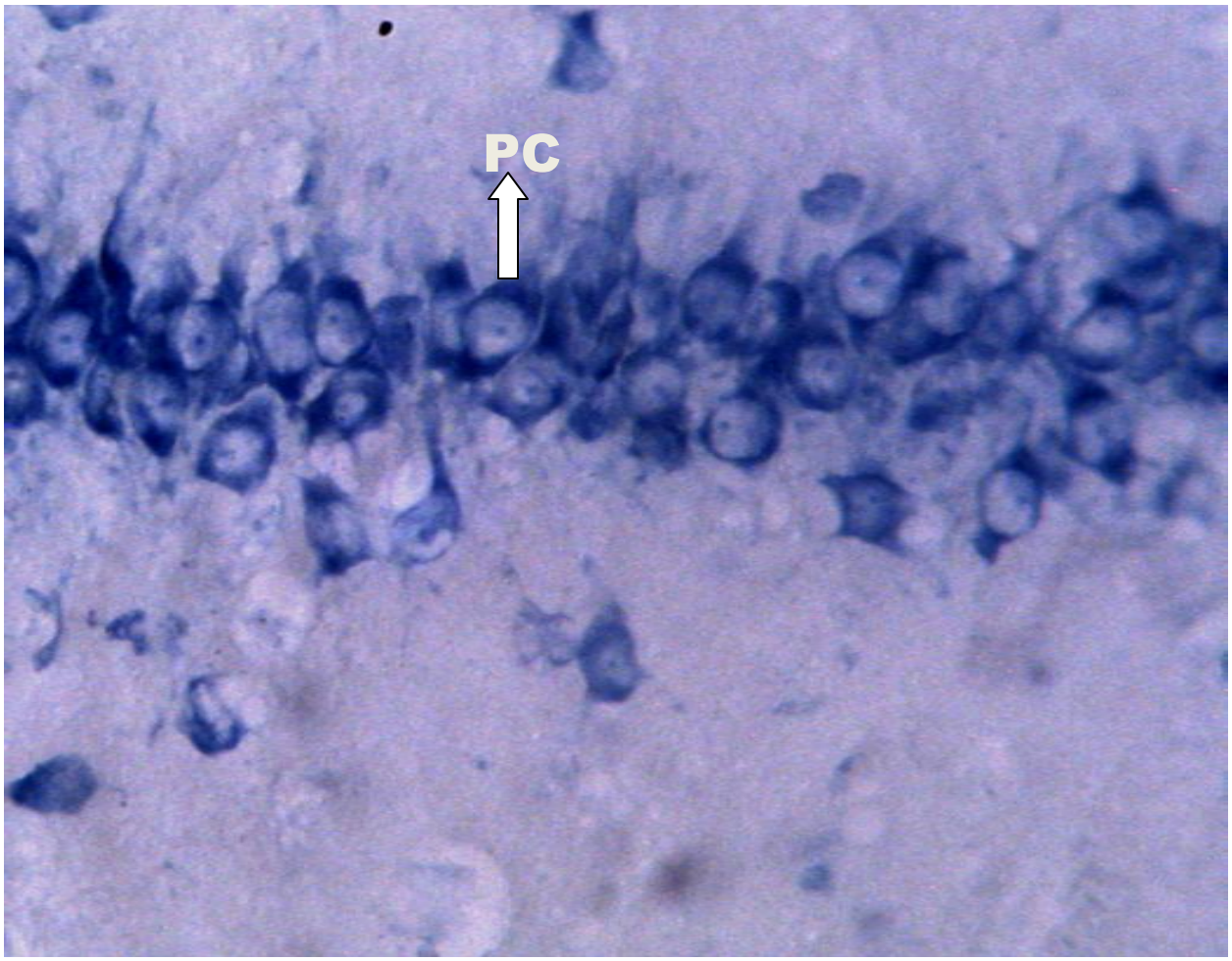



**Plate XXXVIII: Section of the hippocampus (CA3) of Group 3 Wistar rat (1000mg/kg bwt of ethanolic extract of *Psidium guajava* leaves) showing normal orientation of CA3 region, Pyramidal cell (PC) with intact nuclei (Cresyl violet Stain × 250).**

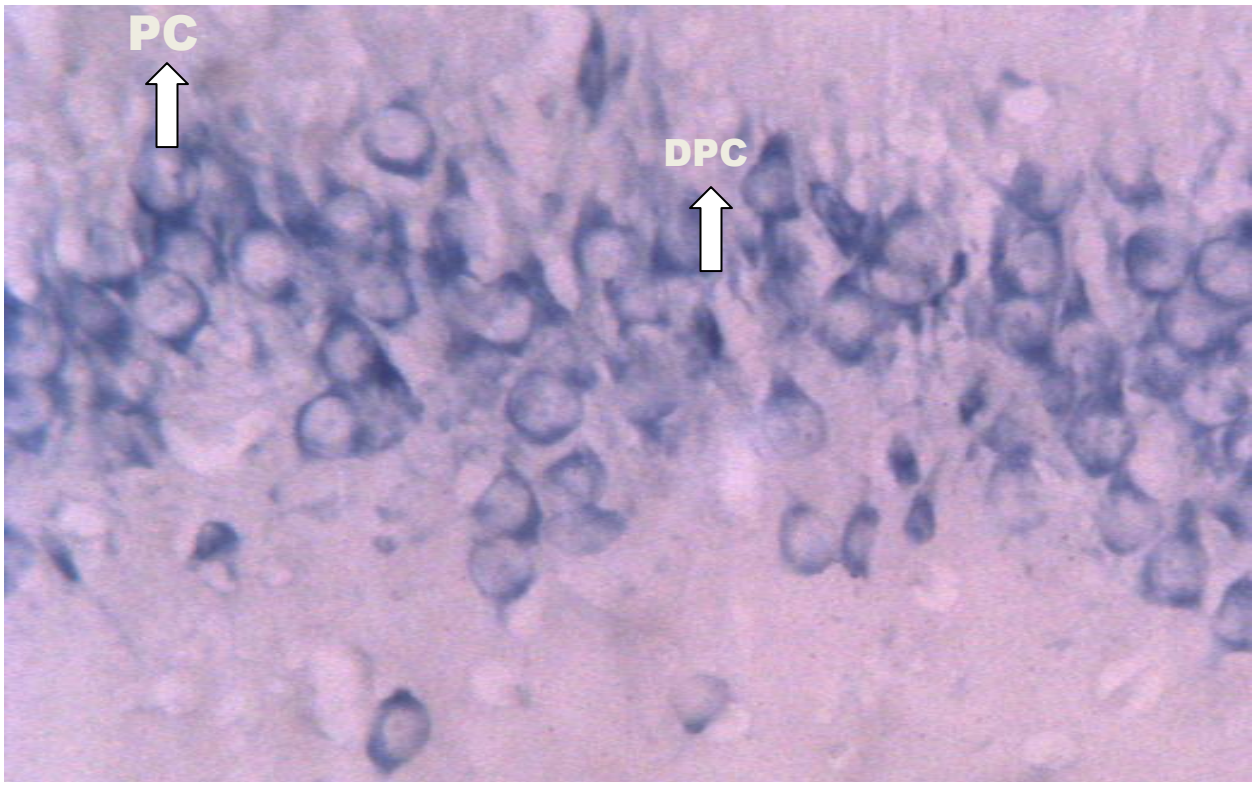



**Plate XXXIX: Section of the hippocampus (CA3) of Group 4 animals (41.5mg/kg$HgCl_2$ and distilled water) showing some normal Pyramidal cells (PC)and degenerating cell (DPC) (Cresyl violet Stain; X250).**

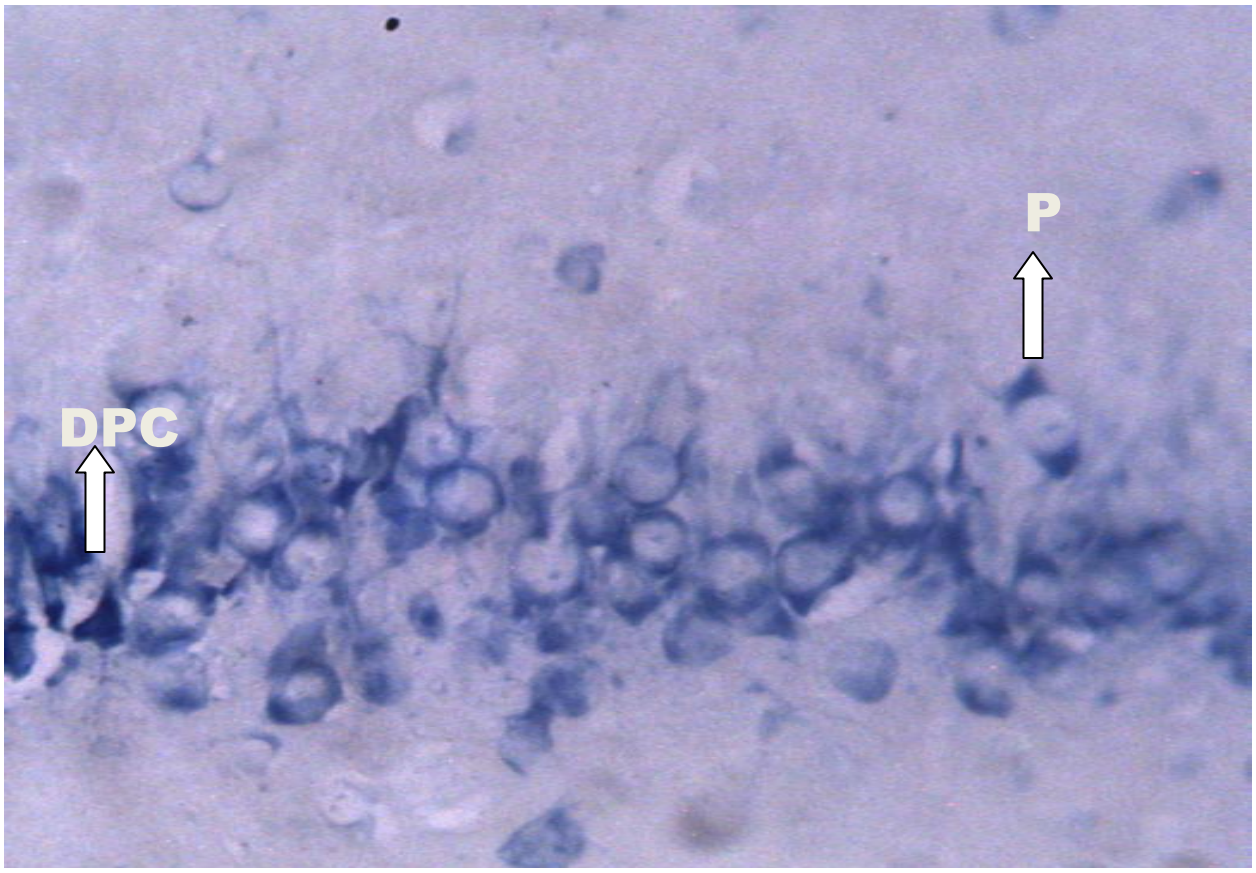



**Plate XXXX: Section of the hippocampus (CA3) of Group 5 Wistar rat (41.5mg/kg mercuric chloride and 500mg/kg ethanolic extract of *Psidium guajava* leaves) showing normal Pyramidal cells (PC) and some evidence of degenerated Pyramidal Cell (DPC) (Cresyl violet Stain × 250).**

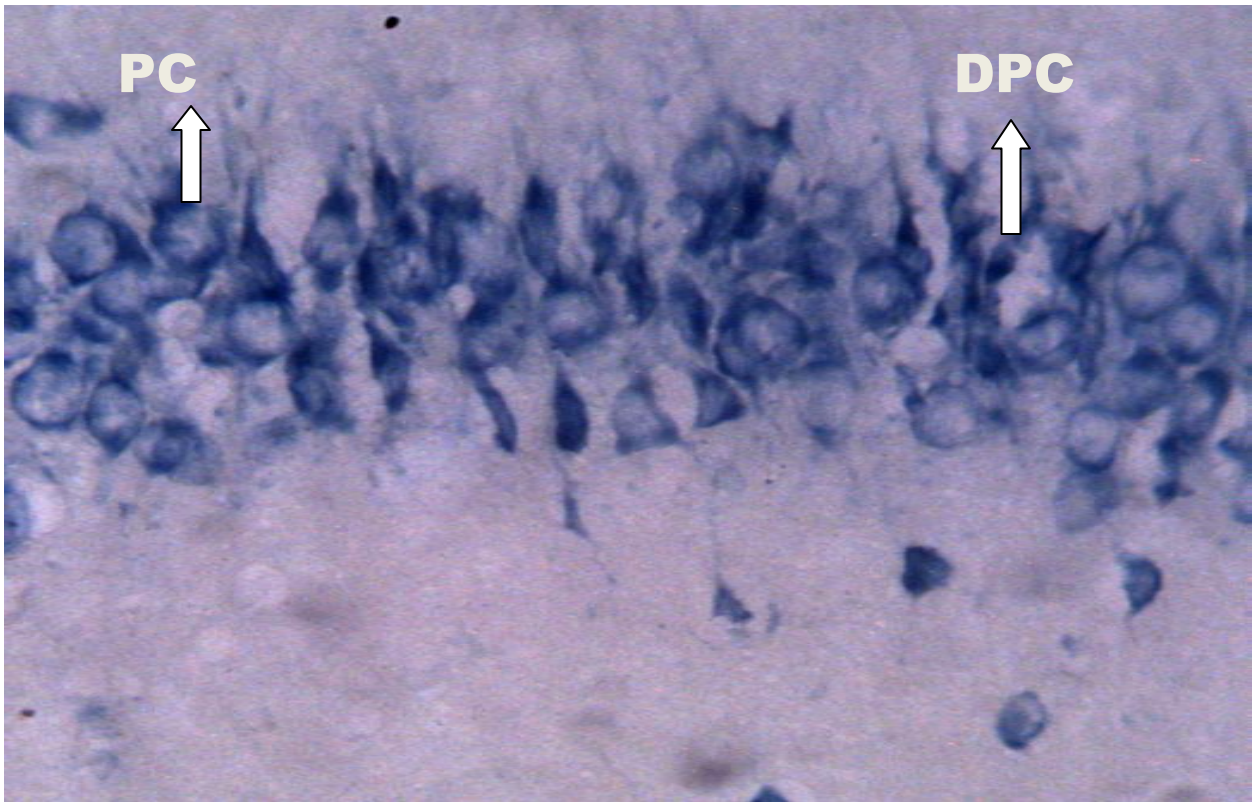



**Plate XXXXI: Section of the hippocampus (CA3) of Group 6 Wistar rat (41.5mg/kg $HgCl_2$and 1000mg/kg ethanolic extract of *Psidium guajava* leaves) showing normal CA3 region, Pyramidal cell (PC) and some evidence of degenerating Pyramidal cells (PC) (Cresyl violet Stain × 250).**

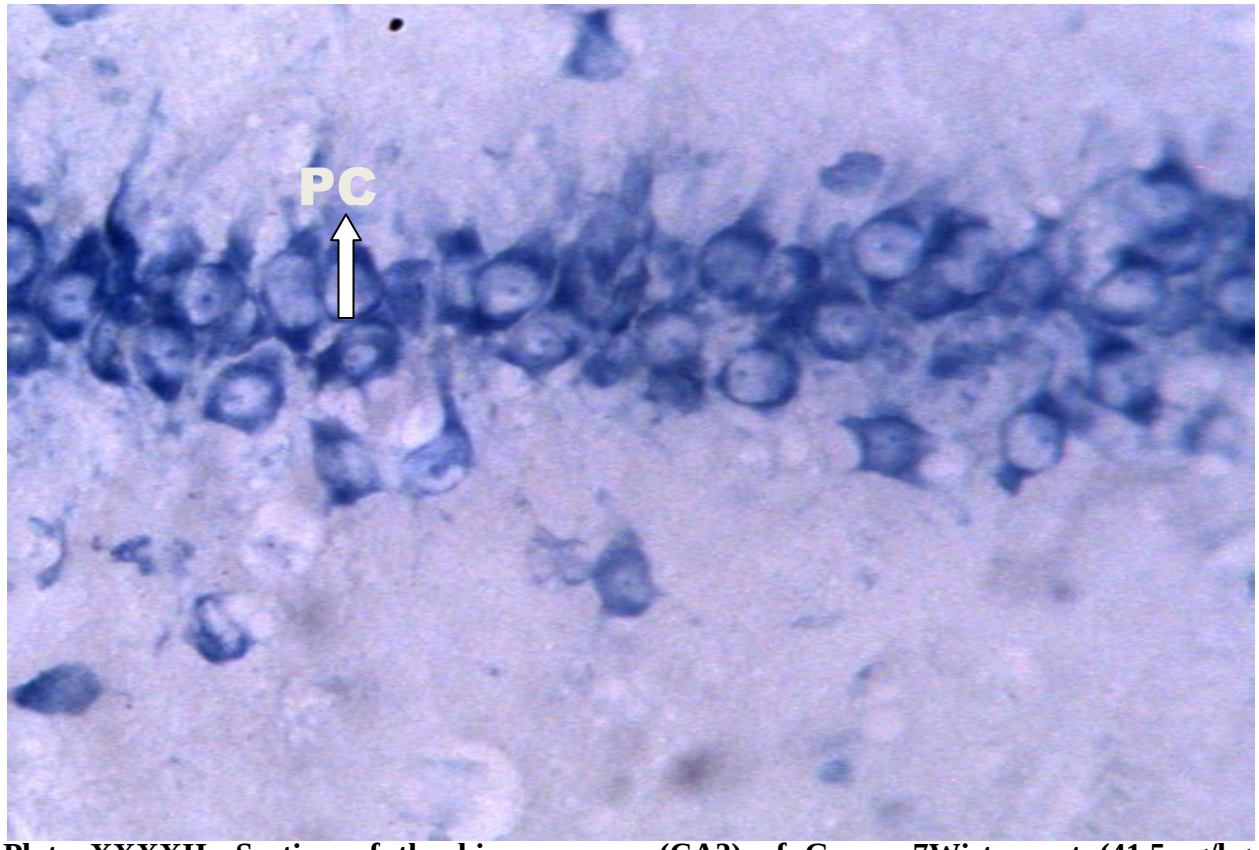



**Plate XXXXII: Section of the hippocampus (CA3) of Group 7Wistar rat (41.5mg/kg mercuric chloride and 1000mg/kg of ascorbic acid) showing CA3 region and Pyramidal cell (PC) (Cresyl violet Stain × 250).**

# CHAPTER 5

## 5.0 DISCUSSION

The result of physical observation showed that animals were very active with no visible physical changes observed in Group 1(Control) while decreased physical activity, gnawing and restlessness, scratching of the mouth and the nostrils, frequent urination and watery feces were noticed in animals treated with Mercury. This could be attributed to the effect of mercuric chloride that might have caused behavioural changes in the animals exposed to mercury as mercury poisoning especially through diet or environment has been reported to cause severe symptoms such as anxiety, restlessness, irritability, mood changes, depression and physical tremors and also put the body at unnecessary risk. The report obtained agrees with the findings of Ibegbu *et al*., (2014) who reported a decrease in physical activity and restlessness following mercury administration.

### 5.1 Body Weight Assessment of Animals

The present study showed progressive increase in the mean body weight of the animals throughout the period of administration of mercuric chloride and *Psidium guajava* leaves extract. However, the degree of weight gains by the animals treated with only mercury throughout the experiment was less than that of the animals treated with *Psidium guajava*leaves extract and ascorbic acids and animals in the Control group. The weight of animals treated with only mercury was significantly lower than the weight of animals in the Control Group and this could be attributed to the effect of mercuric chloride that might have slowed down the degree of weight gain in animals exposed to mercury. Ibegbu *et al.*(2013, 2014), and Animoku*et al.*(2016) reported that the body weight of rats, treated with mercuric chloridewas decreased significantly

lower than their Control during six weeks and eight weeks of exposure respectively. Mariana *et al. (*2016) had reported that the body weight of rats, treated with mercury decreased significantly than their Control Group during the 12 and 48 hours of exposure respectively. Animokun *et al*., (2015) had observed that exposure of rats, rabbits and dogs to mercury vapor for 7h/day, 5 days/week for 72-83 weeks, resulted significantly in weight decrease. In the present study the degree of weight gains by animals exposed tomercuric chloride and treated with *Psidium guajava* did not show any significant difference when compared to the Control group suggesting the ameliorative effect of *Psidium guajava*leaves extract on mercuric induced changes on the body weight of the animals.

**5.2 Neurobehavioral Studies**

The present study showed significant increase in the meantime taken by the experimental animals to locate the hidden platform in Morris water maze test for memory and learning during the weeks of mercuric chloride administration. This could be associated with memory loss which could be as a result of neuronal degeneration and distortion in the general morphology of the pyramidal cells and CA3 region of the hippocampus. These alterations imply that, activity such as memory and learning abilities from the brain region that projects into the pyramidal layer and CA3 region of the hippocampus were lost. (Sadeeq*et al.,* 2013). However, Mutter *et al.*(2010), had earlier reported that short term occupational exposure to high levels of mercury induced slight cognitive deficits and that, mercury has no effect on memory as observed from cognitive test for memory, which conversely does not support the findings of Pablo *et al.(*2014) reported short and long term memory impairment following exposure of experimental animals to mercuric chloride.

The results of the present study showed significant decrease in the meantime taken by the animals to locate the hidden platform in Morris water maze test for spatial learning and memory during the weeks of *Psidium guajava*leavesand ascorbic acid administration. Thus, the study has revealed the ameliorative effects of *Psidium guajava*leaves on spatial learning and memory in the experimental animals induced with mercuric chloride. Administration of *Psidium guajava*leaves extract has shown some improvement in the histology of the cerebral cortex and hippocampus of animals when compared with rats exposed to mercuric chloride only, this could be attributed to its antioxidant activity. Iliyasu *et al* (2015)had reported that *Psidium guajava* leaves extract ameliorated the effect of lead acetate induced toxicity and may likely be beneficial to population in endemic areas exposed to lead poisoning and other heavy metals. Hu *et al* (2007), had reported that *Psidium guajava* produced significant improvement in memory of young and aged rodents and reversed successfully the amnesia induced by scolpolamine and diazepam, this effect was attributed to its anti-oxidant activity and acetyl cholinesterase inhibitory activity.

**5.3 Beam walking test**

Wistar rats exposed to mercuric chloridetoxicity showed higher mean value of latency when compared to the Control Group in the beam walking test. Bruna*et al*. (2012) also reportedthat mercury exposure induces changes in the central nervous system potentially resulting in irritability, fatique, behavioural changes, tremors, incoordination and even death. Pablo *et al. (*2014) also reported motor deficits in rats treated with mercuric chloride.Groups treated with Vitamin C and high dose *psidium gujava leaves* extract did not show significant difference from

the Control Group. The action observed in animals treated with *Psidium guajava* leaves extract may be due to synergism of quercetin with other component of the extract.

**5.4Elevated plus maze studies**

The elevated plus maze test is a typical test widely employed to study the effect of potential anxiogenic and antidepressant compound in animal model. Anxiety like behaviour is measured as preference for closed arm or less activity (entry or duration) in the open arm (Acevedo*et al*., 2012). The results showed anxiolytic behaviour in animals treated with mercury alone, although there was no significant difference in the mercury treated group when compared to the Control. This observation was similar to the finding Pablo*et al*.(2014), who reported anxiety –related behaviours following chronic intoxication with inorganic mercury. A finding by Cargi *et al*(2012), showed that treatment with cadmium and mercuric chloride on offspring of Wistar rats at all doses increased anxiety like behaviours of offspring in the elevated plus maze as indicated by reduced number of entry into the closed arm. Highest doses of cadmium and mercury resulted in increased anxiety like behaviour indicated by a reduced number of entries and time spent in margins and reduced number of entries and time spent in the open arm. The present study showed that animals treated with *Psidium guajava* leaves extract following mercury exposure did not exhibit anxiolytic behaviour. Gurpeet*et al*.(2016) who reported that the ethanolic extract of *Psidium guajava* leaves showed anti-anxiety effect on mice, *Psidium guajava*leaves extract was found to be more effective in decreasing anxiety like behaviour when compared to the standard drug diazepam.

## 5.5Oxidative Stress Studies

Catalase is a common enzyme found in nearly all living organisms, it catalyses hydrogen peroxide into water and oxygen and protects the organism from free radicals**.** The oxidative stress study showed significant reduction in catalase concentration in animals treated with mercuric chloride alone when compared to the control group, as values obtained were significantly lower. Treatment with *Psidium guajava*leaves extract in the present study was observed to be associated withimprovement in the concentration of Catalase in the brain tissues supporting its role as a potent antioxidant. This finding from this study is in agreement with the work the of Vijay and Ramasamy (2016) also reported increase in Catalase activity following treatment with ethanolic extract of *Psidium guajava* leaves on streotozotocin induced diabetic rats as result obtained showed that the ethanolic extract of *Psidium guajava* leaves regulates the antioxidant status in streotozotocin induced diabetic treated groups.

Malondialdehyde, a secondary product of lipid peroxidation is a good marker of free radical mediated damage and oxidative stress. Mercury exposure from day 21 to 42 was observed to be associated with a significant increase in the concentration of malondialdehyde levels in animals treated with mercury alone when compared to the Control Group, thereby buttressing the fact that exposure to mercury is associated with oxidative damage particularly the central nervous system (Huang*et al*.,2010). Treatment with *Psidium guajava*leavesin this study was observed to be ameliorative in mercury treated rats as values obtained were not significant when compare to the Control Group. These findings also support the fact that antioxidants protect against oxidative damage.Vijay and Ramasamy (2016) reported a decrease in malondialdehyde level of

stretozotocin induced diabetic rats and this marked decrease was attributed to the antioxidant activity of *Psidium guajava* leaves extract.

Gluthathione is a key component in metal scavenging due to the high affinity of metals to its thiol group and as a precursor of phytochelatins (Pcs). A reduction in the concentration of reduced glutathione was observed in mercury treated groups. Khan *et al. (*2012) reported a decrease in glutathione level following exposure to mercuric chloride in Wistar rats. This could be due to the interaction of reduced state glutathione and mercuric chloride to form oxidised glutathione or mercuric-glutathione complex. Treatment with *Psidium guajava*leaves extract was observed to be therapeutic as values obtained were not significant when compared with the Control group.Neeraj *et al.* (2012) also reported a significant restoration activities of oxidative stress markers; of Catalase, Superoxide dismutase and glutathione inarsernic induced oxidative stress and cytological damage in Wistar rats after treatment with *Psidium guajava* leaves. This suggests that *Psidium guajava* could provide specific protection from oxidative injury and to some extend on tissue damage.

The activity of SOD is a sensitive index in oxidative damage as it scavenges the superoxide anion from hydrogen peroxide leading to the reduction in the toxic effects (Khan *et al*., 2012). In the present study tissue SOD level decreased in the mercuric chloride treatedGroups when compared with the control; however, there was a remarkable increase in the SOD level of rats treated with *Psidium guajava* leaves extract.There was no significant difference between the Groups treated with *Psidium guajava* leaves extract when compared to the Control suggesting its ameliorative effect. Neeraj *et al.* (2012) had reported a significant restoration activity of oxidative stress markers; of Catalase, Superioxide dismutase and glutathione in arsernic induced

oxidative stress and cytological damage in Wistar rats after treatment with *Psidium guajava* leaves.

## 5.6 HISTOLOGICAL STUDIES

The findings from this study revealed that mercury exposurecaused degenerative changes, distortion of cellular layers of the cerebral cortex, hippocampus and cerebellum of adult Wistar rats. The Pyramidal cells in the hippocampus showed changes ranging from degeneration and reduction in the number of pyramidal cells when compared to animals in the Control Group suggesting the toxic effect of mercury exposure to the animals. This implies that the activities of the hippocampus in memory formation and learning, storage and retrieval of information could be impaired. Wolf *et al.*(2009) in his studies also observed that rats exposed to mercury vapour produced neurodegenerative changes in the hippocampus which was related to impaired memory deficit in the animals.

The cerebral cortex showed neuronal changes involving loss of pyramidal cells with vacuolated cell bodies and necrosis of cells in rats treated with mercury. These neurodegenerative changes could invariably affect learning, memory and hearing abilities associated with the cerebral cortex. Ibegbu *et al.,* (2013) also reported clumping of cerebral cortical cells and necrosis of cells in adultWistar rats following administration of different doses of mercuric chloride for a period of three weeks.

The cerebellum showed neuronal degeneration and vacuolation of cell bodies. These changes could result in disruption of smooth and balanced muscular activity. Other researchers reportedthat many heavy metals such as mercury, lead,cadmium and other organic compounds

have thecapacity to damage the nervous tissues (Farina*et al.,* 2011; Ibegbu *et al.*, 2014; Animoku*et al.,* 2015). It has beenreported that cerebral dysfunction may occur inassociation with exposure to a wide variety oftoxins including heavy metals such as mercury,lead, thallium, manganese, drugs and solvents whilepermanent abnormalities areinduced only by sustained use and exposure tothese chemicals in large quantity (Farina *et al.,* 2011).

The present findings showed that *Psidium guajava*leaves ameliorated the distortions on the normal cytoarchitecture of the cerebral cortex, hippocampus and cerebellum caused by mercuric chlorideand studies indicated that plants rich in antioxidants couldhelp to reduce oxidative stress and to some extent protect tissues from damages (Neeraj *et al*.,2012: Illiyas*et al.*,2015) Hence, *Psidium guajava*leaves extractserved as an antioxidant that played a significant role in the reversal of the toxicity caused by mercuric chloride.

# CHAPTER SIX

# SUMMARY, CONCLUSION AND RECOMMENDATION

## 6.1 Summary

The present study showed that mercury exposure has significantly resulted in a decreased weight in animals exposed to mercuric chloride while animals treated with *Psidium guajava*leaves extract showed significant increase in body weight during the periods of treatment, suggesting the ameliorative effect of *Psidium guajava* against mercury exposure. The findings from the present study revealed that *Psidium guajava* administration was able to reverse to some extent the toxic effect of mercury on spatial memory and learning using Morris water maze navigation test. The result showed significant decreasein the meantime taken by the animals to locate the hidden platform during the weeks of *Psidium guajava*leaves extract administration as opposed to animals treated with mercuric chloride only. The present day study also revealed that *Psidium guajava* administration has ameliorative effect on motor coordination in rats treated with mercuric chloride. Wistar rats exposed to mercuric chloride toxicity in the beam walking test showed higher mean value of latency when compared to the Control group. While there was significant reduction in the mean value of latency for animals treated with *Psidium guajava* after exposure to mercuric chloride. The elevated plus maze test showed anxiolytic behaviour in animals treated with mercury alone. However, animals treated with *Psidium guajava* following mercury exposure did not exhibit anxiety-like behaviour suggesting the anti-anxiety effect of *Psidium guajava*leaves.

The histological changes observed from the present study ranges from degeneration of hippocampal cells, cerebral cortical cells, Pukinje cells, loss of cells, vacuolation and eventually cell death following exposure to mercuric chloride. These changes in the histological studies as a result of mercury exposure in rats affected the neuronal cells in the hippocampus, cerebral cortical cells and cerebellar cells could be related to neurobehavioral deficiencies and or memory impairment. However, *Psidium guajava*leaves extract have shown some level of amelioration against theneurotoxicity induced by mercuric chloride.

## 6.2 Conclusion

From the observation from the study, we concluded that;

i. The present study observed that there was deficit in spatial learning and memory and motor activities in adult Wistar rats exposed to mercuric chloride.
ii. Mercuric chloride induced oxidative stress in adult Wistar rats.
iii. There were histoarchitectural changes in the cerebrum, cerebellum and hippocampus of animals exposed to mercuric chloride.
iv. The study has shown that ethanolic extract of *Psidium guajava* leaves ameliorated the changes induced by mercuric chloride in behaviour, oxidative stress markers and histoarchitectural of adult Wistar.
v. Thus, ethanolic extract of *Psidium guajava* leaves may be useful therapy in mercuric exposed individuals.

## 6.3 Recommendations

The present study has shown the ameliorative effect of *Psidium guajava* leaves on the body weight, neurobehavioral, biochemical and histological parameters of adult Wistar rats treated with mercuric chloride. I recommend that;

1. Further studies involving immune-histochemical methods should be carried out to elucidate the deposits of mercury in brain tissues.

2. Further work should be done on the effect of *Psidium guajavaon* the development of the central nervous tissue using electron microscopy for a better resolution.

3. Cell culture should be employed in the studies of the effectof*Psidium guajava* onthe development of the central nervous system to minimize the use of animal.

4. The consumption ethanolic extract of *Psidium guajava* leavesshould be encouraged to populace who may be exposed to increased risk of mercury poisoning.

## 6.4 Contribution to Knowledge

1. Administration of ethanolic extract of *Psidium guajava* leaves at a dose of 1000mg/kg bwt (14.0±2.09) improved learning and memory in Wistar rats exposed to mercuric chloride compared with Vitamin C (15.33±3.18).
2. Administration of ethanolic extract of *Psidium guajava* leaves at a dose of 1000mg/kg bwt (124.0±2.09) the antioxidant activity in Wistar rats exposed to mercuric chloride compared with Vitamin C (118.33±3.18).
3. *Psidium guajava*leaves ameliorated the toxicity caused by mercuric chloride in the Cerebrum, Cerebellum and Hippocampus of Wistar rats.

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## APPENDIX 1

## MERCURIC CHLORIDE, *PSIDIUM GUAJAVA* AND ASCORBIC ACID DOSE CALCULATIONS

$LD_{50}$of $HgCl_2$ - - - - - - 166mg/kg body weight

25% of $LD_{50}$of $HgCl_2$ - - - - - 41.5mg/kg body weight

Average weight of Wistar rats - - - - 160grams

41.5mg of $HgCl_2$ - - - - - 1000grams of Wistar rat

Xmg - - - - - - - 160grams of Wistar rat

Xmg = 6.64mg/160gbwt

6.64mg of $HgCl_2$ - - - - 1000mls or 1Litre of Dist. $H_2O$

Hence, 0.00664mg of $HgCl_2$ - - - 1ml of solution

$LD_{50}$of EEPGL- - - - 5000mg/kg body weight

10% of $LD_{50}$ofEEPGL - - - - 500mg/kg body weight

Average weight of Wistar rats - - - - 160grams

500mg of EEPGL - - - - - 1000grams of Wistar rat

Xmg - - - - - - - 160grams of Wistar rat

Xmg = 80mg/160gbwt

80mg of EEPGL- - - - 1000mls or 1Litre of Dist. $H_2O$

Hence, 0.08mg of Vit. C - - - 1ml of solution.

$LD_{50}$of Vit. C- - - - - - 11, 900mg/kg body weight

10% of $LD_{50}$of Vit. C - - - - - 1190mg/kg body weight

Average weight of Wistar rats - - - - 160grams

1190mg of Vit. C - - - - - 1000grams of Wistar rat

Xmg - - - - - - - 160grams of Wistar rat

Xmg = 190.4mg/160gbwt

190.4mg of Vit. C - - - - 1000mls or 1Litre of Dist. $H_2O$

Hence, 0.190mg of Vit. C - - - 1ml of solution.

NB: 1 tablet contains 100mg of Vit. C.

APPENDIX II

PREPARATION OF STAINING SOLUTION AND STAINING

**1. Haematoxylin and Eosin Stain**

A. Haematoxylin (Harris (1990) Haematoxylin)

- Haematoxylin - 1 g
- Absolute alcohol - 10 ml
- Ammonium or potassium alum - 20 g
- Distilled water - 200 ml
- Mercuric oxide - 0.5 g

Haematoxylin was dissolved in alcohol and alum which was dissolved in water was added. The mixture was then boiled and mercuric acid added when solution turn dark purple. It was then cooled rapidly.

**B. Eosin**

- Distilled water
- Crystal of thymol 5 %

Eosin was made by dissolving 5 g of eosin in 100 mlof distilled water. To prevent growth of mould in the stock solution, a crystal of thymol was added to obtain 1% staining solution, 20 ml of the stock solution was diluted by making it up to 100 ml.

**C. Staining**

- Removal of wax with xylene
- Dewaxing I, 3 minutes
- Dewaxing II, 3 minutes
    - **Hydration with graded alcohol**
        - Absolute alcohol, 1 minute
        - 95% alcohol, 1 minute
        - 70% alcohol, 2 minutes
        - 50% alcohol, 2 minutes
        - 30% alcohol, 1 minute
    - **Staining Proper**
        - Haematoxylin, 10 – 15 minutes
        - Distilled water (washing)
        - 35 % alcohol, 1 minute
        - Alcohol, 30 seconds (for differentiation between nucleus and cytoplasm)
        - Distilled water, 1 minute
        - Alkaline – alcohol, 2 minutes (for bluing of the nucleus)
        - Distilled water, 1 minute
        - Staining in 1% eosin, 2 minutes
        - Dehydration in alcohol
        - 95% alcohol, 10 -15 seconds
        - Absolute alcohol, 1 minute
    - **Clearing**

- Xylene – alcohol, 2 minutes
- Pure xylene I, 3 minutes
- Pure xylene II, 3 minutes

- **Mounted in DPX**

(Drury *et al*., 1967)

2. CRESYL FAST VIOLET STAINING

- Fixation in 10% formal saline

➢ **Section**

- **Technique**
- Dewax using xylene and take section to water
- Staining for 1 – 6 hours 0.2% cresyl fast violet
- Rinse in distilled water and different in 95% alcohol
- Dehydrate, clear and mount in mountant.

(Drury *et al*., 1967)