EVALUATION OF THE PROTECTIVE EFFECT OF AQUEOUS AND ETHANOL FRUIT EXTRACTS OF *Phoenix dactylifera L.* ON MERCURY - INDUCED NEUROTOXICITY IN WISTAR RATS

BY

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TITLE PAGE

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BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (Ph.D) DEGREE IN HUMAN ANATOMY

DEPARTMENT OF HUMAN ANATOMY

FACULTY OF MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

APRIL, 2017.

DECLARATION

I, Abel Nosereme AGBON, hereby declare that the work in the thesis entitled "Evaluation of the protective effect of aqueous and ethanol fruit extracts of *Phoenix dactylifera L.* on mercury - induced neurotoxicity in Wistar rats" has been carried out by me in the Department of Human Anatomy.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at this or any other institution.

Abel Nosereme AGBON _____ Signature _____ Date

CERTIFICATION

This thesis entitled "EVALUATION OF THE PROTECTIVE EFFECT OF AQUEOUS AND ETHANOL FRUIT EXTRACTS OF *Phoenix dactylifera L.* ON MERCURY - INDUCED NEUROTOXICITY IN WISTAR RATS" by Abel Nosereme AGBON meets the regulations governing the award of the degree of Doctor of Philosophy of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

THIS THESIS IS DEDICATED

to

MY LOVING PARENTS

And to

MY DEARLY BELOVED BROTHERS AND SISTER

And to

MY ENTHUSIASTIC RESEARCH STUDENTS

And to

ALL NEUROANATOMIST AND UPCOMING NEUROSCIENTIST IN NIGERIA

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ABSTRACT

In this study, evaluation of the protective effect of aqueous and ethanol fruit extracts of *Phoenix dactylifera L.* on mercury-induced neurotoxicity in Wistar rats, phytochemical screening of *P. dactylifera* fruit extracts (aqueous and ethanol) revealed the presence of flavonoids, saponins, tannins and alkaloids. The protective effect of the extracts were assessed on mercury-induced cerebral, cerebellar and hippocampal neurotoxicity in Wistar rats. Evaluation of the protective effect of *P. dactylifera* fruit extracts involved the following investigations: brain morphological studies; histometry of pyramidal cells of cerebral cortex- *layer V* and CA1 and CA3 hippocampal regions, and Purkinje cells of the cerebellar cortex; routine histological and histochemical studies of cerebral and cerebellar cortices, and hippocampus (CA1 and CA3 regions); neurobehavioural studies employing elevated plus maze (EPM) to assess anxiety-related behavior, transfer latency (TL) on EPM open arm and Morris water maze (MWM) to evaluate learning and memory, forelimb grip strength (FGS) test to assess muscular strength in the forelimbs and beam walking test (BWT) to monitor motor coordination and balance of Wistar rats; neurochemical analysis to assess the concentration of neuro-trace elements (copper, Cu; iron, Fe; manganese, Mn and zinc, Zn) in brain-tissue homogenate and biochemical analysis to assess lipid peroxide levels and antioxidant enzyme activity (malondialdehyde, MDA; superoxide dismutase, SOD; catalase, CAT and glutathione peroxidase, GPx) in blood serum and brain-tissue homogenate, and acetylcholinesterase (AchE) activity for endogenous enzyme in brain-tissue homogenate of Wistar rats. Seventy-two (72) Wistar rats (male and female) were divided into two *experimental categories*: **1** (twenty-four (24) rats, consisting of three groups; I - III *served as the common groups*) and **2** (forty-eight (48) rats, subdivided into *treatment categories*: A and B of twenty-four (24) rats each, consisting of three groups; IV - VI). Thus, each category consisted of six groups (I - VI) of eight rats each. Group I served as control and was administered distilled water (0.5 ml) while, groups II - VI were treatment groups. Neurotoxicity was induced in rats by the administration of mercury – mercuric chloride ($HgCl_2$). Group II was administered $HgCl_2$ (5 mg/kg); group III was administered vitamin C (100 mg/kg) as reference drug; groups IV, V and VI were administered fruit extract of *P. dactylifera* (250 mg/kg, 500 mg/kg and 1,000 mg/kg,

respectively) followed, concomitantly, by $HgCl_2$ (5 mg/kg) for a period of two weeks. Rats in *category A* were administered *aqueous* fruit extract of *P. dactylifera* (AFPD), while *category B* were administered *ethanol* fruit extract of *P. dactylifera* (EFPD); all administrations were via oral route. Results revealed that, exposure to $HgCl_2$ resulted in neurotoxicity in rats, which was evident from morphologic, histometric, histologic and histochemical alterations in brain; $HgCl_2$ induced anxiety-related responses, short- and long-term memory impairments and motor deficits in neurobehavioural assessment and, altered the levels of neurochemical and biochemical constituent in rats. However, administration of AFPD and EFPD revealed the potentials of the extracts as neuroprotective agent in the following ways: brain morphologic features and histometric characteristic of brain regions (cerebral and cerebellar cortices and hippocampus) were preserved relative to the control; histologic and histochemical alterations in cerebral and cerebellar cortices and hippocampal regions (CA1 and CA3) were ameliorated relative to the control; anxiety-related responses, short- and long-term memory impairments, and motor coordination and balance deficits in neurobehavioural assessment were ameliorated relative to the control; Alterations in the concentration levels of neuro-trace elements (Fe, Mn, Cu and Zn), MDA and endogenous antioxidants (SOD, CAT, GPx), and endogenous enzyme (AchE) activity levels were moderately ameliorated relative to the control. The neuroprotective property of extracts, relative to the reference (vitamin C), is rather similar, and is attributed to antioxidant properties of constituent phytochemicals, such as flavonoids. Neuroprotective activity was dose dependent; 500 and 1,000 mg/kg doses possessing maximal activity for both extracts – with EFPD more efficacious. In conclusion, findings suggest that, AFPD and EFPD are potentially efficacious in ameliorating mercury-induced alterations in the brain (cerebral and cerebellar cortices and hippocampus) of Wistar rats and could be potential candidates for application in the management and treatment of reactive oxygen species-induced neurodegenerative diseases.

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LIST OF ABBREVIATIONS

A.A	Ascorbic acid
ACh	Acetylcholine
AchE	Acetylcholinesterase
AD	Alzheimer's disease
AFP	Alpha fetoprotien
AFPD	Aqueous fruit extract of *Phoenix dactylifera*
AFR	Ascorbate free radical
AGEs	Advanced glycation end products
ALP	Alkaline phosphatase
ALS	Amyotrophic lateral sclerosis
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ANS	Autonomic nervous system
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
ATSDR	Agency for Toxic Substances Disease Registry
BDH	British Drug Houses
bw	Body weight
BWT	Beam walking test
CA	*Cornu Ammonis*
CAM	Complementary Alternative System of Medicine
CARB	California Air Resources Board
CAT	Catalase

CFS	Chronic fatigue syndrome
CFV	Cresly Fast Violet
CNS	Central nervous system
CNs	Cranial nerves
Cu	Copper
DHA	Dehydroascorbate
DNA	Deoxyribonucleic acid
EFPD	Ethanol fruit extract of *Phoenix dactylifera*
EMMI	Environmental Monitoring Methods Index
EPA	Environmental Protection Agency
EPD	Extract of *Phoenix dactylifera*
EPM	Elevated plus maze
Fe	Iron
FGS	Forelimb grip strength
GABA	Gamma aminobutyric acid
GLUT	Glucose transporter
GPx	Glutathione peroxidase
GSH	Glutathione
GSSG-R	Glutathione reductase
H&E	Haematoxylin and Eosin
HDL	High density lipoproteins
Hg^0	Elemental mercury
$HgCl_2$	Mercuric chloride
IDCM	Idiopathic dilated cardiomyopathy
IL-1	Interleukin I

kg	Kilogram
LDH	Lactate dehydrogenase
LDL	Low density lipoproteins
LSD	Least significant difference
MDA	Malondialdehyde
MeHg	Methylmercury
mg	Milligram
ml	Milliliters
Mn	Manganese
MS	Multiple sclerosis
MWM	Morris water maze
NMDA	N-Methyl-D-Aspartate
NRC	National Research Council
OEHHA	Office of Environmental Health Hazard Assessment,
P. dactylifera	*Phoenix dactylifera*
PD	Parkinson's disease
PFC	Prefrontal cortex
PNS	Peripheral nervous system
RER	Rough endoplasmic reticulum,
RNA	Ribonucleic acid
R-O-O-H	Organic peroxides
ROS	Reactive oxygen species
SH	Sulfhydryl groups
SOD	Superoxide dismutase
SVCT1	Sodium-dependent vitamin C transporter-type 1

T cells	T lymphocytes
TBARS	Thiobarbituric acid-reactive-substances
TL	Transfer latency
TLC	Thin layer chromatography
TNF	Tumor Necrosis Factor
TPC	Total phenolic content
UNEP	United Nations Environment Programme
WHO	World Health Organisation
Zn	Zinc

CHAPTER ONE

3.0 Introduction

1.1 Background Information

Mercury is a widespread environmental and industrial pollutant that exerts toxic effect on a variety of vital organs; it induces severe alterations in the tissues (Lund *et al.,* 1993; Mahboob *et al.,* 2001; Sener *et al.,* 2007; Wadaan, 2009; Xu *et al.,* 2012), causes numerous neurological abnormalities (Auger *et al.,* 2005; Kingman *et al.,* 2005) and produces peripheral neuropathy (Boyd *et al.,* 2000; Chuu *et al.,* 2007) in experimental animals and human beings.

The central nervous system (CNS) is one of the most vulnerable organs affected by mercury toxicity. Within the CNS, two of the most often affected areas are the cerebral cortex (Eto *et al.,* 2001; Ferraro *et al.,* 2009) and the cerebellum (Fonfria *et al.,* 2005; Korogi *et al.,* 2011).

Nature is, and will still serve as, the man's primary source for the cure of his ailments. However, the potential of higher plants as sources for new drugs is still largely unexplored (Oke, 2002). About 80% of people in the developing countries rely on phytomedicine for primary healthcare for man and livestock (Harvey, 1999). In traditional practices of medicine, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as Alzheimer's disease (AD) and other memory related disorders (Kumar and Khanum, 2012).

Phoenix dactylifera (date palm) fruits, an important component of diet in the arid and semiarid regions of the world (Biglari *et al.*, 2008) are a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium, and a significant amount of calcium (Abdel-Hafez *et al.*, 1980; Usama *et al.*, 2009). Dates contain vitamins and are widely used in traditional medicine for the treatment of various disorders e.g. memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders (Nadkarni, 1976). It is also used in the treatment for sore throat, to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication (Barh and Mazumdar, 2008; Al-daihan and Bhat, 2012).

Several pharmacological studies have been conducted on *Phoenix dactylifera* and it has been demonstrated to have antiulcer activity; anticancer activity; anti-diarrhoeal activity; hepatoprotective activity; antimutagenic activity; antiinflammatory activity; *in vitro* antiviral activity; effect on reproductive system; antihyperlipidemic activity; nephroprotective activity and antioxidant activity (Vyawahare *et al.*, 2009; Agbon *et al.*, 2013).

Several researchers have also documented the antioxidant property of *Phoenix dactylifera* (Mohamed and Al-Okbi, 2004; Allaith and Abdul, 2005; Al-Qarawi *et al.*, 2008). Of late, more attention has been paid to the role of natural antioxidants mainly phenolic compounds, which may have more antioxidant activity than vitamins C, E, acarotene (Vinson *et al.*, 1998; Haslam, 2006).

1.2 Mercury

Mercury is a heavy metal contaminant with potential for global mobilization following its give off from anthropogenic activities or natural processes (Gochfeld, 2003). In nature, elemental mercury (Hg^0) can be biotransformed and converted to methylmercury (MeHg), which is the most toxic form of mercury in the environment (Malm, 1998; Eisler, 2004; Clarkson and Magos, 2006). The studies about MeHg toxicity became ubiquitous and diversified since the outbreak of environmental catastrophes such as those in Minamata (1950s) and Niigata (1960s). In such episodes, as a consequence of MeHg exposure, the exposed individuals exhibit severe forms of neurological disease which include a collection of cognitive, sensory, and motor disturbance (Takeuchi *et al.,* 1979; Eto, 2000).

The studies on MeHg toxicity have tried to evaluate its impact on several ecosystems around the world, including places in Japan, Irak, Canada, Africa, including Brazilian Amazon, and India (Malm, 1998; Harada *et al.,* 2001; Agarwal *et al.,* 2007) as well as to understand its toxicological effect on biological systems. MeHg was firstly recognized as a potent neurotoxicant for the adult nervous system in studies performed on exposed workers of a chemical factory in England (Hunter *et al.,* 1940; Hunter and Russell, 1954). Later, its importance as a neurotoxicant for the nervous system during development was recognized in the Minamata's outbreak (Takeuchi *et al.,* 1979; Eto, 2000). Since then, several studies of exposed human populations as well as experiments with laboratory animals demonstrated that exposure to toxic levels of MeHg during pre- and post-natal life causes neurological abnormalities, cognitive impairment, and behavioural disturbance (Steuerwald *et al.,* 2000; Cordier *et al.,* 2002).

Mercury exists in three predominant forms: elemental (Hg^0), organic (such as, methylmercury –MeHg), and inorganic (mercuric chloride – $HgCl_2$) mercury (Clarkson and Magos, 2006). Each form has its own effects, routes of absorption and tissue specificity. Organic mercury is the most deadly of the mercury compounds, probably due to its ability to penetrate cells (Abdel-Salam *et al.*, 2010).

Mercury poisoning can result from inhalation, ingestion or absorption through the skin and may be highly toxic and corrosive once absorbed into blood stream (Wadaan, 2009). The major sources of mercury load in humans are food contamination, drug and vaccine preservatives, dental amalgams, or occupational exposure (Clarkson, 2002). Both acute and chronic exposure to mercury is also known to cause a variety of neurological or psychiatric disorders (Choi *et al.*, 1988; Meyer-Baron *et al.*, 2002; Clarkson *et al.*, 2003; Mutter *et al.*, 2005).

The central nervous system (CNS) (developing and adult) is one of the most vulnerable organs affected by mercury toxicity. Within the CNS, two of the most often affected areas are the cerebral cortex (Eto *et al.*, 2001; Ferraro *et al.*, 2009) and the cerebellum (Fonfria *et al.*, 2005; Korogi *et al.*, 2011). In the adult brain, MeHg poisoning damages the so-called primary areas of the cerebral cortex, affecting the visual, auditory, somatic sensory, and motor cortex, as well as the hippocampus and the granule layer of the cerebellum, causing a remarkable loss of neurones in these brain regions.

On the other hand, in the developing brain there is a widespread neuronal loss throughout the CNS, what has been interpreted as due to the high MeHg sensitivity of the immature CNS (Steuerwald *et al.*, 2000). The neural disease due to MeHg neurotoxicity includes

such symptoms as visual field constriction, somatic sensory disturbance, hearing disturbance, cerebellar ataxia, dysarthria, and mnemic deficits (Lakowicz and Anderson, 1980; Carta *et al.,* 2003; Silveira *et al.,* 2003).

The mechanisms underlying mercury-induced toxicity remain largely unknown. However, several studies have shown toxic effects of organic MeHg on the CNS as it can easily cross the blood–brain barrier and accumulate in the brain at high concentrations (Clarkson and Magos, 2006; Farina *et al.,* 2011). MeHg has been reported to interact with a wide range of cellular targets and affect multiple cellular functions. These toxic effects include, but are not limited to, the inhibition of neuronal ion channels (Pekel *et al.,* 1993; Sirois *et al.,* 1996; Peng *et al.,* 2002), disruption of presynaptic transmitter release and postsynaptic receptor function (Castoldi *et al.,* 2001; Yuan and Atchison, 2003; Yuan and Atchison, 2005), damage to neuronal cytoskeleton components and DNA structures (Castoldi *et al.,* 2000; Eto *et al.,* 2001; Juarez *et al.,* 2005) and alteration of Na^+/K^+ ATPase and mitochondrial function (Atchison and Hare, 1994; Insug *et al.,* 1997; Aschner *et al.,* 2000; Limke and Atchison, 2002).

1.3 *Phoenix dactylifera L.* (Date Palm)

Medicinal herbs are indispensible part of traditional medicine practiced all over the world due to easy access, low cost, least risk and low side effect profile (Sujith *et al.,* 2012). The cultural use of medicinal plants is wide spread in Africa (Ashafa and Olunu, 2011). The World Health Organization (WHO) estimates that up to 80% of the world's population relies on traditional medicinal system for some aspect of primary health care (Farnsworth *et al.,* 1985).

Phoenix dactylifera L. (date palm) a diploid with 2n = 36, is a member of the monocot family Arecaceae classified as a dioecious tall evergreen tree (Zohary and Hopf, 1993). The date palm is known to be one of the oldest cultivated trees in the world (Dowson, 1982; Abdulla, 2008). Date palm has been an important crop in arid and semiarid regions of the world. It has always played an important part in the economic and social lives of the people of these regions. The fruit of the date palm is well known as a staple food. It is composed of a fleshy pericarp and seed. Date palms have been cultivated in the Middle East over at least 6000 years ago (Copley *et al.*, 2001).

Date palm is believed to have been introduced into Nigeria in the early 8th century by Arab traders from North Africa. Date fruits are a highly valued delicacy among many communities in Nigeria, especially during ceremonies and festivals. The national consumption of dates in 2009 is estimated at 8,958 metric tons which placed the country among the world top 10 consumers of date (Sani *et. al.*, 2010).

Phoenix dactylifera Linn. is known in *Arabic* as Nakl; fruit as Balah and Tamr; in *English* as Date palm, in *French* as Dattier and in *Hausa* (Nigeria) as Dabino. *P. dactylifera L* is well known in northern Nigeria (with almost desert-like climate; the states in this axis are Jigawa, Borno, Kebbi, Yobe, Sokoto, Katsina and Zamfara) (Okere *et. al.*, 2010).

The various parts of this plant are widely used in folk medicine for the treatment of various ailments. In fact, Muslims believe that "*He who eats seven dates every morning will not be affected by poison or magic on the day he eats them*" (Miller *et al.,* 2003). Dates are widely used in traditional medicine for the treatment of various disorders e.g. memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders

(Nadkarni, 1976), and as a detersive and astringent in intestinal troubles. It is also used in the treatment for sore throat, colds, bronchial asthma, to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication (Barh and Mazumdar, 2008). Dates are a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium, and a significant amount of calcium (Abdel-Hafez *et al.*, 1980). Dates contain at least six vitamins including a small amount of vitamin C, and vitamins B_1 (thiamine), B_2 (riboflavin), nicotinic acid (niacin) and vitamin A (Al-Shahib and Marshall, 1993).

Recent studies indicate that the aqueous extracts of dates have potent antioxidant activity (Mansouri *et al.*, 2005); several researchers have also documented the antioxidant property of *P. dactylifera* (Mohamed and Al-Okbi, 2004; Allaith and Abdul, 2005; Al-Qarawi *et al.*, 2008). The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic, sinapic acids, flavonoids and procyanidins (Gu *et al.*, 2003).

1.4 Statement of the Research Problem

Mercury is present in various environmental media and food, and has caused a variety of documented, significant adverse impacts on human health and wildlife throughout the world (Xu *et al.*, 2012).

Despite massive efforts in search of new drugs that counteract mercurial toxicity, there are no effective treatments available that completely abolish its toxic effects. The use of chelating agents assists the body's ability to eliminate mercury from the tissues (Carvalho *et al.*, 2007). However, these drugs appear to be of limited use, because of their adverse

side effects (Tchounwou *et al.,* 2003) and limited ability to cross the blood-brain barrier (Aposhian *et al.,* 1995; Martins *et al.,* 2009).

Some studies have focused their efforts on the protective effects of natural compounds in various neuropathological conditions (Martins *et al.,* 2009). However, the neuroprotective potential of higher plants as sources for new drugs is still largely unexplored.

There is paucity of literature on the potential beneficial effects of fruit extracts of *P. dactylifera* against the neurotoxicity induced by mercury in Wistar rats.

1.5 Significance of the Study

1. The present study is of importance in the identification and evaluation of available natural agents as alternatives to currently used antioxidant drugs, which are frequently not free from adverse effects.
2. Results of this study could lead to the identification of safe and more effective agent that is affordable and readily available which is key to combating the problem of brain related disorders especially in developing countries.
3. Knowledge gained from this study could provoke the minds of indigenous researchers unto directing their research focus into the field of neuroscience, ethnopharmacology and medicinal plants.

1.8 Study Hypothesis

Aqueous and ethanol fruit extracts of *Phoenix dactylifera* has protective effect on mercury-induced neurotoxicity in Wistar rats.

1.9 Aim and Objectives of the Study

1.7.1 Aim

The aim was to evaluate of the protective effect of aqueous and ethanol fruit extracts of *Phoenix dactylifera L* on mercury-induced neurotoxicity in Wistar rats.

1.7.2 Objectives

The objectives were to:

1. Morphologically, histologically and histochemically investigate the protective effect of aqueous and ethanol fruit extracts of *Phoenix dactylifera L* on mercury-induced cerebral, cerebellar and hippocampal neurotoxicity in Wistar rats.
2. Investigate the cognitive effects of aqueous and ethanol fruit extracts of *P. dactylifera* on mercury-induced hippocampal toxicity in Wistar rats using Elevated Plus Maze test and Morris Water Maze test for spatial memory and learning.
3. Investigate the effect of aqueous and ethanol fruit extracts of *P. dactylifera* on mercury-induced cerebellar and cerebral toxicity in Wistar rats using Beam Walking test for motor coordination and balance and Forelimb Grip Strength test for sensory-motor activity.
4. Neurochemically investigate the protective effect of aqueous and ethanol fruit extracts of *P. dactylifera* on mercury-induced neurotoxicity in Wistar rats using biochemical analysis for metal (copper, Cu; iron, Fe; manganese, Mn and zinc, Zn) estimation, lipid peroxide levels and antioxidant enzyme activity (malondialdehyde – MDA, superoxide dismutase – SOD, catalase – CAT and glutathione peroxidase – GPx), and endogenous enzyme (acetylcholinesterase –AchE) activities.

5. Evaluate the neuroprotective effect of aqueous and ethanol fruit extracts of *P. dactylifera* on mercury-induced neurotoxicity in Wistar rats using an established antioxidant, vitamin C, as a standard.

CHAPTER TWO

4.0 Literature Review

2.1 Mercury

Severe illness and sudden death in human beings have been implicated on environmental pollution, centuries before now. Pollution can be in air, water or on land and can result from mining, automobile exhaust, agricultural and industrial activities among others. Classified among major causes of environmental pollution, are heavy metals.

Heavy metals occur as natural constituents of the earth crust, and are persistent environmental contaminants since they cannot be degraded or destroyed. Although these elements are lacking in abundance they are not lacking in significance (Chen and Chen, 2001). Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water (specific gravity of water is 1 at 4°C) (Adedokun *et al.,* 1989; Galadima *et al.,* 2011). Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; lead, 11.34; and mercury, 13.546 (Galadima and Garba, 2012).

Environmental mercury levels have increased considerably since the on-set of the industrial age. Mercury is now present in various environmental media and food (especially fish) all over the globe at levels that adversely affect humans and wildlife. Widespread exposures are occurring due to human generated sources, and past practices have left a legacy of mercury in landfills, mine tailings, contaminated industrial sites, soils and sediments. Even regions with no significant mercury releases, such as the Arctic, are

adversely affected due to the transcontinental and global transport of mercury (United Nations Environmental Programme–UNEP, 2002).

The most significant releases of mercury pollution are emissions to air, but mercury is also released from various sources directly to water and land. Once released, mercury persists in the environment where it circulates between air, water, sediments, soil and biota in various forms. Current emissions add to the global pool– mercury that is continuously mobilized, deposited on land and water, and remobilized (UNEP, 2002).

Therefore, mercury is a potent environmental contaminant that exerts toxic effect on a variety of vital organs in the human body. It exists in three predominant forms: elemental (Hg^0), organic (such as methylmercury - H_3CHg), and inorganic (mercuric chloride - $HgCl_2$) mercury (Clarkson and Magos, 2006; Xu *et al.,* 2012). Each form has its own effects, routes of absorption and tissue specificity. Organic mercury is the most deadly of the mercury compounds, probably due to its ability to penetrate cells. Within the cell it can destroy various components selectively or in total by releasing lysosomes, damaging DNA and rupturing the cell membrane (Abdel-Salam *et al.,* 2010).

The major sources of mercury load in humans are food contamination, drug and vaccine preservatives, dental amalgams, or occupational exposure (Clarkson, 2002). Both acute and chronic exposure to mercury is also known to cause a variety of neurological or psychiatric disorders (Mutter *et al.,* 2005; Xu *et al.,* 2012).

2.1.1 Definition

Mercury is a chemical element with symbol *Hg* and atomic number 80. It is commonly known as quicksilver and was formerly named *hydrargyrum*. A heavy, silvery d-block element, mercury is the only metallic element that is liquid at standard conditions for temperature and pressure. Mercury occurs in deposits throughout the world mostly as cinnabar (mercuric sulfide). The red pigment vermilion, a pure form of mercuric sulfide, is mostly obtained by reaction of mercury (produced by reduction from cinnabar) with sulfur. Mercury poisoning can result from exposure to water-soluble forms of mercury (such as mercuric chloride or methylmercury), inhalation of mercury vapor, or eating seafood contaminated with mercury (Hammond, 2005).

Mercury is used in thermometers, barometers, manometers, sphygmomanometers, float valves, mercury switches, mercury relays, fluorescent lamps and other devices, though concerns about the element's toxicity have led to mercury thermometers and sphygmomanometers being largely phased out in clinical environments in favour of alternatives such as alcohol- or galinstan-filled glass thermometers and thermistor- or infrared-based electronic instruments. Likewise, mechanical pressure gauges and electronic strain gauge sensors have replaced mercury sphygmomanometers. Mercury remains in use in scientific research applications and in amalgam material for dental restoration in some locales. It is used in lighting: electricity passed through mercury vapor in a fluorescent lamp produces short-wave ultraviolet light which then causes the phosphor in the tube to fluoresce, making visible light (Hammond, 2005).

2.1.2 Chemistry

The chemical and physical properties of mercury are shown on the table below.

Table 2.1: Chemical and physical properties of mercury

CHEMICAL PROPERTIES	
Atomic number	80
Standard atomic weight	200.592(3)
Element category	transition metal
Group, block	group 12, d-block
Period	period 6
Electron configuration	[Xe] $4f^{14}$ $5d^{10}$ $6s^2$
Per shell	2, 8, 18, 32, 18, 2
ATOMIC PROPERTIES	
Oxidation states	4, 2 (mercuric), 1 (mercurous) (a mildly basic oxide)
PHYSICAL PROPERTIES	
Phase	liquid
Melting point	234.3210 K (−38.8290 °C, −37.8922 °F)
Boiling point	629.88 K (356.73 °C, 674.11 °F)

(Lide, 2005).

2.1.3 History

From ancient time, the history of mercury has been connected with that of the medicine and chemistry. Mercury therefore contributes to the history of science throughout times (Norn *et al.,* 2008). Mercury was found in Egyptian tombs that date from 1500 BC (Federal Government of Canada, 2004). In China and Tibet, mercury use was thought to prolong life, heal fractures, and maintain generally good health, although it is now known that exposure to mercury vapor leads to serious adverse health effects (Center for Environmental Health Sciences, 2012). The first emperor of China, Qín Shǐ Huáng Dì—allegedly buried in a tomb that contained rivers of flowing mercury on a model of the land he ruled, representative of the rivers of China—was killed by drinking a mercury and powdered jade mixture formulated by Qin alchemists (causing liver failure, mercury poisoning, and brain death) who intended to give him eternal life (Wright, 2001; Shihuang, 2003).

The ancient Greeks used mercury in ointments; the ancient Egyptians and the Romans used it in cosmetics which sometimes deformed the face. In Lamanai, once a major city of the Maya civilization, a pool of mercury was found under a marker in a Mesoamerican ballcourt (Pendergast, 1982). By 500 BC mercury was used to make amalgams (Medieval Latin *amalgama*, "alloy of mercury") with other metals (Hesse, 2007).

The largest natural source of mercury is cinnabar, it's only known ore, and the richest deposits are found in Spain and Italy. This reddish mineral containing mercury and sulfur has been used as a pigment since prehistoric times. Cinnabar dating from 500 BC has been

identified at a Mayan site in Peru, and prehistoric skulls painted with cinnabar have been found in Italy (Sloane, 2012).

Alchemists thought of mercury as the 'First Matter' from which all metals were formed. They believed that different metals could be produced by varying the quality and quantity of sulfur contained within the mercury. The purest of these was gold, and mercury was called for in attempts at the transmutation of base (or impure) metals into gold, which was the goal of many alchemists (Stillman, 2003).

Historically, preparations of mercury have been in used in treatment of diseases. Mercury (I) chloride (also known as calomel or mercurous chloride) has been used in traditional medicine as a diuretic, topical disinfectant, and laxative. Mercury (II) chloride (also known as mercuric chloride or corrosive sublimate) was once used to treat syphilis (along with other mercury compounds), although it is so toxic that sometimes the symptoms of its toxicity were confused with those of the syphilis it was believed to treat (Pimple *et al.*, 2002). It is also used as a disinfectant. Blue mass, a pill or syrup in which mercury is the main ingredient, was prescribed throughout the 19th century for numerous conditions including constipation, depression, child-bearing and toothaches (Mayell, 2007). In the early 20th century, mercury was administered to children yearly as a laxative and dewormer, and it was used in teething powders for infants. The mercury-containing organohalide merbromin (sometimes sold as Mercurochrome) is still widely used but has been banned in some countries such as the U.S.

Although mercury's mystique held the promise of power, many of the ancients also knew it to be toxic. It was in the mining of the element where mercury first became associated with

human illness beginning as tremors and progressing to severe mental derangement (Sloane, 2012).

The first recorded account of animal experimentation on the toxicity of mercury comes from Rhazes (al-Razi) in the 9th century and in the 11th century Avicenna (Ibn Sina) had the foresight to recommend the use of mercury only as an external remedy, and quicksilver ointments were used by the Arabs in the treating of skin diseases (Norn *et al.,* 2008).

2.1.4 Toxicity

Mercury, used by many cultures for thousands of years, is one of the most toxic nonradioactive heavy metals known (Chang and Hartmann, 1972). It has been noted for inducing public health disasters in Minamata Bay, Japan (Environmental Health Department, 2002) and in Iraq (Bakir *et al.,* 1973; Skerfving and Copplestone, 1976; Clarkson *et al.,* 1981). Mercury, a systemic assassin so poisonous it can injure or kill any cell with which it comes into contact, its vapor passes easily through the skin and the oral mucosa where the metal is quickly absorbed into the blood and transported to every cell in the body (Chang and Hartmann, 1972).

It exists in several forms: inorganic mercury, which includes metallic mercury and mercury vapor and mercurous or mercuric salts; and organic mercury, which includes compounds in which mercury is bonded to a structure containing carbon atoms (methyl, ethyl, phenyl, or similar groups). There is some interconversion *in vivo* between the various forms of mercury. Inhaled elemental mercury vapor, for example, is easily absorbed through mucus membranes and the lung and rapidly oxidized to other forms (but not so quickly as to prevent considerable deposition of elemental mercury in the brain). Methyl mercury is

easily absorbed through the gut and deposits in many tissues, but does not cross the blood-brain barrier as efficiently as elemental mercury; however, on entering the brain it is progressively demethylated to elemental mercury (Berlin *et al.*, 2007). Mercury salts, in contrast, tend to be insoluble, relatively stable, and poorly absorbed. Human toxicity varies with the form of mercury, the dose and the rate of exposure. The target organ for inhaled mercury vapor is primarily the brain (Berlin *et al.*, 2007).

Dissolved mercury penetrates the blood-brain barrier (Chang and Hartmann, 1972) and accumulates in the motor regions of the brain and central nervous system (CNS) (Arvidson, 1984; Monnet-Tschudi *et al.*, 1996; Huggins and Levy, 1998) where the metal damages nerve cells (Lorscheider *et al.*, 1995) and generates high levels of reactive oxygen species (free radicals) (Hussain *et al.*, 1997; Bulat, 1998). A potent neurotoxin that inhibits the production of neurotransmitters (Soleo *et al.*, 1983; Albers *et al.*, 1998), mercury creates serious imbalances in the development of the brain (Ronnback *et al.*, 1992).

Mercurous and mercuric salts chiefly damage the gut lining and kidney, while methyl mercury is widely distributed throughout the body (Berlin *et al.*, 2007). The poison also disrupts the endocrine system (Kawada *et al.*, 1980; Veltman and Maines, 1986; Gerhard *et al.*, 1998) and causes serious kidney damage by selectively destroying cells in the main filtering structures of the kidney (Nylander *et al.*, 1987). Mercury is also a powerful reproductive and developmental toxin that can impair the transport of oxygen and nutrients to the fetus (Lee, 1975; Ogura *et al.*, 1996). Exposure to maternal mercury in the developing fetus and nursing child can lead to severe neurological and learning impairments, including autism (Marlowe *et al.*, 1985). The effect of mercury toxicity on

children can be extensive and involve the entire cortex and frontal region of the brain, resulting in a reduction in brain mass and functionality (Null, 2004). Chronic exposure in adults has been shown to cause cardiovascular disease and increase the risk of heart attack (Salonen *et al.,* 1995; 2000). It also increases the prevalence of opportunistic bacterial, viral, and fungal infections. Damage to the immune system from mercury toxicity results in several autoimmune disorders, leading some researchers to suggest that, mercury toxicity should be classified as an autoimmune disease (Abraham *et al.,* 1984; Snapp *et al.,* 1989; Zamm, 1990).

2.1.4.1 Sources of Mercury Exposure

The principal sources of exposure to mercury in the general population are ingestion and inhalation of mercury compounds from dental amalgams, and ingestion of fish (fresh water and marine) and seafood which contain mercury, primarily as methylmercury (WHO, 1991; Richardson, 1996). Adults and children may also be exposed to small amounts of elemental mercury from broken thermometers, barometers, fluorescent lights, electric switches and inhalation of atmospheric mercury (Children's Environmental Health Protection Act, 2001).

In the environment, mercury comes from natural and anthropogenic sources (WHO, 1991). Mercury can be released into the air through geothermal activity and the weathering of mercury ore-containing rock. Anthropogenic sources of mercury, primarily from the combustion of fossil fuels, and incineration of medical and municipal waste, contribute greater than 80% of the mercury emitted from point sources according to the Agency for Toxic Substances Disease Registry (ATSDR, 1999) and 25% of overall (natural and

anthropogenic) mercury emissions to the atmosphere (U.S. Environmental Protection Agency, EPA, 1997). Based on the most recent inventory by the California Air Resources Board (CARB, 2000), annual statewide industrial emissions of mercury in 1998 were 9,714 lbs, and ambient air levels were 1.6 ng/m^3.

Once in the environment, interconversion between the different forms of mercury can occur. Conversion of inorganic mercury to methylmercury occurs primarily in microorganisms especially in aquatic systems. In the methylated form, mercury bioaccumulates up the food chain and can reach very high concentrations in some fish, many of which are consumed by humans. The majority of exposure to mercury is dietary. The 50 th percentile of mercury ingestion for the U.S. population is 1.4 μg/day (National Research Council, NRC, 2000) while exposures via inhalation are on the order of 30 to 40 ng/day (1.6 ng/m^3 X 10 to 20 m^3/day), based on the average ambient concentration. The ratio of the average statewide ambient concentration to Office of Environmental Health Hazard Assessment, OEHHA's chronic Reference Exposure Level is 0.02. Exposures near sources of emissions may be higher.

In all, there are over 3000 industrial uses for mercury and its various compounds. From pulp and paper to floor waxes and vaccine preservatives, use of this powerful environmental poison is widespread. Ironically, despite grave concern about its toxicity, the greatest source of human exposure to mercury is from mercury-amalgam fillings that are still used for the vast majority of tooth restorations. Each year in the United States, over 100 million mercury amalgams are placed into the mouths of Americans—without a scintilla of evidence of safety (WHO, 1991).

2.1.4.2 Mercury Poisoning

Known as the Great Masquerader, mercury is a poison whose chameleon-like effects are frustratingly unpredictable. The pervasive nature of its toxicity explains why those affected do not react in the same manner or develop the same symptoms of disease. The metal's solubility in fats confers a special affinity for those tissues and organs that have high lipid content, including the kidneys; brain and CNS; the liver; and the glandular tissues of the pancreas, testes, ovaries and prostate. Only through understanding the mechanisms of toxicity within these target organs can we have a better understanding of the confusing kaleidoscope of symptoms associated with mercury toxicity. It also helps to clarify why patients with seemingly disparate neurological, immunological and systemic disorders (including rheumatoid arthritis, fibromyalgia, eczema, chronic fatigue syndrome (CFS), multiple sclerosis (MS), lupus, amyotrophic lateral sclerosis (ALS), thyroiditis, glomerulonephritis, and emotional/ mood disorders), often improve significantly or recover completely after mercury is removed from the body (Wentz, 2004a). Mercury affects the body through three principal means: systemic, immunological and neurological.

2.1.4.2.1 Systemic Disruptions

By binding with proteins on the surfaces of cell membranes, mercury disrupts the transport of materials in and out of cells. This is particularly damaging to the cells of the blood-brain barrier as it facilitates the penetration of other toxic substances into the brain and CNS (Chang and Hartmann, 1972), thereby enhancing the overall toxic challenge. The poison even finds its way to the very heart of the life process, where it interferes with cellular respiration by disrupting mitochondrial production of adenosine triphosphate (ATP), the

energy currency of the cell. By disabling certain enzymes controlling the respiratory process, including those of the Kreb's Cycle and the cytochrome oxidase system, mercury deprives the cell of its ability to replenish vital ATP stores—a systemic interference with energy production that may explain the profound fatigue often experienced with mercury poisoning.

- **Blood and Circulation**

Mercury's ability to block the active sites of haemoglobin, the oxygen-carrying pigment of red blood cells, reduces the ability of the circulatory system to deliver oxygen to the tissues and further compromises the body's energy demands. The poison also destroys red blood cell membranes and damages the lining of blood vessels. Its accumulation in the heart damages the heart muscle and the valves which regulate blood flow. Consequently, it is no surprise that mercury poisoning is well associated with irregularities in blood haemoglobin, chest pains and tachycardia (abnormally rapid heartbeat). Mercury levels in the heart tissues of individuals who have died from idiopathic dilated cardiomyopathy (IDCM) have been found to contain mercury levels 22,000 times those of individuals who died from other forms of heart disease (Haley, 2001). Many of the victims were well-conditioned athletes who dropped dead during sporting events.

- **Protein Damage**

The ability of mercury to bind to the sulfhydryl groups (-SH) of proteins results in the inactivation of sulfur-containing enzymes and the production of sulfur metabolites in the body. Disruption of sulfur metabolism may, in turn, be related to the release of inflammatory cytokines, common in many degenerative processes, including Parkinson's

disease (PD), amyotrophic lateral sclerosis (ALS), lupus, rheumatoid arthritis and Alzheimer's disease (AD) (Heafield *et al.*, 1990; Pean *et al.,* 1994; Wilkinson and Waring, 2002). According to noted toxicologist, Boyd Haley, "Mercury should be considered as a causal contributor to Alzheimer's disease since the mercury can produce two pathological hallmarks of the disease and inhibits the same thiol-sensitive enzymes that are dramatically inhibited in the AD brain" (Haley, 2001).

- **Oxidative Stress**

Researchers at the National Center for Toxicological Research found that superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px/ GPx) are both impaired by exposure to mercury. A significant loss of SOD and GSH-Px antioxidant activity, and a consequent increase in free radical formation in the cerebellum and brain stem, follows exposure to mercury. The findings led the authors to conclude that oxidative stress related to mercury poisoning is a major contributor to the development of neurodegenerative disorders (Hussain *et al.,* 1997). Similar findings also reveal impairment of other antioxidant enzymes in the blood caused by chronic mercury exposure (Perrin-Nadif *et al.,* 1996). Additionally, the fact that mercury also depletes several minerals essential for proper enzyme function and it is not a stretch to see how mercury effectively antagonizes and diminishes the body's antioxidant and detoxification mechanisms (Quig, 1998; Zabinski, 2000).

Mercury's ability to generate free radicals is prodigious, and its presence in the body also results in an increase in the production of advanced glycation end products (AGEs). These troublesome protein/ sugar complexes accelerate aging and cause certain nerve cells in the

brain to pump out damaging superoxide free radicals. Unquenched oxidation, in turn, increases the formation of AGEs and creates a vicious cycle which leads to cell death. The substantia nigra, a region of the brain which manufactures the neurotransmitter dopamine, is susceptible to AGE-related oxidative damage induced by mercury's presence. Loss of dopamine production in the hippocampus, a region of the brain responsible for converting short-term memory to more permanent memory and for recalling spatial relationships, results in a profound degradation of voluntary control. Damage to the hippocampus has been shown to be the likely cause of PD (Zabinski, 2000).

2.1.4.2.2 Immunological Reactions

Even at very low levels, mercury can depress the number of T lymphocytes (T cells) in the blood. One of the body's frontline defenses against infection, T cells are white blood cells that attack other cells which the body recognizes as invaders. A low T cell count, indicative of a compromised immune system, diminishes the ability of the body to meet even the slightest challenge. When amalgam is placed in a person's mouth the number of T cells in their blood declines markedly (Shenker *et al.,* 1992). Alteration of the ratio of T cells in the blood as a consequence of insertion of amalgam fillings (Eggleston, 1984) is now believed to play a central role in the development of several diseases related to immune suppression, including lupus, inflammatory bowel syndrome, anemia, multiple sclerosis (MS) and eczema.

Mercury also impairs the function of neutrophils, another type of white blood cell important in the immune response. Individuals exposed to moderate levels of mercury exhibit an impaired resistance to infections such as *Candida albicans,* a recurring yeast

infection that can spread throughout the body (Perlingeiro and Queiroz, 1994). *Candida*, in turn, causes a conversion of mercury into an extremely toxic organic form, methylmercury, which can cross the blood brain barrier and accumulate in the CNS. According to noted immunologist, Myron Wentz, through depression of the immune system and its promotion of *Candida* infections, mercury exposure from amalgam appears to be a major contributor to the development of chronic fatigue syndrome (Wentz, 2004b).

Mercury poisoning also impairs phagocytic immune cells (B lymphocytes and macrophages), and interfers with the production of cytokines, specialized proteins that prompt the white blood cells to swing into action, thereby crippling the immune system's early warning defense against viral infection (Omura and Beckman, 1995; Christensen *et al.*, 1996). By reducing the ability of cells to manufacture antibodies and the anti-viral protein, Interferon, mercury exposes the individual to opportunistic infections (Christensen *et al.*, 1996). From common challenges, such as *Herpes simplex*, a virus which causes fever blisters and cold sores, to *Chlamidia trachomatis* and other sexually transmitted diseases, the individual burdened with a heavy accumulation of mercury becomes an easy target.

- **Autoimmune Responses**

It has been estimated that up to 35 percent of those individuals who have amalgam fillings may be sensitized to mercury exposure. In such individuals, chronic exposure can lead to autoimmune disease, a severe allergic response which causes the body to attack its own tissues (Wentz, 2004c). Glomerulonephritis, an acute inflammation of the kidneys, is an autoimmune response to mercury exposure and is, perhaps, the most insidious effect of

early mercury toxicity. By attaching to the cells of the glomeruli, the microscopic filtering structures in the kidneys, mercury "fools" the body into thinking the kidney cells are foreign pathogens. The body consequently manufactures antibodies that attack and destroy the tissue—and with it the ability of the kidneys to function.

Mercury also triggers the release of Interleukin I (IL-1) and Tumor Necrosis Factor (TNF) from white blood cells. These inflammatory cytokines cause inflammation in joint tissue (Windham, 2004) and chronic stimulation can cause cartilage damage, bone destruction and contribute to cardiovascular disease. Similar autoimmune damage, induced by mercury poisoning, occurs in the protective myelin sheaths of nerve cells. Destruction of the myelin sheath leads to severe neurological damage and motor nerve dysfunction, as seen in MS.

Mercury's facile ability to incite autoimmune responses is a consequence of the toxin's capacity to bind with sulfur-containing enzymes and proteins, thereby changing their structure and functionality. The body, no longer recognizing the proteins, activates T cells to destroy them (Shenker *et al.,* 1992), resulting in an autoimmune response. Dramatic evidence of this is provided in a 1994 Swedish study, in which mercury amalgam was implanted in the peritoneal cavities of mice. The results reveal an unsettling litany of immunosuppressive effects as well as evidence of stimulatory autoimmune responses (Hultman *et al.,* 1994). Many researchers now argue that tests for mercury toxicity should become a standard practice in the diagnosis of autoimmune disease (Sehnert *et al.,* 1994).

2.1.4.2.3 Neurological

Mercury is a potent neurotoxin, consequently neurological problems are among the most common and most serious effects of chronic exposure. Adverse behavioural effects of

exposure to mercury vapor include the inability to make trivial decisions, resolve doubts, resist temptations and perform intellectual tasks. Because the effects are so unpredictable, mercury poisoning can be expressed as dementia, schizophrenia, neurosis, psychosis—or simply dismissed as nervous "jitters."

- **Inactivation of Neurotransmitters**

Acetylcholine (ACh) is an important neurotransmitter that transports nervous impulses across the synaptic gap, a tiny space separating nerve cells. Exposure to mercury elevates Ach (Hastings *et al.*, 1975; Miszta and and Dabrowski, 1989; Devi and *Fingerman*, 1995), which can lead to aggressive, violent and erratic behaviour.

Conversely, noradrenalin, an excitatory neurotransmitter produced by the adrenal glands, is depressed by exposure to the poison. This is likely a consequence of inhibition of the enzyme responsible for its manufacture. A loss of noradrenalin activity deepens depressive states and enhances mood disorders.

Exposure to mercury also suppresses serotonin, another neurotransmitter associated with depression and other mood disorders. A study conducted at Colorado State University found that general health problems—particularly those related to mental and emotional health—were 45 percent greater in those patients with mercury amalgam fillings (Silberud, 1998). Unexplained anger, irritability, anxiety and depression subsided or disappeared within one year after subjects had their amalgams removed.

- **Excitotoxicity**

Mercury dramatically diminishes astrocyte activity in the brain (Silberud, 1998), and the loss of these ancillary nerve cells causes a toxic buildup of glutamate, an excitatory neurotransmitter (Ronnback and Hansson, 1992; Walum *et al.,* 1995). Glutamate-induced toxicity causes cells in certain regions of the brain to become so over stimulated that they undergo apoptosis, a process of cellular suicide. It appears that the excess glutamate binds with receptors on the surfaces of nerve cells, causing a rapid inflow of calcium, which is lethal to the cell. This leads to a degradation of motor nerve function similar to that observed in ALS and a gradual extinction of brain cells in the hippocampus. Loss of these cells appears to be an underlying cause of AD (Mader, 2003). Glutamate-induced neurotoxicity and related calcium toxicity may also be principal factors in the neural degeneration observed in MS and PD (Clauw, 1995).

- **Disruption of Microtubules**

Microtubules are microskeletal-like structures that are vital to proper cell structure. Chronic mercury exposure has been shown to disrupt the production of microtubules in brain cells— an insidious effect with grave consequences for the cell (Pendergrass *et al.*, 1997). Canadian researchers uncovered convincing visual evidence of mercury-induced disintegration of microtubules in brain cells. This, in turn, disrupted cell membrane structure and caused the formation of neurofibrillary tangles, characteristic of AD (Leong *et al.,* 2001). The findings directly implicate mercury as a causative factor in the development of AD.

Similar observations are reported in a Swiss study that showed nerve cells exposed to mercury increased their production of amyloid protein. This sticky protein makes up the tangled plaques found in the autopsied brains of Alzheimer's patients (Olivieri *et al.,* 2000). Noted toxicologist, Boyd Haley, comments: "Mercury is the toxicant behind Alzheimer's disease. It may not be the only one, but mercury's role in the development of Alzheimer's disease is clear" (O'Brian, 2001). There are myriad ways for mercury to harm us, consequently is no predictable pattern to the symptoms or to the level of a person's sensitivity to the poison. There is, however, one thing of which we can be certain: mercury, in any form, is poisonous and if you are exposed to it for long enough you are going to be hurt.

Mercury pollution has significant impacts at local, national, regional and global levels. Numerous actions implemented in Europe, North America and elsewhere to address the situation at each of these levels, targeting reductions in uses, releases and exposures have been successful. However, inventories are still incomplete in these regions, and some releases are still significant (UNEP, 2002).

Despite massive efforts in search of new drugs that counteract mercurial toxicity, there are no effective treatments available that completely abolish its toxic effects. In MeHg poisoning, supportive care is given when necessary to maintain vital functions. In addition, the use of chelating agents assists the body's ability to eliminate mercury from the tissues (Pingree *et al.,* 2001; Carvalho *et al.,* 2007). However, these drugs appear to be of limited use, because of their adverse side effects (Tchounwou *et al.,* 2003) and limited ability to cross the blood-brain barrier (Aposhian *et al.,* 1995). Some studies have focused their

efforts on the protective effects of plants or natural compounds in various neuropathological conditions (Martins *et al.,* 2009).

2.2 Plant

2.2.1 Medicinal Plants

From the very beginning of human existence, man has familiarized himself with plants and used them in a variety of ways throughout the ages. In search of food and to cope successfully with human suffering, primitive man began to distinguish those plants suitable for nutritional purpose from others with definitive pharmacological action. This relationship has grown between plants and man, and many plants came to be used as drugs (Shinwari and Gilani, 2003).

Plant-based medicines enjoy a respectable position today, especially in the developing countries, where modern health service is limited. Indigenous remedies which are more effective, safe and inexpensive are gaining popularity among both rural and urban areas. Information from ethnic groups or indigenous traditional medicine has played a vital role in the discovery of novel products from plants as chemotherapeutic agents (Katewa *et al.,* 2004).

The World Health Organization has emphasized the importance of the traditional indigenous medicines, since a large majority of rural people in the developing countries still use these medicines as the first defence in health care (Goleniowski *et al.,* 2006). Globally, about 85% of all medications for primary health care are derived from plants (Farnsworth, 1988).

Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants. These plants can be extremely useful as lead structure for synthetic modification and optimization of bioactivity. Besides widespread use of botanicals as medicinal products in developing countries, such products are also becoming part of the integrative health care systems of industrialized nations known as "complementary alternative system of medicines" (CAM). Safety and efficacy of natural herbal product is therefore a cause of concern to promote and rationalize their use (Tijani *et al.,* 2008).

2.2.1.1 Neuroprotective Plants

Plant extracts have a wide range of medicinal actions, and throughout history, they have been used to treat many different types of diseases. More recently, however, scientists have begun investigating the biological activities of medicinal plants, including their neuroprotective actions (Uddin *et al.,* 2013). Of particular importance, the beneficial effects of plants/ natural compounds against metal-induced neurotoxicity have been reported under both *in vitro* and *in vivo* conditions (Xu *et al.,* 2005; Gupta and Flora, 2006; Franco *et al.,* 2007).

Farina *et al.* (2005) have shown the protective effects of the hydroalcoholic extract of *Polygala paniculata* – a plant traditionally used in folk medicine as a sedative and an anti-inflammatory (Lee *et al.,* 2004) – against methyl mercury (MeHg) induced neurotoxicity in mice. Plants of the genus Polygala (*Polygala paniculata*) have been shown to possess protective effects against neuronal death and cognitive impairments in neurodegenerative disorders related to excitotoxicity (Park *et al.,* 2002; Lee *et al.,* 2004).

Findings from studies conducted by Lucena *et al.* (2007), the *in vivo* protective effects of *Cipura paludosa* against MeHg-induced neurotoxicity, have encouraged studies concerning the beneficial effects of *Cipura paludosa* on neurological conditions related to excitotoxicity and oxidative stress. Similarly, findings from studies conducted by Sumathi *et al.* (2012) strongly implicated that *Bacopa monniera* has potential to protect brain from oxidative damage resulting from MeHg-induced neurotoxicity in rat. *B. monniera* extract is claimed to have antioxidative function and had been proved to improve human cognitive function (Pandit, 2011).

The study of antioxidants has gained great attraction recently due to their health implications. The central nervous system is vulnerable to oxidative stress as a consequence of high metabolic rate and deficient antioxidant system. The increasing amount of experimental evidence shows the involvement of reactive oxygen species in neurodegenerative diseases. Antioxidants neutralize free radicals and are effective in suppressing or preventing these disorders. Ayurveda, an ancient system of Indian medicine, has defined a number of plants with therapeutic benefits for the treatment of neurodegenerative disease, having antioxidant activities. *Ginkgo biloba* is reported to reduce free radical levels. Extracts of *Withania somnifera* improves antioxidant status in oxidative stress induced neurodegeneration. It has been proved that consumption of antioxidant rich diet may reduce lipid peroxide and increase antioxidant levels in plasma. There are still a large number of plants that need to be examined for their potential neuroprotective properties (Pandit, 2011).

2.2.2 *Phoenix dactylifera* (Date Palm)

Phoenix dactylifera L. (*P. dactylifera*) a diploid with 2n = 36, is a member of the monocot family Arecaceae classified as a dioecious tall evergreen tree (Zohary and Hopf, 1993). *P. dactylifera* have been cultivated in the Middle East since at least 6000 BC (Copley *et al.,* 2001). It is considered as native to countries around the Arabian Gulf (Chandra *et al.,* 1992). The Palm family is a symbol of prosperity and love to Muslims and its legend dates back to Judeo-Christian mythology (Al-Qarawi *et al.,* 2004). *P. dactylifera* fruits have been an important component of the diet in most of the arid and semiarid regions of the world (Biglari *et al.,* 2008).

P. dactylifera is believed to have been introduced into Nigeria in the early 8th century by Arab traders from North Africa. *P. dactylifera* fruits are a highly valued delicacy among many communities in Nigeria, especially during ceremonies and festivals (Sani *et al.,* 2010).

The various parts of this plant are widely used in traditional medicine for the treatment of various disorders which include memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders (Nadkarni, 1976; Anonymus, 1985). The fruits of *P. dactylifera* are used as a detersive and astringent in intestinal troubles, treatment for sore throat, colds, bronchial asthma, to relieve fever, cystisis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication (Seelig, 1974). It is also scientifically proved to possess a variety of pharmacological activities which indicate its usefulness in various kinds of diseases and disorders.

2.2.2.1 Taxonomy

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Liliopsida*
Order	*Arecales*
Family	*Arecaceae*
Genus	*Phoenix*
Species	*Phoenix dactylifera*
Binomial name	*Phoenix dactylifera Linn*

(Vyawahare *et al.*, 2009).

2.3.2.2 Botanical Description

P. dactylifera is dioecious, medium-sized tree, 15–25m tall, often clumped with several trunks from a single root system, but often growing singly as well, that is, is a multi-stemmed palm from which the suckers are usually removed to create single stemmed specimens. Trimmed in this manner the date palm will grow to heights of 100 ft (45.7m).

The broad gray trunk is patterned with diamond-shaped leaf scars and is up to 16 inches in diameter. The large greenish or bluish gray pinnate leaves are typically 18-20 ft (5.5-6.1m) long by 2 ft (0.6m) wide. They are arranged in a thick canopy up to 40 feet wide.

Leaflets are 1-2 ft (0.3-0.6m) long and arranged in V-shape ranks that run the length of the leaf stem. Leaflets near the base are modified into sharp 3-4 in (7.6-10.2cm) spines. The yellow orange to red fruit, called 'dates', are oblong and about 1.5 in (3.8cm) in length.

The *P. dactylifera* fruit is a berry consisting of a single seed surrounded by a fibrous, parchment-like endocarp, a fleshy mesocarp and the fruit skin (pericarp). The fruit is attached to the spikelet by a perianth (calyx or cap) (Barreveld, 1993). (*See* **Figures 2.1** and **2.2** in **appendix VI**).

2.2.2.3 Varieties of Date Palm

Dates are oval-cylindrical, 3–7cm long, and 2–3cm diameter, and when unripe, range from bright red to bright yellow in colour, depending on variety. (*See* **Table 2.1** and **Figure 2.3** in **appendix VI**).

2.2.2.4 Nutritional Composition

Dates are of important nutritional value (*See* **Tables 2.2** and **2.3** in **appendix VI**).

2.2.2.5 Phytochemistry

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves but recent researches demonstrate that they can also protect humans against diseases.

Phytochemically, the whole plant (*Phoenix dactylifera*) contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. The phenolic profile of the plant revealed the presence of mainly cinnamic acids (ferulic, sinapic and coumaric acids and their derivatives, such as 5-o-caffeoylshikimic acid also called as dactyliferic acid), flavonoid glycosides (luteolin, methyl luteolin, quercetin, and methyl quercetin), flavanols (catechin, epicatechin) (Seelig, 1974; Dowson, 1982; Biglari *et al.,* 2008).

Four free phenolic acids (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) and nine bound phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, and o-coumaric acid) were tentatively identified (Ziouti *et al.,* 1996; Eong *et al.,* 2006).

The thin layer chromatography (TLC) analysis revealed the presence of steroids namely cholesterol, stigmasterol, campesterol and α-sitosterol. Anthocyanins were detected only in fresh dates. The TLC analysis also showed that the major carotenoid pigment present in dates is lutein followed by α-carotene (Al-Farsi *et al.,* 2005; Boudries *et al.,* 2007).

Moreover the gas liquid chromatography of the date seed oil revealed the presence of oleic, lauric, palmitic, capric, myristic, myristoleic, palmitoleic, stearic, linoleic and linolenic

acids. Dates contain at least six vitamins including a small amount of vitamin C, and vitamins B_1 (thiamine), B_2 (riboflavin), nicotinic acid (niacin) and vitamin A (Al-Shahib and Marshall, 1993).

Enzymes such as phytase, invertase and peroxidase have been isolated in dates (Nadkarni, 1976). Presence of estrone has also been reported in the dried date seeds and pollens (Heftmann and Bennett, 1965; Bennett and Heftmann, 1966).

The date palm pollen grains showed the presence of α-amirin, triterpenoids saponins and a crude gonadotropic substance (Mahran *et al.,* 1976). Other isolated chemical constituents include α-D glucan, heteroxylon, and galactomannans (Hussein *et al.,* 1998; Tabeta *et al.,* 2002; Ishurd *et al.,* 2003).

2.2.2.6 Medicinal Applications of *Phoenix dactylifera*

The water in which fresh dates are soaked for a while is a drink given to relieve alcohol intoxication. The milk in which clean and fresh dates are infused is a very nourishing and restorative drink to the children as well as adults especially during convalescence from fevers and smallpox. The sweet pulp of the date fruits is useful in dysentry.

Date fruits are used as an ingredient in various aphrodiasiac and tonic confections. They are also useful in asthma. Paste made from ground seeds of date is said to be applied for opacity of cornea and to the head to relieve headache and hemicrania. The smoke produced from burning of date seeds in powder form is a useful fumigatory for piles (Anonymus, 1985).

Some doctors advise dates as consumptives as they promote expectoration, soothe the chest and prevent constipation (Anonymus, 1985). Dried fruit pounded and mixed with

almonds, quince seeds, pistachia nuts, spices and sugar forms a nutritious formulation. Seeds roasted and ground into powder make a beverage like coffee called “date coffee”. A fine paste of the seed of date fruit and the roots of *Achyranthus aspera*, applied to betel leaves like lime and made into small packets with clove, cardamom, catechu and betelnut powder is a popular antiperiodic remedy among Vaidyas for the prevention of ague which is preceeded by severe shivering. A gum or juice obtained from the stem and named “laghi” (Khajurnidaru) is used as a demulcent, diuretic and refrigerant in genitourinary infections (Nadkarmi, 1976).

The liquid distillate obtained from spathe of *P. dactylifera* locally known as ‘Maa Al-liqah’ or ‘Maa Al-Tiltal’, water of the spathe is believed to have certain medical uses as it relieves abdominal gases and pain especially after heavy meal and claimed to have anti-spasmodic activity (Nadkarmi, 1976). The flower of the plant is used as a purgative (Zoghet *et al.*, 2000). The pollen grains of date palm have been used in Egyptian local practice to improve fertility in women (Amin *et al.*, 1969).

Owning to the above mentioned medicinal applications of date palm, several researches have been conducted to scientifically validate these folk medicinal claims. Recently, many pharmacological studies (*in vitro and in vivo*) conducted on *P. dactylifera* have been demonstrated to have the following activities:

- Anti-gastric ulcer activity
- Anticancer activity
- Anti-diabetic activity
- Anti-fungal activity

- Antimutagenic activity
- Antiinflammatory activity
- Antihyperlipidemic activity
- Antioxidant activity
- Haematoprotective activity
- Effect on gastrointestinal transit
- Hepatoprotective activity
- Gonadotropic activity (effect on reproductive system)
- Immunostimulatory activity
- *In vitro* antiviral activity
- Nephroprotective activity
- Neuroprotective activity (Vyawahare *et al.*, 2009).

A summary of some findings of the pharmacological studies listed above are presented below.

- **Antiulcer Activity**

Al-Qarawi *et al.* (2005) carried out a study on the ameliorative effect of dates (*Phoenix dactylifera L*) on ethanol-induced gastric ulcer in rats. The work aimed at testing, in a rat model of ethanol-induced gastric ulceration, a local folk medicinal claim that dates are beneficial in gastric ulcers in humans. Aqueous and ethanolic extracts from date fruit and pits were given orally to rats at a dose of 4 ml/kg for 14 consecutive days. Gastric ulcer was induced by the administration of ethanol, 80% (1 ml/rat) by gastric intubation.

The results indicated that the aqueous and ethanolic extracts of the date fruit and, to a lesser extent, date pits, were effective in ameliorating the severity of gastric ulceration and mitigating the ethanol-induced increase in histamine and gastrin concentrations, and the decrease in mucin gastric levels. The ethanolic undialyzed extract was more effective than the rest of the other extracts used. It is postulated that the basis of the gastroprotective action of date extracts may be multi-factorial, and may include an antioxidant action.

- **Haematoprotective**

Abdul-Wahab *et al.* (2010) carried out a work to investigate the Preventive Effects of Ethanolic Extract of *Phoenix dactylifera l.* on Lead Induced Haematotoxicity in Rats. The results showed that lead caused a significant decrease in haematocrit, RBC, WBC, haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and lymphocyte and monocyte count; and significant increase in neutrophil count. These were, however, prevented in the extract of *Phoenix dactylifera* (EPD) treated groups. It was therefore concluded that oral admistration of EPD promotes blood's health and annuls lead induced haematotoxicity.

- **Hepatoprotective Activity**

Pre and post treatment with aqueous extract of date flesh or pits significantly reduced CCl_4 induced elevation in plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) enzymes and bilirubin concentration and ameliorated morphological and histological liver damage in rats. This study suggests that CCl_4-induced liver damage in rats can be reversed by treatment of extracts from date

flesh or pits. Moreover it can also be used prophylactically as a dymnamic liver support (Al-Qarawi *et al.,* 2004).

In another study, the date flesh or pit extracts not only normalised the elevated glutamylγplasma activities of AST, ALT, ALP, lactate dehydrogenase (LDH), GT, enzymes and plasma concentration of bilirubin but also γtransferase (exhibited an enormous increase in the reduced serum levels of testosterone, alpha fetoprotien (AFP) and glucose in the thioacetamide induced chirrotic rats. The extracts also showed significant reduction in oxidative stress evidenced by significant rise in the hepatic malonaldehyde (MDA) levels and decline in hepatic glutathione levels by normalising them (Mohamed *et al.,* 2008). This data suggest that the daily oral consumption of an aqueous extract of the flesh of dates, and as a part of the daily diet ad libitum, was prophylactic to thioacetamide poisoning.

- **Antioxidant Activity**

Phytochemicals from fruits have been shown to possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of degenerative diseases in human (Javanmardi *et al.,* 2003). Various *in vitro* and *in vivo* antioxidant activity studies have been carried out on various extracts of different parts of *Phoenix dactylifera*. Studies conducted on antioxidant activity and phenolic content of various fruits of *Phoenix dactylifera* cultivated in Iran, Algeria and Bahrain demonstrated a linear relationship between antioxidant activity and the total phenolic content (TPC) of date fruit extract (Allaith, 2005). Aqueous date extract was found to inhibit significantly the lipid peroxidation and protein oxidation and also exhibited a potent superoxide and hydroxyl

radical scavenging activity in a dose-dependent manner in an *in vitro* study (Dammak *et al.*, 2008).

Methanolic extract of *Phoenix dactylifera* seeds showed a significant increase in plasma levels of vitamin C, E and A, β-carotene and significant decrease in the elevated MDA levels due to the lipid peroxidation in adjuvant arthritis in rats (Mohamed *et al.*, 2004). These findings suggests its possible use in diseases such as scurvy, ataxia and night blindness caused due to the deficiency of vitamins C, E and A respectively. Date seed oil was found to limit oxidative injuries induced by hydrogen peroxide in human skin organ culture which confirmed the potent free radical scavenging activity of the plant (Dammak *et al.*, 2008).

- **Anti-inflammatory Activity**

Oral administration of the methanolic and aqueous extracts of edible portion of *Phoenix dactylifera* fruits suppressed the swelling in the foot significantly by 67.8 and 61.3% respectively, while the methanolic extract of date seeds showed significant reduction by 35.5% in adjuvant arthritis in rats by mechanistically reducing ESR and plasma fibrinogen and normalizing the plasma level of antioxidants. Administration of the extracts also produced significant increase in body weight gain and food efficiency ratio (Mohamed *et al.*, 2004).

- **Nephroprotective Activity**

Al-Qarawi *et al.* (2008) studied the effect of the extracts of the flesh and pits of *Phoenix dactylifera* on gentamicin-induced nephrotoxicity in rats, in which the extracts significantly reduced the increase in plasma creatinine and urea concentrations induced by

gentamycin nephrotoxicity and ameliorated the proximal tubular damage. Antioxidant components in the date (e.g., melatonin, vitamin E, and ascorbic acid) were suggested to be the basis of the nephroprotection (Al-Qarawi *et al.,* 2008).

- **Neuroprotective Activity**

Phoenix dactylifera provides a physical benefit that is crucial to the brain, called the neuroprotective or cerebroprotective effect. In general, this neuroprotective effect protects the brain from the destructive activity of the reactive oxygen species (ROS) that could come from the cell metabolism itself or from exogenous sources (Steinbrenner and Sies, 2009).

Date palm is an excellent antioxidant agent and this is due to the high concentrations of phenolic compounds, flavonoids and anthocyanins as well as the presence of selenoproteins (Baliga *et al.,* 2011). Antioxidant effects have been implicated for neuroprotective activity of *P. dactylifera* (Asadi-Shekaari *et al.,* 2008; Zangiabadi *et al.,* 2011; Kalantaripour *et al.,* 2012).

Some of the known neuroprotective agents of aqueous fruit extract of *Phoenix dactylifera* (AFPD) are: Melatonin, a potent antioxidant and free radical scavenger with special tendency to brain (Al-Qarawi *et al.,* 2008), Phenolic compounds consist of cinnamic acids and different flavonoids with strong antioxidant properties (Mansuri *et al.,* 2005; Biglari *et al.,* 2008), Vitamin C, a known antioxidant compound (Aydin *et al.,* 2005), Magnesium, an antagonist of N-methyl-D-aspartate receptors (Lyden and Wahlgren 2000), Vitamin B_3, a watersoluble Vitamin with neuroprotecive property (Sakakibara *et al.,* 2002). Considering the existence of the different compounds, AFPD is a unique natural complex

neuroprotective agent (Majid *et al.*, 2008). This basic antioxidant effect is studied in the brain of the rats in order to evaluate its benefit to the brain.

2.3 Brain

The nervous system, for purposes of description, is divided into two main parts: the *central nervous system* (CNS), which consists of the brain and spinal cord, and the *peripheral nervous system* (PNS), which consists of the cranial and spinal nerves and their associated ganglia (*see* **Figure 2.4** in **appendix VI**).

In the CNS, the brain and spinal cord are the main centers where correlation and integration of nervous information occur. Both the brain and spinal cord are covered with a system of membranes, called meninges, and are suspended in the cerebrospinal fluid; they are further protected by the bones of the skull and the vertebral column. The CNS is composed of large numbers of excitable nerve cells and their processes, called neurones, which are supported by specialized tissue called neuroglia. The long processes of a nerve cell are called axons or nerve fibers (*see* **Figure 2.5** in **appendix VI**). The interior of the CNS is organized into gray and white matter. Gray matter consists of nerve cells embedded in neuroglia; it has a gray color. White matter consists of nerve fibers embedded in neuroglia; it has a white color due to the presence of lipid material in the myelin sheaths of many of the nerve fibers (Snell, 2010).

In the PNS, the cranial and spinal nerves, which consist of bundles of nerve fibers or axons, conduct information to and from the CNS. Although the nerves are surrounded by fibrous sheaths as they run to different parts of the body, they are relatively unprotected and are commonly damaged by trauma (Snell, 2010).

2.3.1 Embryology

2.3.1.1 Origin of the Nervous System

The nervous system develops from the *neural plate*, a thickened area of embryonic ectoderm. It is the notochord and paraxial mesenchyme that induce the overlying ectoderm to differentiate into the neural plate. Signaling molecules involve members of the transforming growth factor Î² family, Shh, and BMPs. The neural folds, neural tube, and neural crest are formed from the neural plate.

- The neural tube differentiates into the CNS, consisting of the brain and spinal cord.
- The neural crest gives rise to cells that form most of the PNS and autonomic nervous system (ANS), consisting of cranial, spinal, and autonomic ganglia, and many other structures.

Neurulation- formation of the neural plate and neural tube-begins during stage 10 of development (22-23 days) in the region of the fourth to sixth pairs of somites. At this stage, the cranial two thirds of the neural plate and tube, as far caudal as the fourth pair of somites, represent the future brain, and the caudal one third of the neural plate and tube represents the future spinal cord. Fusion of the neural folds and formation of the *neural tube* proceeds in cranial and caudal directions until only small areas of the tube remain open at both ends. Here the lumen of the neural tube-*neural canal*-communicates freely with the amniotic cavity. The cranial opening, the *rostral neuropore*, closes on approximately the 25th day and the *caudal neuropore* 2 days later. *Closure of the neuropores* coincides with the establishment of a blood vascular circulation for the neural tube. The walls of the neural tube thicken to form the brain and the spinal cord (*see* **Figure**

2.6 in **appendix VI**). The neural canal forms the ventricular system of the brain and the central canal of the spinal cord (Moore and Persaud, 2008 a).

2.3.1.1.1 Development of the Brain

The *neural tube* cranial to the fourth pair of somites develops into the brain. Fusion of the neural folds in the cranial region and closure of the rostral neuropore form three primary brain vesicles from which the brain develops. The three *primary brain vesicles* form:

- The forebrain (prosencephalon)
- The midbrain (mesencephalon)
- The hindbrain (rhombencephalon)

During the fifth week, the forebrain partly divides into two *secondary brain vesicles*, the *telencephalon* and *diencephalon*; the midbrain does not divide; the hindbrain partly divides into the *metencephalon* and *myelencephalon*; consequently, there are five secondary brain vesicles.

- **Brain Flexures**

During the fourth week, the embryonic brain grows rapidly and bends ventrally with the head fold. This produces the *midbrain flexure* in the midbrain region and the *cervical flexure* at the junction of the hindbrain and spinal cord. Later, unequal growth of the brain between these flexures produces the *pontine flexure* in the opposite direction. This flexure results in thinning of the roof of the hindbrain.

Initially, the primordial brain has the same basic structure as the developing spinal cord; however, the brain flexures produce considerable variation in the outline of transverse sections at different levels of the brain and in the position of the gray and white matter (substance). The *sulcus limitans* extends cranially to the junction of the midbrain and forebrain, and the alar and basal plates are recognizable only in the midbrain and hindbrain (Moore and Persaud, 2008b).

- ***Forebrain***

As closure of the rostral neuropore occurs, two lateral outgrowths-*optic vesicles*-appear, one on each side of the forebrain. The optic vesicles are the *primordia of the retinae and optic nerves*. A second pair of diverticula, the *telencephalic vesicles*, soon arises more dorsally and rostrally. They are the *primordia of the cerebral hemispheres*, and their cavities become the *lateral ventricles*. The rostral or anterior part of the forebrain, including the primordia of the cerebral hemispheres, is the *telencephalon*, and the caudal or posterior part of the forebrain is the *diencephalon*. The cavities of the telencephalon and diencephalon contribute to the formation of the third ventricle, although the cavity of the diencephalon contributes more (Moore and Persaud, 2008b).

- **Hippocampus**

The hippocampus is phylogenetically one of the oldest parts of the brain; it resides in the temporal lobe, and forms part of the limbic system. It consists of the complex interfolded layers of the dentate gyrus and cornu ammonis (Williams and Warwick, 1980; Standring *et al.*, 2008); which develop from morphogenetically discrete components that differ in their proliferative activity: the ammonic neuroepithelium and the primary dentate

neuroepithelium (Bayer and Altman, 1974). Each of the regions of the hippocampus, including their various subfields, as well as the different lamellae has been empirically assumed to carry out different tasks in hippocampal functions (Leutgeb *et al.,* 2004), of which the two best known examples are: the ability to represent current self-location in spatial and other cognitive dimensions, and the involvement in the storage and retrieval of episodic memory (the ability to recall personal, spatial, and temporal context of events) (Samsonovich and Ascoli, 2005). Also, the hippocampus has been implicated in other areas such as learning, motivation, and the control of appetite (Tracy *et al.,* 2001).

- **Diencephalon**

Three swellings develop in the lateral walls of the third ventricle, which later become the *thalamus, hypothalamus*, and the *epithalamus*. The thalamus is separated from the epithalamus by the epithalamic sulcus and from the hypothalamus by the hypothalamic sulcus. The latter sulcus is not a continuation of the sulcus limitans into the forebrain and does not, like the sulcus limitans, divide sensory and motor areas (Moore and Persaud, 2008b).

- ***Midbrain***

The *midbrain* (mesencephalon) undergoes less change than any other part of the developing brain, except for the caudal part of the hindbrain. The *neural canal* narrows and becomes the *cerebral aqueduct*, a channel that connects the third and fourth ventricles. Neuroblasts migrate from the alar plates of the midbrain into the *tectum* (roof) and aggregate to form four large groups of neurones, the paired superior and inferior colliculi, which are concerned with visual and auditory reflexes, respectively. Neuroblasts from the

basal plates may give rise to groups of neurones in the tegmentum of midbrain (red nuclei, nuclei of third and fourth cranial nerves (CNs), and the reticular nuclei). The *substantia nigra*, a broad layer of gray matter adjacent to the cerebral peduncle, may also differentiate from the basal plate, but some authorities believe that it is derived from cells in the alar plate that migrate ventrally. Fibers growing from the cerebrum form the stemlike *cerebral peduncles* anteriorly. The cerebral peduncles become progressively more prominent as more descending fiber groups (corticopontine, corticobulbar, and corticospinal) pass through the developing midbrain on their way to the *brainstem* and spinal cord (Moore and Persaud, 2008 b).

- ***Hindbrain***

The *cervical flexure* demarcates the hindbrain from the spinal cord. Later, this junction is arbitrarily defined as the level of the superior rootlet of the first cervical nerve, which is located roughly at the foramen magnum. The *pontine flexure,* located in the future pontine region, divides the hindbrain into caudal (myelencephalon) and rostral (metencephalon) parts. The *myelencephalon* becomes the *medulla oblongata* (often called the medulla), and the *metencephalon* becomes the *pons* and *cerebellum*. The cavity of the hindbrain becomes the *fourth ventricle* and the *central canal* in the medulla (Moore and Persaud, 2008b).

- **Mylencephalon**

The caudal part of the myelencephalon (closed part of medulla) resembles the spinal cord, both developmentally and structurally. The neural canal of the neural tube forms the small *central canal* of the myelencephalon. Unlike those of the spinal cord, neuroblasts from the alar plates in the myelencephalon migrate into the marginal zone and form isolated areas of

gray matter-the *gracile nuclei* medially and the *cuneate nuclei* laterally. These nuclei are associated with correspondingly named tracts that enter the medulla from the spinal cord. The ventral area of the medulla contains a pair of fiber bundles-the *pyramids*- that consist of corticospinal fibers descending from the developing cerebral cortex (Moore and Persaud, 2008b).

- **Metencephalon**

The walls of the metencephalon form the *pons* and *cerebellum*, and the cavity of the metencephalon forms the *superior part of the fourth ventricle*. As in the rostral part of the myelencephalon, the pontine flexure causes divergence of the lateral walls of the pons, which spreads the gray matter in the floor of the fourth ventricle. As in the myelencephalon, neuroblasts in each basal plate develop into motor nuclei and organize into three columns on each side (Moore and Persaud, 2008b).

The *cerebellum* develops from thickenings of dorsal parts of the alar plates. Initially, the cerebellar swellings project into the fourth ventricle. As the swellings enlarge and fuse in the median plane, they overgrow the rostral half of the fourth ventricle and overlap the pons and medulla. Some neuroblasts in the intermediate zone of the alar plates migrate to the marginal zone and differentiate into the neurones of the cerebellar cortex. Other neuroblasts from these plates give rise to the central nuclei, the largest of which is the *dentate nucleus*. Cells from the alar plates also give rise to the *pontine nuclei*, the cochlear and vestibular nuclei, and the sensory nuclei of the trigeminal nerve.

The structure of the cerebellum reflects its phylogenetic (evolutionary) development:

- The *archicerebellum* (flocculonodular lobe), the oldest part phylogenetically, has connections with the vestibular apparatus.
- The *paleocerebellum* (vermis and anterior lobe), of more recent development, is associated with sensory data from the limbs.
- The *neocerebellum* (posterior lobe), the newest part phylogenetically, is concerned with selective control of limb movement.

Nerve fibers connecting the cerebral and cerebellar cortices with the spinal cord pass through the marginal layer of the ventral region of the metencephalon. This region of the brainstem is called the *pons* (*L*. bridge) because of the robust band of nerve fibers that crosses the median plane and forms a bulky ridge on its anterior and lateral aspects (Moore and Persaud, 2008b).

2.3.2 Gross Anatomy

The brain lies in the cranial cavity and is continuous with the spinal cord through the foramen magnum. It is surrounded by three meninges: the *dura mater*, the *arachnoid mater*, and the *pia mater*; these are continuous with the corresponding meninges of the spinal cord. The cerebrospinal fluid surrounds the brain in the subarachnoid space.

The brain is conventionally divided into three major divisions. These are, in ascending order from the spinal cord, the hindbrain, the midbrain, and the forebrain. The hindbrain may be subdivided into the medulla oblongata, the pons, and the cerebellum. The forebrain may also be subdivided into the diencephalon (between brain), which is the central part of the forebrain, and the cerebrum. The brainstem (a collective term for the medulla oblongata, pons, and midbrain) is that part of the brain that remains after the cerebral

hemispheres and cerebellum are removed (Snell, 2010). Thus, the brain is composed of the cerebrum, cerebellum, and brainstem. When the calvaria and dura are removed, gyri (folds), sulci (grooves), and fissures (clefts) of the cerebral cortex are visible through the delicate arachnoid-pia layer. Whereas the gyri and sulci demonstrate much variation, the other features of the brain, including overall brain size, are remarkably consistent from individual to individual (Moore *et al.,* 2014).

2.3.2.1 Cerebrum

The cerebrum (*L.* brain) includes the cerebral hemispheres and basal ganglia. The cerebral hemispheres, separated by the falx cerebri within the longitudinal cerebral fissure, are the dominant features of the brain. Each cerebral hemisphere is divided for descriptive purposes into four lobes, each of which is related to, but the boundaries of which do not correspond to, the overlying bones of the same name. From a superior view, the cerebrum is essentially divided into quarters by the median longitudinal cerebral fissure and the coronal central sulcus. The central sulcus separates the frontal lobes (anteriorly) from the parietal lobes (posteriorly). In a lateral view, these lobes lie superior to the transverse lateral sulcus and the temporal lobe inferior to it. The posteriorly placed occipital lobes are separated from the parietal and temporal lobes by the plane of the parieto-occipital sulcus, visible on the medial surface of the cerebrum in a hemisected brain. The anteriormost points of the anteriorly projecting frontal and temporal lobes are the frontal and temporal poles. The posteriormost point of the posteriorly projecting occipital lobe is the occipital pole. The hemispheres occupy the entire supratentorial cranial cavity. The frontal lobes occupy the anterior cranial fossae, the temporal lobes occupy the lateral parts of the middle

cranial fossae, and the occipital lobes extend posteriorly over the tentorium cerebelli (Moore *et al.*, 2014).

The diencephalon, almost completely hidden from the surface of the brain (Snell, 2010), is composed of the epithalamus, dorsal thalamus, and hypothalamus and forms the central core of the brain (Moore *et al.*, 2014).

2.3.2.1.1 Hippocampus

The hippocampus (named after its resemblance to the seahorse, from the *Greek* "seahorse" from *hippos*, "horse" and *kampos*, "sea monster") is a major component of the brains of humans and other vertebrates. Humans and other mammals have two hippocampi, one in each side of the brain. It belongs to the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation. The hippocampus is located under the cerebral cortex (Wright, 1997); and in primates it is located in the medial temporal lobe, underneath the cortical surface. It contains two main interlocking parts: Ammon's horn and the dentate gyrus (Pearce, 2001).

Anatomically, the hippocampus is an elaboration of the edge of the cerebral cortex. The structures that line the edge of the cortex make up the so-called limbic system (Latin *limbus = border*): These include the hippocampus, cingulate cortex, olfactory cortex, and amygdala. The hippocampus is anatomically connected to parts of the brain that are involved with emotional behaviour—the septum, the hypothalamic mammillary body, and the anterior nuclear complex in the thalamus—therefore play a role as a limbic structure (Amaral and Lavenex, 2006).

- **Role in memory**

Psychologists and neuroscientists generally agree that the hippocampus plays an important role in the formation of new memories about experienced events (episodic or autobiographical memory) (Eichenbaum and Cohen, 1993; Squire and Schacter, 2002). Part of this function is hippocampal involvement in the detection of novel events, places and stimuli (VanElzakker *et al.*, 2008). Some researchers regard the hippocampus as part of a larger medial temporal lobe memory system responsible for general declarative memory (memories that can be explicitly verbalized—these would include, for example, memory for facts in addition to episodic memory) (Squire, 1992).

- **Role in spatial memory and navigation**

Studies conducted on freely moving rats and mice have shown that many hippocampal neurones have *"place fields"*, that is, they fire bursts of action potentials when a rat passes through a particular part of the environment. In humans, cells with location-specific firing patterns have been reported in a study of patients with drug-resistant epilepsy who were undergoing an invasive procedure to localize the source of their seizures, with a view to surgical resection. The patients had diagnostic electrodes implanted in their hippocampus and then used a computer to move around in a virtual reality town (Ekstrom *et al.*, 2003).

Place responses in rats and mice have been studied in hundreds of experiments over four decades, yielding a large quantity of information (Moser *et al.*, 2008). Place cell responses are shown by pyramidal cells in the hippocampus proper, and granule cells in the dentate gyrus. These constitute the great majority of neurones in the densely packed hippocampal layers. Inhibitory interneurones, which make up most of the remaining cell population,

frequently show significant place-related variations in firing rate that are much weaker than those displayed by pyramidal or granule cells.

The discovery of place cells in the 1970s led to a theory that the hippocampus might act as a cognitive map—a neural representation of the layout of the environment (O'Keefe and Nadel, 1978). Several lines of evidence support the hypothesis. It is a frequent observation that without a fully functional hippocampus, humans may not remember where they have been and how to get where they are going: getting lost is one of the most common symptoms of amnesia (Chiu *et al.*, 2004). Studies with animals have shown that an intact hippocampus is required for initial learning and long-term retention of some spatial memory tasks, in particular ones that require finding the way to a hidden goal (Morris *et al.*, 1982; Sutherland and McNaughton, 2001; Clark *et al.*, 2005). The *"cognitive map hypothesis"* has been further advanced by recent discoveries of head direction cells, grid cells, and border cells in several parts of the rodent brain that are strongly connected to the hippocampus (Solstad *et al.*, 2008).

2.3.2.2 Midbrain

The midbrain is the narrow part of the brain that connects the forebrain to the hindbrain. The narrow cavity of the midbrain is the cerebral aqueduct, which connects the third and fourth ventricles (Snell, 2010). The midbrain, the rostral part of the brainstem, lies at the junction of the middle and posterior cranial fossae. Cranial nerve (CN) III and IV are associated with the midbrain (Moore *et al.*, 2014).

2.3.2.3 Pons

The pons is the part of the brainstem between the midbrain rostrally and the medulla oblongata caudally and situated on the anterior surface of the cerebellum; it lies in the anterior part of the posterior cranial fossa. The pons, or bridge, derives its name from the large number of transverse fibers on its anterior aspect connecting the two cerebellar hemispheres (Snell, 2010). Cranial nerve (CN) V is associated with the pons (Moore *et al.,* 2014).

2.3.2.4 Medulla

The conically shaped medulla oblongata (medulla) is the most caudal subdivision of the brainstem that is continuous with the spinal cord; it lies in the posterior cranial fossa. CN IX, X, and XII are associated with the medulla, whereas CN VI-VIII are associated with the junction of pons and medulla (Snell, 2010; Moore *et al.,* 2014).

2.3.2.5 Cerebellum

The cerebellum is the large brain mass lying posterior to the pons and medulla and inferior to the posterior part of the cerebrum. It lies beneath the tentorium cerebelli in the posterior cranial fossa. It consists of two lateral hemispheres that are united by a narrow middle part, the vermis (Moore *et al.,* 2014). The cerebellum is connected to the midbrain by the superior cerebellar peduncles, to the pons by the middle cerebellar peduncles, and to the medulla by the inferior cerebellar peduncles. The peduncles are composed of large bundles of nerve fibers connecting the cerebellum to the remainder of the nervous system (Snell, 2010).

2.3.3 Brain Histology

2.3.3.1 Cerebrum

The cerebral hemispheres consist of a convoluted cortex of grey matter overlying the central medullary mass of white matter which conveys fibres between different parts of the cortex and to and from other parts of the CNS.

Histologically, the neurones of the cerebral cortex are divided into five different morphological types which are arranged in several layers. In submammalian species, the major function of the cortex concerns the sense of olfaction (smell) and the neurones are arranged into three layers. In mammals, there has evolved the so-called *neocortex* consisting of six layers of neurones. The neocortex includes the *sensory* and *motor areas* of the cortex as well as the *association cortex* and in humans constitutes about 90% of the cerebral cortex. The primitive three-layered pattern persists only in the olfactory cortex and cortical part of the limbic system in the temporal lobe (Young *et al.*, 2007).

2.3.3.2.1 Neurone Types in the Cerebral Cortex

There are five characteristic types of cortical neurones; the pyramidal and stellate cells are by far the most common type.

- **Pyramidal cells**, as their name implies, have pyramid-shaped cell bodies, the apex being directed towards the cortical surface. A slender axon arises from the base of the cell and passes into the underlying white matter, though in the case of small superficially located cells, the axon may synapse in the deep layers of the cortex. From the apex, a thick branching dendrite passes towards the surface where it has an array of fine dendritic branches. In addition, short dendrites arise from the edges of

the base and ramify laterally. The size of the pyramidal cells varies from small to large, the smallest tending to lie more superficially. The huge upper motor neurones of the motor cortex, known as ***Betz cells***, are the largest of the pyramidal cells in the cortex.

- **Stellate** (**granule**) **cells** are small neurones with a short vertical axon and several short branching dendrites, giving the cell body the shape of a star; basket and neurogliaform subtypes are also described. With routine histological methods, the cells look like small granules giving rise to their alternative name.
- **Cells of Martinotti** are small polygonal cells with a few short dendrites; the axon extends towards the surface and bifurcates to run horizontally, usually in the most superficial layer.
- **Fusiform cells** are spindle-shaped cells oriented at right angles to the surface of the cerebral cortex. The axon arises from the side of the cell body and passes superficially. Dendrites extend from each end of the cell body branching into deeper and more superficial layers.
- **Horizontal cells of Cajal** are small and spindle-shaped but oriented parallel to the surface. They are the least common cell type and are only found in the most superficial layer where their axons pass laterally to synapse with the dendrites of pyramidal cells.

In addition to neurones, the cortex contains supporting neuroglial cells, i.e. astrocytes, oligodendroglia and microglia (Young *et al.*, 2007).

2.3.3.1.2 Layers of the Neocortex

The neurones in the neocortex are arranged into six layers, the layers differing in neurone morphology, size and population density. The layers merge with one another rather than being highly demarcated and vary somewhat from one region of the cortex to another depending on cortical thickness and function. The characteristics of each layer are as follows:

I. **Plexiform (molecular) layer.** This most superficial layer mainly contains dendrites and axons of cortical neurones making synapses with one another; the sparse nuclei are those of neuroglia and occasional horizontal cells of Cajal.

II. **Outer granular layer.** A dense population of small pyramidal cells and stellate cells make up this thin layer which also contains various axons and dendritic connections from deeper layers.

III. **Pyramidal cell layer.** Pyramidal cells of moderate size predominate in this broad layer, the cells increasing in size deeper in the layer. Martinotti cells are also present.

IV. **Inner granular layer.** This layer consists mainly of densely packed stellate cells.

V. **Ganglionic layer.** Large pyramidal cells and smaller numbers of stellate cells and cells of Martinotti make up this layer, the name of the layer originating from the huge pyramidal (ganglion) Betz cells of the motor cortex.

VI. **Multiform cell layer.** This is so named for the wide variety of differing morphological forms found in this layer. It contains numerous small pyramidal cells and cells of Martinotti, as well as stellate cells, especially superficially, and fusiform cells in the deeper part.

The synaptic interconnections within the cortex are exceedingly complex, with any one neurone synapsing with several hundred others. However, there are several basic principles of cortical organisation and function:

- Functional units are disposed vertically, corresponding to the general orientation of axons and major dendrites.
- Afferent fibres (their cell bodies lying elsewhere in the CNS) generally synapse high in the cortex with dendrites of efferent neurones, the cell bodies of which lie in deeper layers of the cortex.
- Efferent pathways, typically the axons of pyramidal cells, tend to give off branches which pass back into more superficial layers to communicate with their own dendrites via interneuronal connections involving other cortical cell types (Young *et al.*, 2007).

2.3.3.2 Hippocampus

The hippocampus as a whole has the shape of a curved tube, which has been variously compared to a seahorse, a ram's horn (*Cornu Ammonis*, hence the subdivisions CA1 through CA4), or a banana. It can be distinguished as a zone where the cortex narrows into a single layer of densely packed pyramidal neurones three to six cells deep in rats, which curl into a tight U shape; one edge of the "U," field CA4, is embedded into a backward-facing, strongly flexed, V-shaped cortex, the *dentate gyrus*. It consists of ventral and dorsal portions, both of which are of similar composition but are parts of different neural circuits (Moser and Moser, 1998). In the hippocampus proper, are pyramidal cells and granule cells in the dentate gyrus. Other cells present are interneurones (Amaral and Lavenex, 2006).

2.3.3.3 Cerebellum

The cerebellum, which coordinates muscular activity and maintains posture and equilibrium, consists of a cortex of grey matter with a central core of white matter containing four pairs of nuclei. Afferent and efferent fibres pass to and from the brain stem via inferior, middle and superior cerebellar peduncles linking medulla, pons and midbrain respectively.

The cerebellar cortex forms a series of deeply convoluted folds or *folia* supported by a branching central medulla of white matter. At higher magnification, the cortex is seen to consist of three layers. The outer *molecular layer* contains relatively few neurones and large numbers of unmyelinated fibres. The inner *granular cell layer* is extremely cellular. Between the two is a single layer of huge neurones called *Purkinje cells.*

- Purkinje cells have very large cell bodies, a relatively fine axon extending down through the granular cell layer, and an extensively branching dendritic system which arborises into the outer molecular layer.
- The deep granular cell layer of the cortex contains numerous small neurones, the non-myelinated axons of which pass outwards to the molecular layer where they bifurcate to run parallel to the surface to synapse with the dendrites of Purkinje cells.

In addition to the Purkinje and granular cells, there are three other types of small neurones in the cerebellar cortex, namely *stellate cells* and *basket cells* scattered in the molecular layer and *Golgi cells* scattered in the superficial part of the granular cell layer.

In simple terms, afferent fibres enter the cerebellum from the brain stem and then pass via the white matter core to make complex connections with granular cells, these in turn connect with Purkinje dendrites via basket cells and other neurones of the cerebellar cortex. The only efferent fibres from the cerebellar cortex are the Purkinje cell axons which pass down through the granular cell layer into the white matter where they synapse in the central nuclei of the cerebellum (Young *et al.*, 2007).

2.3.4 Histometry

Histologists, traditionally, have relied on verbal descriptions of tissue structure and have seldom favoured a mathematical approach. However, in recent years, measurement of the microscopic images of tissues and cells has increasingly been used in research and diagnostic work. Morphometry has a wide meaning of measurement of the forms of organisms being defined as, a body of methods for obtaining numerical information about anatomical structure (macroscopic or microscopic) in terms of quantities, such as, volume, surface area, number of components, size of components (Aherne and Dunnill, 1982).

Histometry, on the other hand, refers to measurement of tissues only (Anderson, 1988). Histologic sections are used to define the normal or abnormal appearance of the brain tissue. In this regard histologic descriptions often use terms like “large,” “small,” “many,” “few,” “absent” or “present.” These terms are very helpful and often sufficient to describe features of sections, particularly when there are distinct differences between pathologic and physiologic conditions. However, they are not good enough to test for statistically significant changes in appearance resulting from a disease or an experimental treatment, particularly if differences between pathologic and physiologic conditions are discrete

(Schmitz and Hof, 2005). Histometry provides a sound basis for comparison of histological observation. It offers objectivity, increases precision compared with direct visual appraisal and makes statistical analysis easier. It improves assessment of certain histological change, which though may be recognizable by the eye, are accurately graded and their progression better appreciated by histometric quantization (Huda and Zaid, 2007).

2.3.5 Neurobehavioural Testing

Neurobehavioural testing is a non-invasive method used to evaluate different aspects of the functioning of the central nervous system. Test batteries consist of tasks that measure performance of particular neurologic function, such as ability to learn, reaction time, memory, and coordination (Broadwater, 2010).

Additionally, neurobehavioural evaluations are an important component of screening for neurotoxicological effects. A neurobehavioural test battery is chosen to assess a variety of endpoints, including autonomic, neuromuscular, sensory, and excitability functions (Moser, 1991; 2001).

2.3.5.1 Learning and Memory

- **Morris water maze**

Studies of learning and memory play an important role in the study of neurological disorders with cognitive components, such as schizophrenia and Alzheimer's disease. The most common spatial navigation task is the Morris water maze (Morris, 1984; Brandeis *et al.*, 1989; D'hooge and De Deyn, 2001), which can be used for both rats and mice (with the size of the pool scaled appropriately). In this task, a rodent is placed in a tank of opaque water containing a hidden escape platform. Over the course of repeated trials, the animal

learns to locate the platform using visual cues placed around the room. The rate at which the animal learns to locate the platform can be measured, and learning is confirmed using a probe trial, in which the platform is removed, and the amount of time the animal spends investigating the area of the tank that formerly contained the platform is measured (*see* **Figure 2.7 a** in **appendix VI**).

The Morris water maze was initially used to show that lesions of the hippocampus impair spatial learning (Morris *et al.,* 1982). A variation on the Morris water maze is the *Barnes maze*, in which the animal must locate a hidden escape box on an open platform, which may be less stressful than swimming in the water maze (Harrison *et al.*, 2006).

2.3.5.2 Anxiety-Like Behaviour

- **Elevated plus maze**

The most commonly used tests of anxiety-like behaviour assess exploratory behaviour in different novel environments, and were initially developed based on the efficacy of anxiolytic drugs in altering behaviour in rats, although they have since been adapted for use in mice (Cryan and Holmes, 2005). As foraging species subject to predation, rodents have a natural tendency to explore a novel arena, but are also inclined to avoid open, brightly lit areas. The *open field*, *elevated plus maze*, and *light/dark box* are all designed to assess these competing drives. Mice tend to avoid the more aversive areas of the arena (the center of the open field, open arms of the elevated plus maze, or light area of the light/ dark box), but this behaviour can be altered by drugs or mutations that affect innate anxiety (Cryan and Holmes, 2005). The elevated plus maze is suitable for both rats and mice (though the size of the maze will vary depending on the species). The maze consists of two

perpendicular arms, one of which is open, and other with high walls, located 40-60 cm above the ground (*see* **Figure 2.7 b appendix VI**). The animal is placed in the center, facing an open arm, and the amount of time spent in each arm and entrances into each arm is recorded over 5 min (Pellow *et al.,* 1985; Lister, 1987; Costall *et al.,* 1989). Anxiolytic drugs will increase time spent in the open arms.

2.3.5.3 Beam Walking

The beam-walking test (also known as raised-beam or raised-bridge test) is used to assess deficits in coordination and integration of motor movement, especially in the hindlimb (Puurunen, 2001). In this test, rodents are trained to traverse a series of elevated, narrow beams to reach an enclosed escape platform (*see* **Figure 2.7 c** in **appendix VI**). Protocols are designed to measure footslips and latency to traverse the beam, and a number of other parameters of motor coordination and balance can also be measured (Carter *et al.,* 2001).

Beam walking across bridges of different cross-sections also provides a well-established method of monitoring motor coordination and balance in rodents. The method has been used perhaps most commonly to test sensorimotor function in the rat, for example to assess sensorimotor coordination following cortical injury, such as that induced by unilateral sensorimotor cortex ablation (Gentile *et al.,* 1978; Feeney *et al.,* 1982; Goldstein and Davis, 1990; Boyeson *et al.,* 1992), gamma aminobutyric acid (GABA) infusion into the frontal cortex (Majchrzak *et al.,* 1992), and traumatic brain injury (Soblosky *et al.,* 1996). However, beam walking has also been used to test sensorimotor function following the more general neuronal injury sustained in rodent models of stroke (Alexis *et al.,* 1995; Ohlsson and Johansson, 1995) as well as in the absence of injury to test the effects of daily

spontaneous running on the function of the nigrostriatal pathway (Dluzen *et al.*, 1995) and to characterize development and aging processes in rats (Wallace *et al.*, 1980; Gage *et al.*, 1984).

2.3.5.4 Forelimb grip strength test

Forelimb grip strength test is a measure of the muscular strength in the forelimbs (Olopade *et al.*, 2012). In this test, the rat's forepaws are placed on a horizontally suspended metal wire 2 mm in diameter and placed 1 m above a landing area filled with soft bedding. The length of time the rat was able to stay suspended before falling off the wire is recorded (Tamashiro *et al.*, 2000).

2.4 Vitamin C

Ascorbic acid (vitamin C) is a water-soluble micronutrient required for multiple biological functions or physiological functions in living organisms (Hacisevki, 2009). Ascorbic acid is a strong reducing agent and readily oxidizes reversibly to dehydroascorbic acid.

Studies on the interactions of ascorbic acid with various chemicals and metal ions have indicated that ascorbic acid and its oxidation product dehydroascorbic acid, as well as its intermediate monodehydroascorbic acid free radical, may function as cycling redox couples in reactions involving electron transport and membrane electrochemical potentiation (Tolbert *et al.*, 1975; Packer and Fuchs, 1997). Research on the electron transport and redox coupling reactions has been the subject of numerous biochemical studies. For example, ascorbic acid has been shown to participate in many different neurochemical reactions involving electron transport. Neurones are known to use ascorbic acid for many different chemical and enzymatic reactions, including the synthesis of

neurotransmitters and hormones (Packer and Fuchs, 1997; Arrigoni and De Tulio, 2002). Studies on the interactions of extracellular ascorbic acid with various plasma membrane proteins suggest that ascorbic acid may function as a neuromodulator (Packer and Fuchs, 1997).

In a variety of other functions, the role of ascorbic acid in cellular metabolism can be accounted for by its reducing properties to protect cellular components from oxidative damage. It acts as a scavenger for oxidizing free radicals and harmful oxygen-derived species, such as the hydroxyl radical, hydrogen peroxide, and singlet oxygen (Arrigoni and De Tulio, 2002).

2.4.1 Definition

Ascorbic acid is a naturally occurring organic compound with antioxidant properties. It is a white solid, but impure samples can appear yellowish. It dissolves well in water to give mildly acidic solutions. Ascorbic acid is one form ("vitamer") of vitamin C. It was originally called L-hexuronic acid, but, when it was found to have vitamin C activity in animals ("vitamin C" being defined as a vitamin activity, not then a specific substance), the suggestion was made to rename it. The new name, ascorbic acid, is derived from *a*- (meaning "no") and *scorbutus* (scurvy), the disease caused by a deficiency of vitamin C. Because it is derived from glucose, many animals are able to produce it, but humans require it as part of their nutrition (Lachapelle and Drouin, 2010).

There exists a D-ascorbic acid, which does not occur in nature. It may be synthesized artificially. It has identical antioxidant properties to *L*-ascorbic acid yet has far less vitamin C activity (although not quite zero). This fact is taken as evidence that the antioxidant

properties of ascorbic acid are only a small part of its effective vitamin activity. To be specific, *L*-ascorbate is known to participate in many specific enzyme reactions that require the correct enantiomer (*L*-ascorbate and not *D*-ascorbate) (Davies *et al.,* 1991).

2.4.2 Chemistry

- **IUPAC Name:** (5*R*)-[(1*S*)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one
- **Molecular Formula:** $C_6H_8O_6$ (Safety Data for Ascorbic Acid, 2007).
- **Molecular Structure**

L-ascorbic acid is a dibasic acid with an enediol group built into a five membered heterocyclic lactone ring. The chemical and physical properties of ascorbic acid are related to its structure (Buettner, 1993; Aguirre and May, 2008). The structure of dehydroascorbic acid, the first oxidation product of ascorbic acid (**Figure 2.8**), has been analyzed by x-ray crystallography to be a dimer. Electrochemical studies have indicated that ascorbic acid and dehydroascorbic acid form a reversible redox couple. The ascorbic acid molecule consists of two asymmetric carbon atoms, C-4 and C-5 (Tolbert *et al.,* 1975). Therefore, in addition to *L*-ascorbic acid itself, there are three other stereoisomers: D-ascorbic acid, D-isoascorbic acid, and *L*-isoascorbic acid. These three isomers have very little or no antiscorbutic activity (Winkler, 1987; Mehlhorn, 1991).

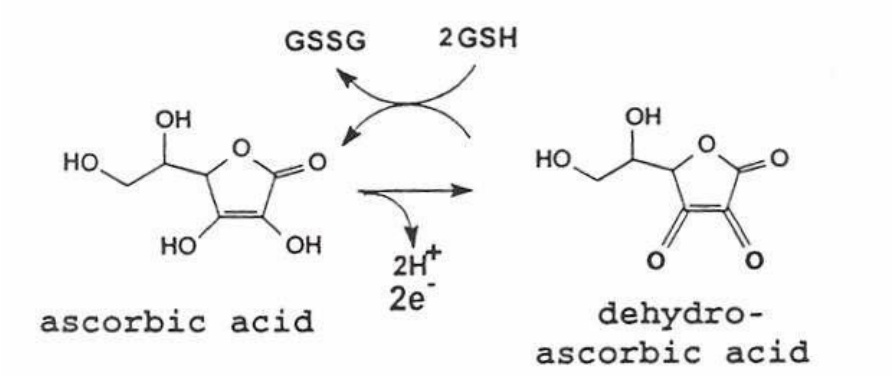


Figure 2.8: Ascorbic acid and dehydroascorbic acid. Ascorbic acid is the reduced form of vitamin C. The oxidized form, dehydroascorbic acid, can be reduced back to ascorbic acid by glutathione (GSH) (Aguirre and May, 2008).

2.4.7 Biosynthesis

The biosynthesis of ascorbic acid in animals is included in the glucuronic acid metabolic pathway. The metabolic pathway is involved in the metabolism of sugars under normal and disease conditions, and in regulation of physiological functions. It is an important pathway for major detoxification processes. The activities of the synthesizing enzymes vary from species to species (Chatterjee *et al.,* 1975).

Most animals can convert *D*-glucose into *L*-ascorbic acid. Humans and other primates, guinea pigs, Indian fruit bats, some fish and birds, and insects are unable to produce ascorbic acid endogenously. Most of research on ascorbic acid synthesis in animals has been carried out using rats. *D*-glucose is converted into *L*-ascorbic acid via *D*-glucuronic acid, *L*-gulonic acid, *L*-gulonolactone and 2-keto-L-gulonolactone as intermediates. Studies with radioactive labeling technique have indicated that, in the synthetic pathway, inversion of C-1 and C-6 takes place between *D*-glucuronic acid and *L*-gulonic acid, while the *D*-glucose chain remains intact. Animals that cannot synthesize ascorbic acid

endogenously lack the oxidizing enzyme *L*-gulono- γ-lactone oxidase. This enzyme is required in the last step of the conversion of *L*-gulono-γ- lactone to 2-oxo-L-gulono-γ-lactone, which is a tautomer of *L*-ascorbic acid and, transforms spontaneously into vitamin C (Nishikimi and Yagi, 1991; Nishikimi *et al.*, 1994).

2.4.8 Metabolism

Ascorbic acid is metabolized in the liver, and to some extent in the kidney, in a series of reactions. The principal pathway of ascorbic acid metabolism involves the loss of two electrons (Packer and Fuchs, 1997; Arrigoni and De Tulio, 2002). The intermediate free radical reversibly forms dehydroascorbic acid, leading to the irreversible formation of the physiologically inactive 2,3-diketogulonic acid (Fituri *et al.*, 1983). Diketogulonic acid may be either cleaved to oxalic acid and threonic acid, or decarboxylated to carbon dioxide, xylose, and xylulose, leading eventually to xylonic acid and lyxonic acid. All of these metabolites and ascorbic acid itself fare excreted in the urine (Kallneri *et al.*, 1985).

2.4.8.1 Redox Metabolism of Ascorbic Acid

In addition to its antiscorbutic action, vitamin C is a potent reducing agent and scavenger of free radicals in biological systems (Rose *et al.*, 1993; Buettner and Jurkiewicz, 1996; Duarte and Lunec, 2005). Briefly, mono-anion form (ascorbate) is the predominant chemical species at physiological pH. Ascorbate readily undergoes two consecutive, yet reversible, one-electron oxidations to generate dehydroascorbate (DHA) and an intermediate, the ascorbate free radical (AFR). AFR is, however, a relatively unreactive free radical, with a reduction potential considerably low compared to the α tocopherol radical, the glutathione radical and virtually all reactive oxygen and nitrogen species that

are thought to be involved in human disease (e.g. superoxide anion, hydroxyl radical, hydroperoxyl radicals, singlet oxygen, nitrogen dioxide, nitroxide radicals and hypochlorous acid) (May *et al.,* 2001; Wilson, 2002; Duarte and Lunec, 2005).

2.4.8.2 Ascorbic Acid Availability and Transport

Ascorbic acid is water-soluble and is well absorbed from the gastrointestinal tract. Mean plasma ascorbic acid levels are 50-60 μM for healthy, well-nourished, non-smoking individuals (May, 1999; Woollard *et al.,* 2002; Duarte and Lunec, 2005). Plasma levels can be increased by long-term vegetarian diet (Astley *et al.,* 2004) and by oral supplementation up to approximately 100 μM; (Brennan *et al.,* 2000; Woollard *et al.,* 2002; Choi *et al.,* 2004). Higher plasma levels are not observed even with supplemental doses higher than 500 mg/day due to efficient vitamin C excretion in the urine (Levine *et al.,* 1996). Some studies have shown that the increase in plasma vitamin C was accompanied by an increase in the intracellular levels of the vitamin (Choi *et al.,* 2004); however, this increase is often not dosedependent (Duarte and Lunec, 2005), presumably due to cellular saturation. Thus it is known that the intracellular vitamin C concentrations of neutrophils, monocytes and lymphocytes saturate at lower supplementation doses than human plasma (Levine *et al.,* 1996). Cellular vitamin C transport has been studied *in vitro* and occurs by two distinct mechanisms (Tsukaguchi *et al.,* 1999; Duarte and Lunec, 2005). Ascorbate enters mammalian cells via a family of specific transporters in a process driven by the sodium electrochemical gradient.

Absorption of vitamin C occurs primarily in the distal intestine via active transport through an ascorbate transporter termed the sodium-dependent vitamin C transporter-type 1

(SVCT1, gene product of slc23a1). This transporter is also present in the renal proximal tubules, where it serves to reabsorb filtered ascorbate. Ascorbate circulates in blood at 30-60 μM, but its concentrations in most cells are considerably higher. This is due to its active transport by another ascorbate transporter isoform, termed the SVCT2 (gene product of slc23a2). The SVCT2 is present in most tissues in the body, including brain, lung, liver, and cardiac and skeletal muscle. Plasma concentrations of ascorbate are limited to about 120 μM due to saturation of absorption, uptake into tissues, and failure of complete reabsorption in the kidney (Tsukaguchi *et al.,* 1999). Notably, the oxidized form (DHA) is transported into the cells faster than the reduced form by facilitated diffusion through several isoforms of the glucose transporter (GLUT) (Vera *et al.,* 1993; Welch *et al.,* 1995; Rumsey *et al.,* 1997), a process that can be inhibited by glucose in some but not all cell types (Duarte and Lunec, 2005).

2.4.9 The Roles of Ascorbic Acid in Biological Pathways

Free radicals are produced through biological processes and in response to exogenous stimuli, and controlled by various enzymes and antioxidants in the body. Oxidative stress occurs when free radical formation exceeds the ability to protect against them, what can lead to tissue injury after trauma, inflammatory events and chronic conditions, such as atherosclerosis, degenerative disease and cancer (Ames *et al.,* 1994). Vitamin E, vitamin C, and β-carotene, often referred to as "antioxidant vitamins", have been suggested to limit oxidative damage in humans, thereby lowering the risk of certain chronic diseases. In epidemiological studies, cardiovascular disease is associated with low plasma concentrations of *L*-ascorbic acid, tocopherol and β-carotene (Ames *et al.,* 1994; Villacorta *et al.,* 2007).

Ascorbic acid is involved in the metabolism of several amino acids, leading to the formation of hydroxyproline, hydroxylysine, norepinephrine, serotonin, homogenistic acid, and carnitine (Barnes, 1975). Hydroxyproline and hydroxylysine are components of collagens, the fibrous connective tissue in animals. Collagens are principal components of tendons, ligaments, skin, bone, teeth, cartilage, heart valves, intervertebral disks, cornea, eye lens, and the ground substances between cells. When collagen is synthesized, proline and lysine are hydroxylated posttranslationally on the growing polypeptide chain. Hydroxyproline and hydroxylysine are required for the formation of a stable extracellular matrix and cross-links in the fibers. The subsequent triple helix quaternary state of physiologically effective collagen can only be achieved if the requisite proline and lysine residues have been hydroxylated. A deficiency of ascorbic acid reduces the activity of two mixed function oxidases, prolylhydroxylase and lysyl hydroxylase, which hydroxylate proline and lysine. The role of ascorbic acid is probably to maintain the iron cofactor in a reduced state at the active sites of the hydroxylases. Some collagen forms in the absence of ascorbic acid, but the fibers are abnormal, resulting in skin lesions and blood vessel fragility, characteristics of scurvy (Desole *et al.*, 1987; Diliberto and Daniels, 1991; Bruick and McKnight, 2001).

Ascorbic acid regulates and participates in enzymatic reactions and transport for neurotransmitters and in hormone biosynthesis (Desole *et al.,* 1987; Diliberto and Daniels, 1991). In the biosynthesis of a variety of neurochemicals, ascorbic acid is involved in many of the hydroxylation and decarboxylation reactions. Tyrosine is normally catabolized to fumaric and acetoacetic acid via homogenistic acid. Animals deficient in ascorbic acid metabolize tyrosine incompletely. In another metabolic pathway, tyrosine is metabolized in

the presence of ascorbic acid to catecholamines by hydroxylation and decarboxylation, forming dopamine, norepinephrine, epinephrine, and adrenocrome. Ascorbic acid is directly involved in the dopamine-β-hydroxylase reaction to produce norepinephrine. The ascorbate free radical may be the primary product of the oxidation. The catecholamine biosynthesis occurs in the adrenal glands and the brain, both with relatively large amounts of ascorbic acid. Ascorbic acid protects catecholamines by direct chemical interactions and by elimination of adrenocrome, a toxic product of catecholamine oxidation, which has been linked to certain mental diseases (Chatterjee *et al.,* 1975; Bruick and McKnight, 2001; Arrigoni and De Tulio, 2002).

The hydroxylation of tyrosine to catecholamines and the hydroxylation of phenylalanine to tyrosine seem to involve the folic acid derivative tetrahydrobiopterin as an electron carrier, and the recycling of ascorbic acid. Ascorbic acid may function to restore this substrate from the oxidized dihydrobiopterin. It has been suggested that dopamine-β-hydroxylase works in conjunction with monodehydroascorbate reductase to recycle tetrahydrobiopterin (Eipper and Mains, 1991; Packer and Fuchs, 1997).

The synthesis of serotonin, a neurotransmitter and vasoconstrictor, involves the hydroxylation and decarboxylation of tryptophan. The initial hydroxylation step, catalyzed by tryptophan hydroxylase, is thought to require ascorbic acid. The cosubstrate for the hydroxylase is tetrahydrobiopterin. Again, it has been suggested that ascorbic acid is able to restore this substrate from its oxidized form, dihydrobiopterin (Chatterjee *et al.,* 1975; Eipper and Mains, 1991).

The chemical and biological properties of *L*-ascorbic acid suggest that it can act as an antioxidant *in vivo* (Rose and Bode, 1993). Lipid peroxidation and oxidative modification of low density lipoproteins (LDL) are implicated in development of atherosclerosis. *L*-ascorbic acid protects against oxidation of isolated LDL by different types of oxidative stress primarily by scavenging reactive oxygen species in the aqueous milieu (Frei *et al.*, 1989; Jialal *et al.*, 1990). In studies done *in vitro*, *L*-ascorbic acid acted as a synergistic antioxidant together with α -tocopherol to prevent LDL oxidation (Sato *et al.*, 1990). In addition, *in vitro* studies have shown that physiological concentrations of *L*-ascorbic acid strongly inhibit LDL oxidation by vascular endothelial cells (Martin and Frei, 1997). Adhesion of leucocytes to the endothelium is an important step in initiating atherosclerosis; leucocyte-endothelial cell interactions induced by cigarette smoke or oxidized LDL is inhibited by *L*-ascorbic acid *in vivo* (Lehr *et al.*, 1994, 1995, 1997).

Ascorbate is a primary antioxidant in that it directly neutralizes radical species. Ascorbate is not very reactive with prevalent cellular oxidants such as hydrogen peroxide and probably reacts mostly with hydrogen peroxide breakdown products (Halliwell, 1999; Aguirre and May, 2008).

2.4.6 Vitamin C and Mercuric Intoxication

Ismail *et al.* (2014) investigated the protective effect of *L*-ascorbic acid (A.A.), against mercury toxicity in albino rats. Twenty four rats were used, divided into three groups; the control was fed a basic diet, while the other two groups were treated either by Hg alone (1g $HgCl_2$/ Kg food) or Hg-ascorbic acid (1g $HgCl_2$/Kg food + 5% ascorbic acid), respectively, for 35 consecutive days. Serum parameters (alkaline phosphatase (ALP), aspartate

aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, uric acid, triglicerides, total cholesterol, HDL-cholesterol and LDL cholesterol) were estimated. Consequent on observed results, Ismail *et al.* (2014) concluded that, ascorbic acid may partially help in the protection against mercury intoxication and it could also be considered a safe nutritional source.

Similarly, Ibegbu *et al.* (2013) conducted a study aimed at evaluating the effect of ascorbic acid on mercuric chloride induced changes on the cerebrum of Wistar rats. Thirty rats were used, divided into six groups of five rats each; group 1 (control) administered distilled water, groups 2 and 3 were administered 52 mg/kg and 26.25 mg/kg of mercuric chloride ($HgCl_2$) respectively, while groups 4 and 5 were administered 52 mg/kg of $HgCl_2$ and 5 mg/kg of ascorbic acid and 26.25 mg/kg of $HgCl_2$ and 5 mg/kg of ascorbic acid respectively, and group 6 was administered 5mg/kg of ascorbic acid, daily for a period of three weeks. Results obtained demonstrated amelioration of mercuric chloride-induced degenerative changes in the cerebrum of Wistar rats with ascorbic acid administration.

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

3.1.1 Plant Material

Dried *Phoenix dactylifera L*. (date palm) fruits were obtained from a local market (Samaru Market) in Zaria, Kaduna, Nigeria, and authenticated and deposited in the Herbarium Unit of Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria with the Voucher Specimen Number of 7130.

3.1.2 Experimental Animals

A total of seventy-two (72) healthy Wistar rats (male and female; 110 to 140 g) were obtained from Animal House of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Kaduna, Nigeria and housed in wired cages in the Animal House of the Department of Human Anatomy, Ahmadu Bello University, Zaria and they were acclimatized for two weeks prior to the commencement of the experiments.

All animals were given food (rat chow – Vital Feeds) and water *ad libitum*. Treatment groups were administered aqueous or ethanol fruit extract of *P. dactylifera/* mercury/ vitamin C in addition to water and rat chow. Rats were weighed at the beginning, during and at the end of the study.

3.1.3 Drugs

- **Mercury:** 70 g of mercuric chloride was obtained from Steve Moore Chemicals, Zaria and used as neurotoxicant for the experiment. The product is manufactured by British Drug Houses (*BDH) chemicals,* Poole, England.

- **Vitamin C:** Vitamin C (Emzor Vitamin C) was obtained from Al-Husna Pharmacy Store, Zaria and used for the experiment as standard antioxidant. The product is manufactured by Emzor Pharmaceutical Industries Limited, Lagos, Nigeria.

3.1.4 Neurobehavioural Set-up

The set-ups used for neurobehavioural studies are as follows:

1. Morris water maze
2. Elevated plus maze
3. Beam walking, and
4. Forelimb grip strength

3.1.5 Animal Feed

Pelletised Vital Feed was obtained and used to feed the animals for the experiment. The product is manufactured by Grand Cereals and Oil Mills Limited (GCOML), Plateau State, Nigeria.

3.1.6 Other Materials

Other materials used in the course of the study include:

Digital weighing balance, digital vernier caliper, centrifuging machine, spectrophotometer and atomic absorption spectrophotometer (ASS), cages, water bottles, saw dust for bedding, animal feed, distilled water, microscope, glass slides, cover slips, stains, fixative (Bouin's fluid and formal saline), bottles for fixing tissues, dissecting set, dissecting boards, orogastric tubes, measuring cylinders, beakers, syringes, blood sample bottles, tissue paper, gloves, methylated spirit, ionized water, phosphate buffer, pH meter, e.t.c.

3.2 Methods

3.2.1 Plant Extract Preparation

Preparation of aqueous and ethanol fruit extracts of *P. dactylifera* were conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria. The method of maceration as reported by Abdul-Wahab *et al.* (2010) and Agbon *et al*. (2013) for the preparation of ethanol and aqueous *P. dactylifera* fruit extract, respectively, were adopted.

The flesh of the dried *P. dactylifera* fruits were manually separated from the pits and pulverized into powder using laboratory mortar and pestle. Preparation of the aqueous extract was in this wise: about 250 g of the powder was soaked in 2 litres of cold distilled water in a conical flask. After 24 hours, the solution (mixture of date palm fruit powder and distilled water) was filtered using filter rag and funnel. The filtrate was allowed to settle for a while, followed by decantation of the supernatant.

The supernatant was heated (steamed) to dryness in an evaporating dish (Royal Worcester; *Made in England*) using H-H Digital Thermometer Water Bath (Mc Donald Scientific International – 22050Hz1.0A International Number) at 60^{o}C. A yield of 28.20% of the extract was obtained.

Preparation of the ethanol extract was in this wise: about 250 g of the powder was soaked in 1 litre of ethanol in a conical flask. After 24 hours, the solution (mixture of date palm fruit powder and distilled water) was filtered using filter rag and funnel. The filtrate was allowed to settle for a while, followed by decantation of the supernatant.

The supernatant was heated (steamed) to dryness in an evaporating dish (Royal Worcester; *Made in England*) using H-H Digital Thermometer Water Bath (Mc Donald Scientific International – 22050Hz1.0A International Number) at 40^{o}C. A yield of 21.28% of the extract was obtained.

3.2.6 *Phoenix dactylifera* Phytochemical Screening

Phytochemical screening of aqueous and ethanol fruit extracts of *P. dactylifera* were conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria. The method of Trease and Evans (1983) for phytochemical screening was adopted.

3.2.7 Dose Preparation of Fruit Extract of *Phoenix dactylifera*

100 g of the extract (aqueous/ ethanol) was measured and dissolved in 800 ml of distilled water. This makes up the working solution. From freshly prepared working solution, little portion was measured out for animal administration.

Different doses of extract were administered to experimental animals by oral gavages using orogastric tube for the treatment period. The method for computing the dosage is indicated in **appendix III.**

3.2.8 Dose Preparation of Mercury

50 g of mercuric chloride was dissolved in 1,000 ml of distilled water to form a solution of mercuric chloride to aid administration of drug easily to the experimental animals by oral gavages. The method for calculating the dosage is indicated in **appendix III**.

3.2.9 Dose Preparation of Vitamin C (Standard Drug)

10 g of ascorbic acid (vitamin C) was dissolved in 300 ml of distilled water to form a solution of vitamin C to aid administration of drug easily to the experimental animals by oral gavages. The method for calculating the dosage is indicated in **appendix III**.

3.2.6 Experimental Design

Seventy-two (72) Wistar rats (male and female) were divided into two experimental categories: **1** (twenty-four (24) rats, consisting of three groups; I - III *served as the common groups*) and **2** (forty-eight (48) rats, subdivided into treatment categories: A and B of twenty-four (24) rats each, consisting of three groups; IV - VI). Thus, each treatment category consisted of six groups (I - VI) of eight rats each. Group I served as control and was administered distilled water (0.5 ml) while, groups II – VI were treatment groups. Neurotoxicity was induced in experimental animals by the administration of mercury – mercuric chloride – as reported by Sheikh *et al.* (2013). Group II was administered mercuric chloride (5 mg/kg; *that is, 12.5% of* LD_{50}*; mercuric chloride* LD_{50} *oral in rats = 40 mg/kg* (Sheikh *et al.*, 2013)) only. Group III was administered vitamin C (100 mg/kg (Raghu Jetti *et al.*, 2014)); groups IV, V and VI were administered fruit extract of *P. dactylifera* (250 mg/kg, 500 mg/kg and 1,000 mg/kg, respectively (*that is, 5%, 10 % and 20% of* LD_{50} *respectively; fruit extract of P. dactylifera* LD_{50} *oral in rats = 5000 mg/kg* (Agbon *et al*., 2014)) followed, concomitantly, by mercuric chloride (5 mg/kg) for a period of 2 weeks. All administrations were via oral route.

Note: Rats of *category A* were administered aqueous fruit extract of *P. dactylifera,* while *category B* were administered ethanol fruit extract of *P. dactylifera* (*see* **Tables 3.1 and 3.2**).

Table 3.1: Treatment of Experimental Animals for Category 'A' Study

Group	Treatment/ day	Duration
I	Distilled water (0.5 ml)	2 weeks
II	$HgCl_2$ (5 mg/kg)	2 weeks
III	Vitamin C (100 mg/kg) + $HgCl_2$ (5 mg/kg)	2 weeks
IV	AFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg)	2 weeks
V	AFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg)	2 weeks
VI	AFPD (1,000 mg/kg) + $HgCl_2$ (5 mg/kg)	2 weeks

n=8; **AFPD=** Aqueous fruit extract of *Phoenix dactylifera*, **$HgCl_2$**=Mercuric chloride. All administrations were via oral route.

Table 3.2: Treatment of Experimental Animals for Category 'B' Study

Group	Treatment/ day	Duration
I	Distilled water (0.5 ml)	2 weeks
II	$HgCl_2$ (5 mg/kg)	2 weeks
III	Vitamin C (100 mg/kg) + $HgCl_2$ (5 mg/kg)	2 weeks
IV	EFPD (250 mg/kg) + $HgCl_2$ (5 mg/kg)	2 weeks
V	EFPD (500 mg/kg) + $HgCl_2$ (5 mg/kg)	2 weeks
VI	EFPD (1,000 mg/kg) + $HgCl_2$ (5 mg/kg)	2 weeks

n=8; **EFPD=** Ethanol fruit extract of *Phoenix dactylifera*, **$HgCl_2$**=Mercuric chloride. All administrations were via oral route.

3.2.7 Experimental Procedure

All experimental procedures were conducted in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, except, otherwise specified.

3.2.7.1 Neurobehavioural/ Cognitive Studies

The following neurobehavioural tests were conducted:

1. Elevated Plus Maze Test

The elevated plus maze (EPM) is a widely used behavioural assay for rodents and it has been validated to assess anti-anxiety effects and to define brain regions and mechanisms underlying anxiety-related behaviour (Walf and Frye, 2007; He *et al.*, 2011).

The EPM set-up was built according to the description of Lister (1987) as reported by John (2010). The apparatus is in the configuration of a '+' and comprised two open arms (50 x 10 cm) across from each other and perpendicular to two closed arms (50 x 10 x 20 cm) with a center platform (10 x 10 cm). The closed arms had a high (20 cm) wall to enclose the arm. The entire apparatus was 50 cm above the floor. The apparatus was made of wooden materials, painted black.

Procedure: Wistar rat was placed in the central square of the Plus-Maze facing an open arm and was then allowed to explore the apparatus for 5 minutes. The maze was then cleaned with methylated spirit and allowed to dry between tests. Behaviours scored were:

i. ***Open arm entries***: Frequency with which the animal entered the open arms. All four of the rat's paws should be in the open arms to be regarded as an entry.

ii. ***Open arm duration***: Length of time the animal spent in the open arms.

iii. *Close arm entries*: Frequency with which the animal entered the closed arms. All four of the rat's paws should be in the closed arms to be regarded as an entry.

iv. ***Close arm duration***: Length of time the animal spent in the closed arms.

v. *Head dipping*: Frequency with which the animal lowered its head over the sides of the open arms towards the floor.

vi. ***Rearing***: Frequency with which the animal stands on its hind legs or leans against wall of the maze with front paws.

Note: Prior to the test (that is, before treatment), rats were habituated to the EPM set-up daily for 3 days.

2. Transfer Latency (TL) on EPM Open Arm

The elevated plus maze served as the exteroceptive behavioural model (wherein the stimulus existed outside the body) to evaluate learning and memory in Wistar rats (Chakravarthi *et al.*, 2012; Khare *et al.*, 2014). The apparatus, as described above, was used for this study.

Procedure: On the first the day, before treatment, each rat was placed at the end of open arm of the EPM, facing away from central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the rat to move from the open arm into any one of the closed arms with all its four paws. TL was recorded on the first day (training session) for the each animal. The rat was allowed to explore the maze for another 2 minutes and returned to its home cage. Retention of this learned task (memory) was examined 24 hours after the first

day trial (that is, after the first day of treatment). Retention of memory was also examined after 7 days and 14 days of treatment (that is, 24 hours after the last dose of 2 weeks duration).

3. Morris Water Maze Test

The method described by Morris *et al.* (1982), as reported by Drapeau *et al.* (2003), for spatial memory and learning was adopted for this study. Rats were tested in a Morris water maze (180 cm diameter, 60 cm high) filled with water. An escape platform was hidden 2 cm below the surface of the water in a fixed location in one of four quadrants halfway between the wall and the middle of the pool.

Procedure: Before the test (that is, before treatment), the rats were habituated to the pool daily for 3 days. During training, animals were required to locate the submerged platform by using distal extramaze cues. They were tested for four trials per day (90 seconds with an inter-trial interval of 30 seconds and beginning from three different start points that varied randomly each day). If an animal did not find the platform, it was set on the platform at the end of the trial. The time to reach the platform (latency in seconds) was recorded.

Rats were tested after 7 days and 14 days of treatment (that is, 24 hours after the last dose of 2 weeks duration).

3.2.7.2 Sensory-motor Activity, Motor Coordination and Balance Studies

1. Forelimb Grip Strength Test

Forelimb grip strength test was assessed using a modified procedure described by Olopade *et al*. (2012).

Procedure: The rat's forepaws were placed on a horizontally suspended metal wire 2.5 mm in diameter, 1 m in length and placed 1 m above a landing area filled with soft bedding. The length of time each rat was able to stay suspended before falling off the wire was recorded; a maximum of 2 minutes was given to each rat.

Note: Prior to the test (that is, before treatment), rats were habituated to the set-up daily for 2 days. Rats were tested after 7 days and 14 days of treatment (that is, 24 hours after the last dose of 2 weeks duration).

2. 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; Karl *et al.*, 2003; Fan *et al.*, 2008). A modification of the beam walking apparatus (BWA) described by Puurunen (2001) and Carter *et al.* (2001) was used.

Procedure: The BWA consisted of an elevated platform connected by a 100 cm long wood beam with a width of 3 cm. The beam was placed horizontally, 50 cm above the floor surface, with one end mounted on a narrow support (connected to start platform 10 x 10 cm (Rodrigues-Alves *et al.*, 2009)) and the other end attached to a goal box (20 x 20 x 20 cm). The start point was placed by a bright light source to motivate the rats to traverse the beam. A sawdust-filled box at the base served as protection for the falling rats.

Beam-walking ability was measured using a 6-point scale described by (Puurunen, 2001): the rat was not able to stay on the beam, *0 point*; the rat did not move, but was able to stay on the beam, *1 point*; the rat tried to traverse the beam, but fell, *2 points*; the rat traversed the beam with more than 50% (footslips $\geq$ 4) footslips of hindlimb, *3 points*; the rat

traversed the beam with more than one footslip, but less than 50%, *4 points*; the rat had only one slip of the hindlimb, *5 points*; the rat traversed the beam without any hindlimb slips, *6 points*.

In addition, the latency until the animal's nose entered the goal box (up to 60 seconds) was recorded as described by Abou-Donia *et al.* (2008). Rats that fell off the beam or did not enter the goal box were assigned latencies of 60 seconds. Beam-walk scores were based on an average of 3 trials, cleaned with methylated spirit at inter-trial intervals.

Note: Prior to the test (that is, before treatment), rats were habituated to the BWA daily for 3 days. Rats were tested after 7 days and 14 days of treatment (that is, 24 hours after the last dose of 2 weeks duration).

3.2.7.3 Morphological Studies

Rats were weighed at the beginning, during and at the end of the study. Rats were humanely killed using chloroform anaesthesia and brain organs harvested for morphometry. Morphometry involved taking brain weight using digital weighing scale, brain-body weight ratio computed, and brain dimensions (cerebral and cerebellar length and width) using digital vernier caliper.

Histometric studies involved measuring the cell area and perimeter of the pyramidal cells (neurones) of the cerebral cortex (layer V) and CA1 and CA3 areas of the hippocampus, and Purkinje cells of cerebellar cortex using a light microscope (HM-LUX, Leitz Wetzlar, Germany) with a 40/ 0.65 x objective (x 400 magnification) and, a micrometer slide (1 mm graduated in 0.01 mm units; that is divided 10x into 100 μm units) and a computer

running imaging soft ware (AmScope MT *version* 3.0.0.5, USA) according to the manufacturer's instruction (*see* **appendix V**).

Cresyl Fast Violet stained micrographs (digital microscopic images) were used for the histometry. Three micrographs were randomly captured (Lai *et al.*, 2011; Kaizaki *et al.*, 2013) in each of the brain regions (*cerebral cortex* - layer V (Toft *et al.*, 2005; Eriksen and Pakkenberg, 2007), *hippocampus* - CA1 and CA3 areas (Akdogan *et al.*, 2002; Lai *et al.*, 2011) and *cerebellar cortex* - Purkinje cell layer (Andersen and Pakkenberg, 2003; Jelsing *et al.*, 2006)) and 5 - 10 cells were randomly selected, measured and averaged in the three micrographs (*see* **Plate 3.1 in appendix V**).

3.2.7.4 Histological and Histochemical Studies

At the end of the experiment, harvested brain organs were fixed in Bouin's fluid and tissues processed (*see* **appendix I**) for light microscopic examination, stained with routine Haematoxylin and Eosin (H&E) and histochemical (Cresyl Fast Violet – CFV and Golgi) stains. Methods of stains preparation and staining protocols are indicated in **appendix II.**

3.2.7.5 Neurochemical and Biochemical Studies

At the end of the experiment, blood samples were collected via jugular vein and rats' brains dissected out, weighed and homogenized in 0.1 M phosphate buffer (pH 7.4) (1 g tissue/ 4 ml (Ige *et al.*, 2011)). The homogenate were then centrifuged and aliquots of the supernatant were obtained for neurochemical/ biochemical analysis.

Neurochemical analysis for metal (Cu, Fe, Mn and Zn) estimation in tissue (brain) homogenate was conducted using Atomic Absorption Spectrophotometer (AAS – AA240FS, Varian) at the Multiuser Laboratory, Department of Chemistry, Ahmadu Bello

University, Zaria. The analytical method for determining metals in biological tissues as reported by Environmental Monitoring Methods Index, EMMI (1997) was adopted. Protocol is indicated in **appendix IV.**

Lipid peroxide levels, malondialdehyde (MDA) and antioxidant enzymatic activity: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) using collected blood samples and tissue (brain) homogenate, and activity of acetylcholinesterase enzyme (AchE) using brain homogenate were conducted in the Department of Chemical Pathology, Faculty of Medicine, Ahmadu Bello University Teaching Hospital, Shika.

The concentration of thiobarbituric acid-reactive substance, malondialhydehyde (MDA), as an index of lipid peroxidation, was evaluated in the samples using an assay kit (Rat Malondialhydehyde (MDA) ELISA Kit; *WKEA Med Supplies Corp, China*) as stated by the manufacturer. The principle was based on the method of Janero (1990) as reported by Okey and Ayo (2015).

Catalase activity was determined using Rat Catalase (CAT) ELISA Kit (*WKEA Med Supplies Corp, China*) according to the manufacturer's instruction. The procedure used was based on the method of Beer and Sizer (1952) as reported by Okey and Ayo (2015).

Superoxide dismutase activity was analyzed using Rat Super Oxidase Dimutase (SOD) ELISA Kit (*WKEA Med Supplies Corp, China*) as stated by the manufacturer. The procedure was based on the method of Martin *et al*. (1987) as reported by Okey and Ayo (2015).

Glutathione peroxidase was analyzed using Glutathione Peroxidase (GPx) ELISA Kit (*WKEA Med Supplies Corp, China*) as stated by the manufacturer. The procedure was based on the method of (Ursini *et al.,* 1995).

Acetylcholinesterase activity was analyzed using Acetylcholinesterase Activity Assay Kit (*Sigma-Aldrich, USA*) as stated by the manufacturer. The procedure was based on the method of Ellman *et al*. (1961) as reported by Okey and Ayo (2015).

3.3 Data Analysis

Results obtained were analysed using the statistical soft ware, Statistical Package for the Social Sciences (SPSS *version 18.0*, SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-641, USA) and Microsoft Office Excel 2007 for charts. Results were expressed as mean ± S.E.M, and presence of significant differences among means of the groups were determined using one way ANOVA with least significant difference (LSD) *post hoc test* for significance. Paired sample *t*–test was employed for the comparison of means as appropriate. Values were considered significant when $p \leq 0.05$.

CHAPTER FOUR

4.0 Results

4.1 Phytochemical Analysis

Phytochemical analysis of aqueous and ethanol fruit extracts of *Phoenix dactylifera* produced positive reaction for each of the following secondary metabolites: Alkaloids, Tannins, Saponins, Flavonoids and Carbohydrates. However, a negative reaction was obtained for Anthraquinone and Cardiac glycoside (**Table 4.1**).

4.2 Physical Observation

During the period of administration, the physical activities of experimental animals (Wistar rats) were observed. Rats in the control group were observed to exhibit normal physical activities, such as movement and playfulness, whereas animals in the treatment groups exhibited decreased activity, especially in mercury ($HgCl_2$) - treated group.

The weights of the rats, in all groups (*categories 'A' and 'B' studies*), were observed to have increased when initial and final weights were compared. Remarkable ($p<0.05$) increases in weights were observed with the control and extract- treated groups, especially, aqueous fruit extract of *P. dactylifera* (250 mg/kg and 500 mg/kg) and ethanol fruit extract of *P. dactylifera* (500 mg/kg and 1,000 mg/kg) doses. However, there were no significant differences ($p>0.05$) in weight changes (difference in initial and final weights) when treated groups were compared with the control (**Table 4.2)**.

Table 4.1: Phytochemical Constituents of the Fruit Extracts of *Phoenix dactylifera*

	Aqueous extract	**Ethanol extract**
Constituents	Inference	Inference
Alkaloids	+	+
Anthraquinones	–	–
Cardiac glycosides	–	–
Carbohydrates	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+

+ = Positive (present)
– = Negative (absent)

Table 4.2: Weight comparison and weight change of Wistar rats *initial* (day 1) and *final* (day 14) of treatment

Group/ treatment	IW (g)	FW (g)	*t*	p-value	FW-IW (g)
Control (H_2O 0.5ml)	116.63±13.28	138.50±12.01	8.919	0.000	21.14±2.70
$HgCl_2$ (5mg/kg)	109.50±20.67	131.00±38.92	2.261	0.073	29.00±10.71
Vit C (100mg/kg) **+ $HgCl_2$** (5mg/kg)	101.40±19.27	111.20±8.55	0.735	0.503	8.57±11.38
AFPD (250mg/kg**) + $HgCl_2$** (5mg/kg)	111.25±12.09	144.75±39.51	3.310	0.045	24.00±3.02
AFPD (500mg/kg**) + $HgCl_2$** (5mg/kg)	104.57±16.55	129.86±13.23	2.881	0.028	25.29±8.78
AFPD (1000mg/kg**) + $HgCl_2$** (5mg/kg)	105.00±16.86	124.20±42.54	1.329	0.255	18.71±7.28
EFPD (250mg/kg) **+ $HgCl_2$** (5mg/kg)	144.25±36.37	159.25±59.36	1.730	0.182	7.29±4.32
EFPD (500mg/kg **)+ $HgCl_2$** (5mg/kg)	117.50±21.42	138.00±21.61	4.889	0.016	20.57±3.17
EFPD (1000mg/kg**) + $HgCl_2$** (5mg/kg)	122.50±25.85	148.00±25.60	6.755	0.007	24.14±2.38

n = 8; mean ± SEM; Paired sample *t*- test; One way ANOVA *LSD post hoc test. p>0.05 when compared with the control.* **AFPD=** *Aqueous fruit extract of Phoenix dactylifera*; **EFPD**= *Ethanol fruit extract of Phoenix dactylifera*; **Vit C**= Vitamin C; **FW**=Final weight; **IW**= Initial weight; **FW-IW**= Weight change.

4.3 Morphological Studies

4.3.1 Morphometry

Morphological studies involved the morphometry of brain dimensions (cerebral and cerebellar length and width) and brain weight. The relative organ weights (brain weight/ body ratio and index) were computed and compared with the control. Relative to the control, there was increase in relative organ weight in all the groups, except in ethanol fruit extract of *P. dactylifera* (1,000 mg/kg)-treated group. However, observed increases were not statistically significant ($p>0.05$), except in vitamin C- treated group, when compared with the control (**Figures 4.1)**.

Rats' brain dimensions revealed striking ($p<0.05$) difference when compared with the control in both studies (that is, in aqueous and ethanol fruit extract of *P. dactylifera* treatments). In category 'A' (aqueous fruit extract of *P. dactylifera* treatment) study, cerebral length showed increase in mercury- and vitamin C- treated groups, and decrease in extract- treated groups. Increase was remarkable ($p<0.001$) in vitamin C- treated group, and decrease in aqueous fruit extract of *P. dactylifera* (AFPD, 250 mg/kg dose) - treated group only when compared with the control. Cerebral width showed decrease in the treatment groups, except in AFPD (500 mg/kg)- treated group. Decrease were only remarkable ($p<0.05$) in vitamin C- and AFPD (250 mg/kg) - treated groups when compared with the control (**Figure 4.2a)**.

In category 'B' (ethanol fruit extract of *P. dactylifera* treatment) study, Wistar rats' cerebral length showed increase in the treatment groups, remarkable ($p<0.05$) in vitamin C- and ethanol fruit extract of *P. dactylifera* (EFPD, 250 mg/kg and 1,000 mg/kg doses) -

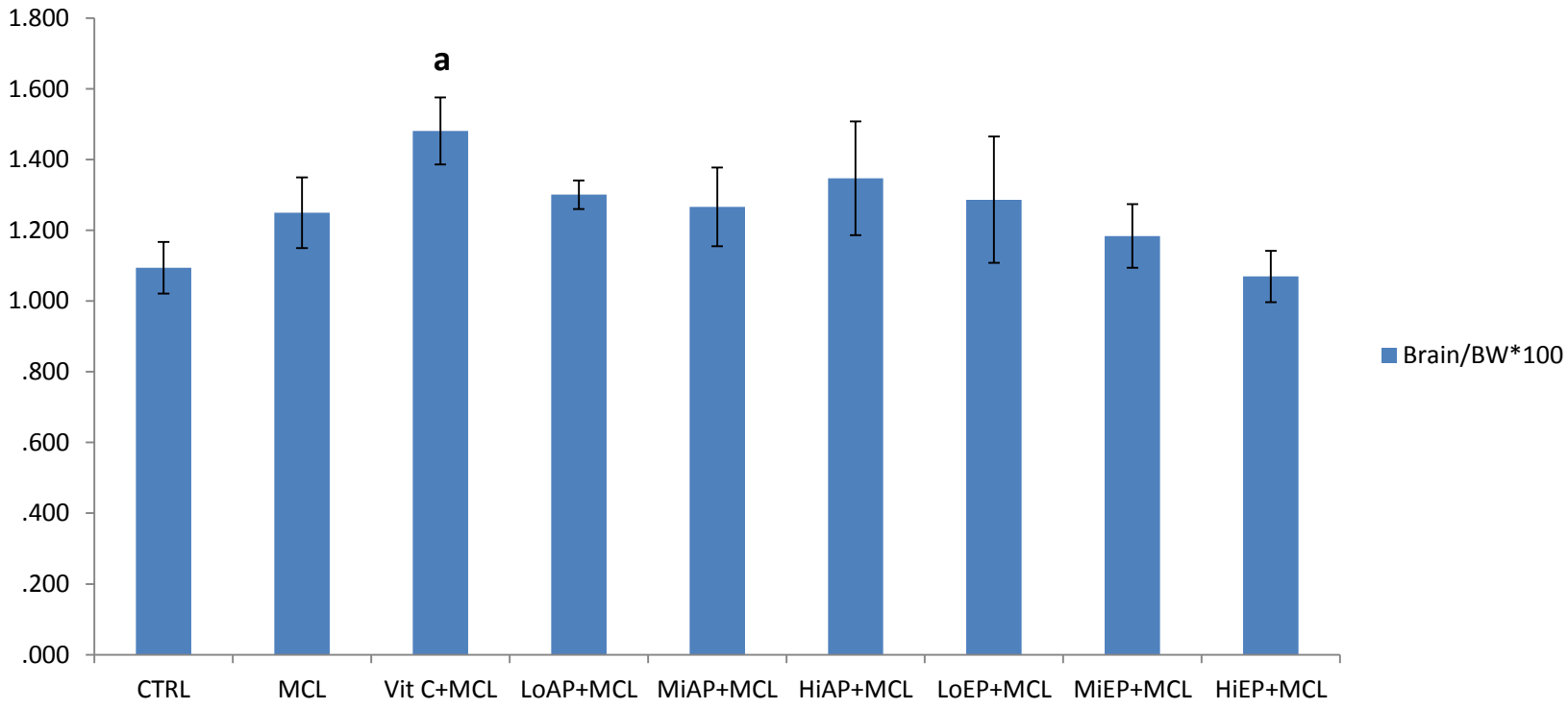


Figure 4.1: Comparison of organ (brain) - body weight index of Wistar rats

n = 8; mean ± SEM; One way ANOVA LSD *post hoc test.* ***a****=p<0.05 when compared with the control.* **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg); **BW=** Body weight.

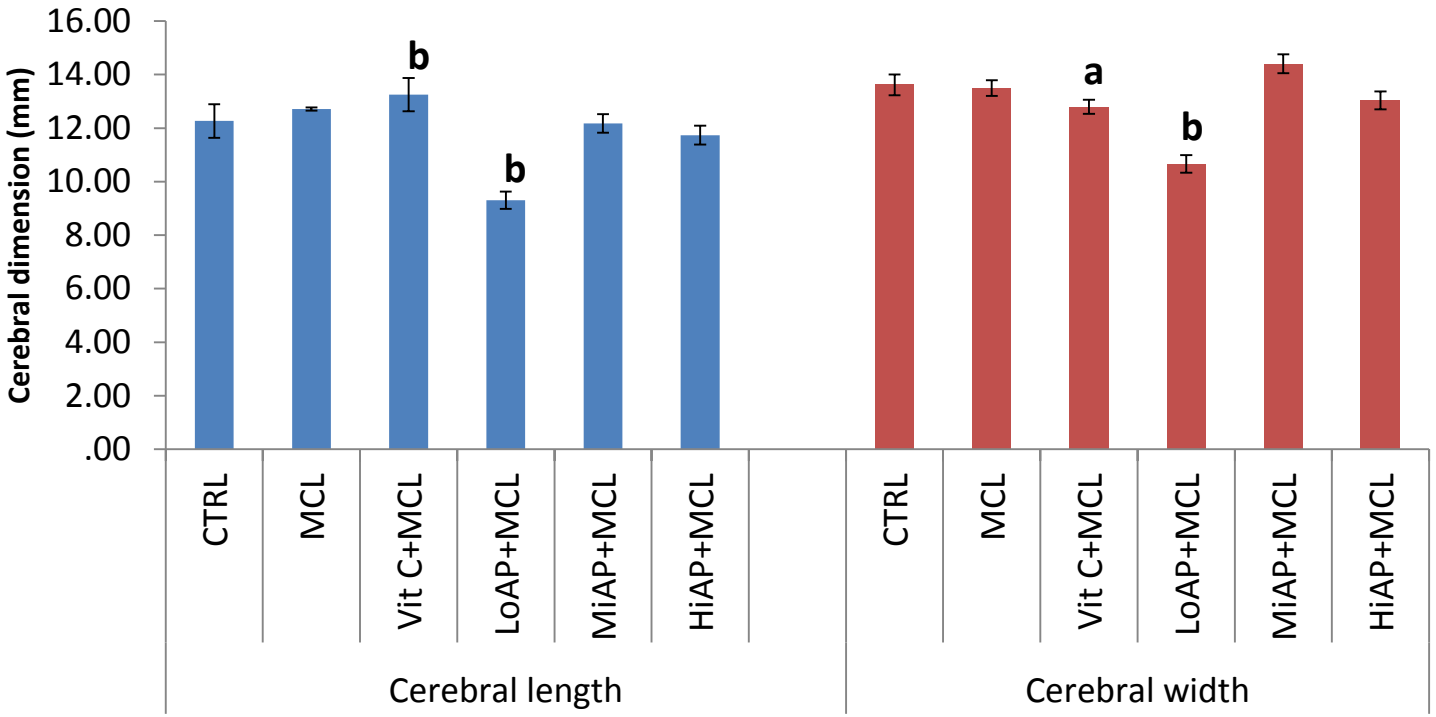


Figure 4.2a: Effect of aqueous fruit extract of *P. dactylifera* on cerebral dimensions of Wistar rats

n = 8; mean ± SEM; One way ANOVA *LSD post hoc test.* ***a****=p<0.05;* ***b****= p<0.001 when compared with the* control. **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

treated groups, when compared with the control. However, cerebral width showed decrease in the treatment groups, remarkable ($p<0.05$) in vitamin C- and EFPD (250 mg/kg) - treated groups, when compared with the control (**Figure 4.2b)**.

In category 'A' study, Wistar rats' cerebellar length showed remarkable ($p<0.05$) decrease in the treatment groups when compared with the control. However, cerebellar width showed increase in the treatment groups, remarkable ($p<0.05$) in vitamin C- and AFPD (500 mg/kg) - treated groups, when compared with the control (**Figure 4.3a)**.

In category 'B' study, Wistar rats' cerebellar length showed remarkable ($p<0.05$) decrease in the treatment groups when compared with the control. However, cerebellar width showed increase in the treatment groups, except in EFPD (250 and 500 mg/kg) - treated groups. Increase and decrease were remarkable ($p<0.05$) in vitamin C- and EFPD (500 mg/kg) - treated groups, respectively, when compared with the control (**Figure 4.3b)**.

4.3.2 Histometric Studies

Histometry involved measuring the cell area and perimeter of neurones (pyramidal cells of cerebral cortex- *layer V* and CA1 and CA3 hippocampal regions, and Purkinje cells of the cerebellar cortex) of the Wistar rats.

4.3.2.1 Cerebral and Cerebellar Cortices Histometric Characteristics

In category 'A' study, histometric analysis of cerebral cortex pyramidal cells revealed decrease in the cell area of all the treated groups, except the mercury-treated group. Cell perimeter increase was observed in all the treated groups, except the AFPD (250 and 1,000 mg/kg)-treated groups. However, differences in the histometric characteristics were not

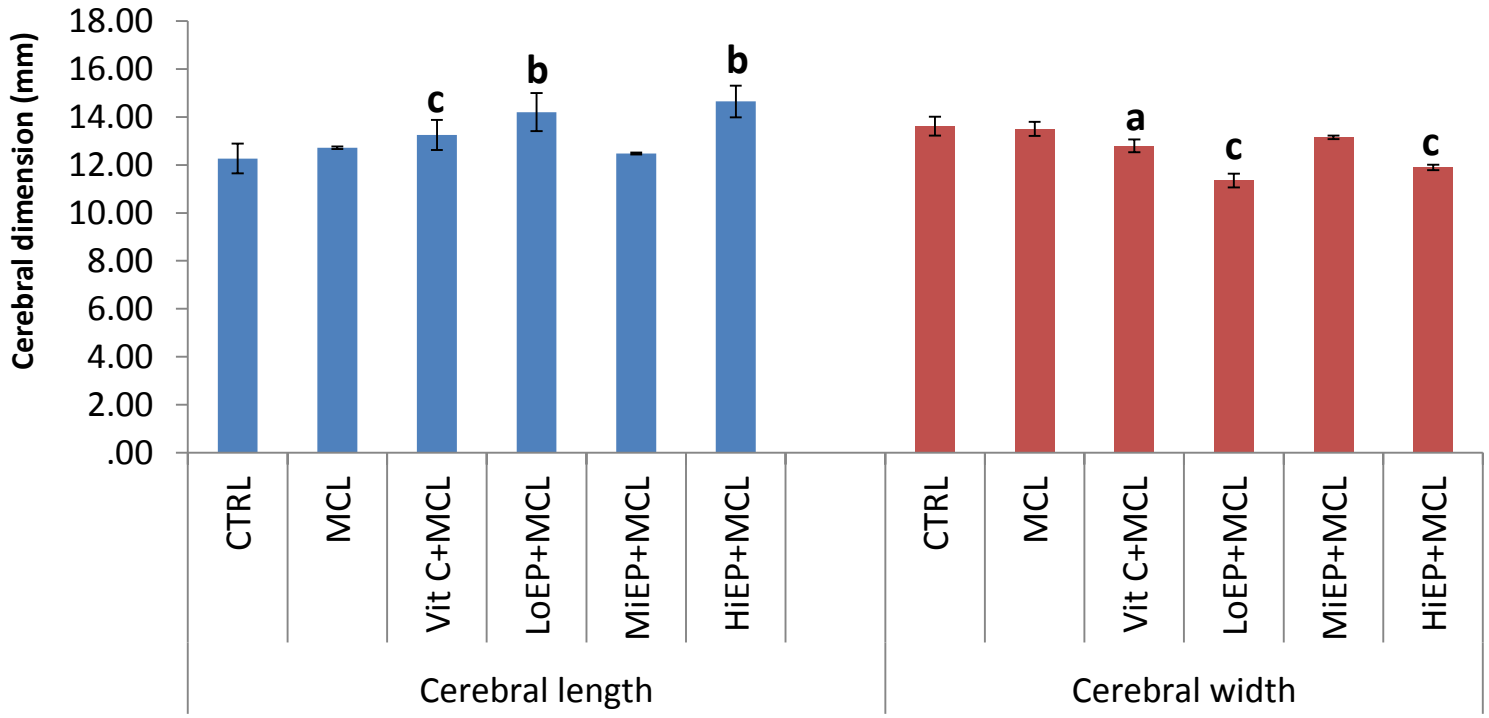


Figure 4.2b: Effect of ethanol fruit extract of *P. dactylifera* on brain cerebral dimensions of Wistar rats

n = 8; mean ± SEM; One way ANOVA *LSD post hoc test.* ***a****=p<0.05;* ***b****= p<0.01;* ***c****= p<0.001 when compared with the* control. **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

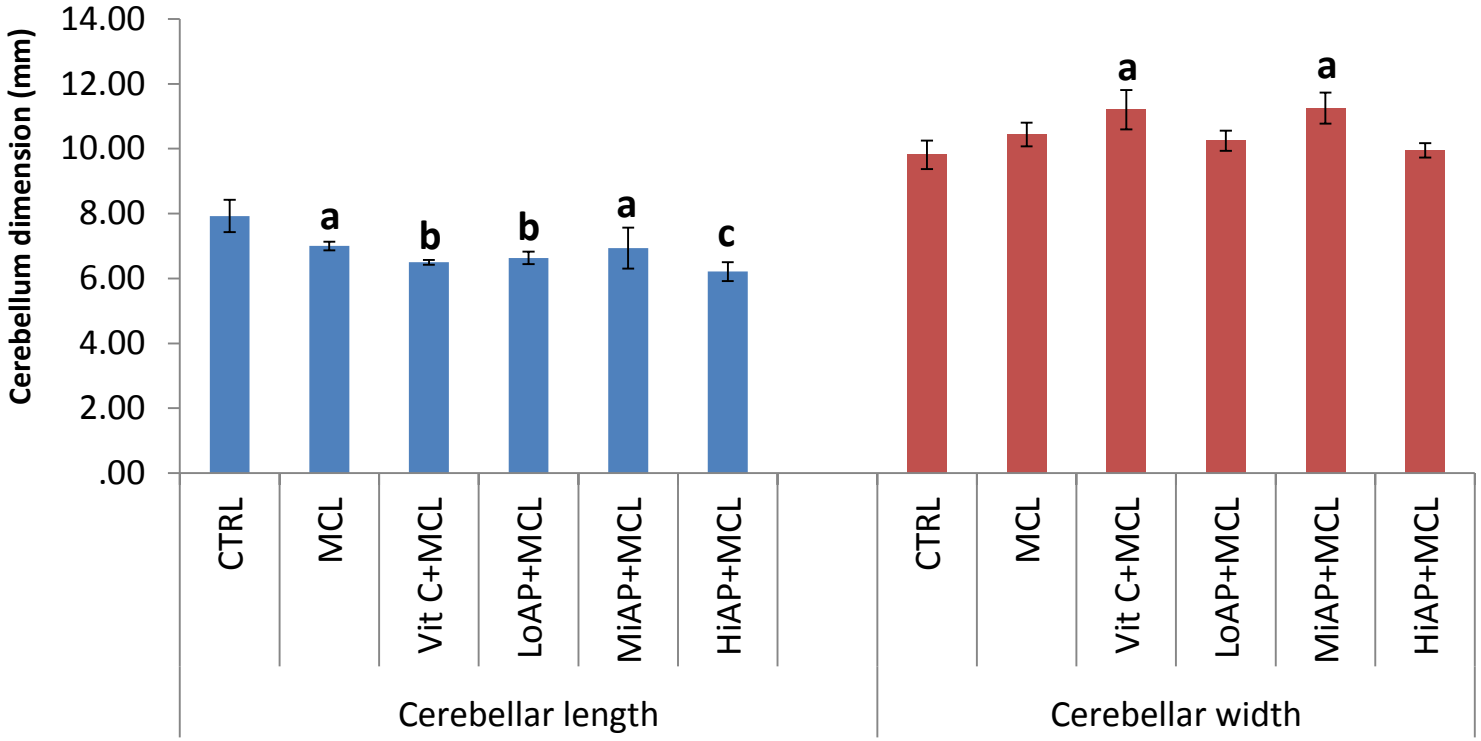


Figure 4.3a: Effect of aqueous fruit extract of *P. dactylifera* on cerebellar dimensions of Wistar rats

n = 8; mean ± SEM; One way ANOVA *LSD post hoc test.* ***a****=p<0.05;* ***b****= p<0.01;* ***c****= p<0.001 when compared with the* control. **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

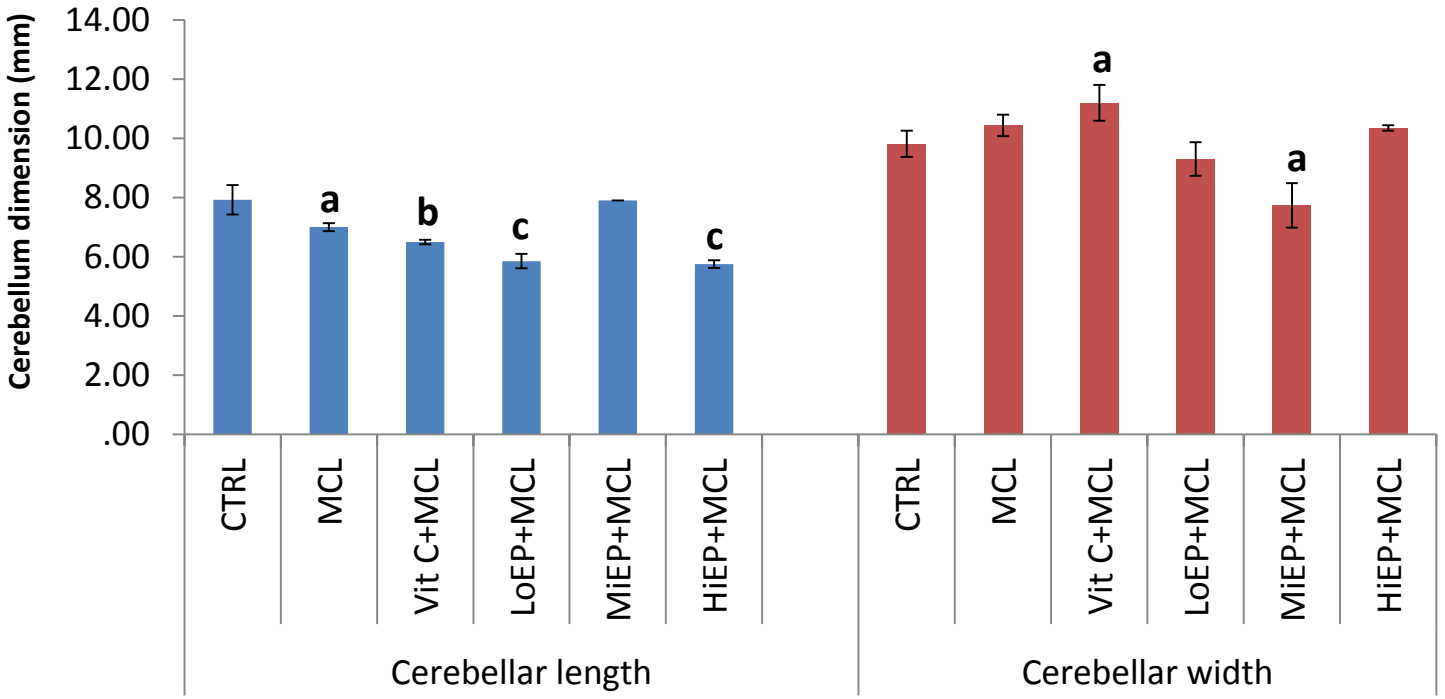


Figure 4.3b: Effect of ethanol fruit extract of *P. dactylifera* on cerebellar dimensions of Wistar rats

n = 8; mean ± SEM; One way ANOVA *LSD post hoc test.* ***a****=p<0.05;* ***b****= p<0.01;* ***c****= p<0.001 when compared with the* control. **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

statistically significant (*p>0.05*) when compared to the control. Cerebellar cortex histometric characteristics of the Purkinje cells showed decrease in the cell area and perimeter of all the treated groups. Differences were only significant (*p<0.01*) in cell area when compared to the control. Besides, remarkable (*p<0.05*) difference in Purkinje cell area was observed in AFPD (250 mg/kg)-treated group when compared to the mercury-treated group (**Table 4.3a**).

In category 'B' study, analysis of histometric characteristics of cerebral cortex pyramidal cells revealed decrease in the cell area of all the treated groups, except the mercury- and EFPD (500 mg/kg)-treated groups. Increase in cell perimeter was observed in all the treated groups, except the EFPD (1,000 mg/kg)-treated group. However, differences in the histometric characteristics were not statistically significant (*p>0.05*) when compared to the control. Histometric characteristics of the Purkinje cells of cerebellar cortex showed remarkable (*p<0.01*) decrease in the cell area of all the treated groups, except in EFPD (250 and 1,000 mg/kg)-treated groups when compared to the control. Cell perimeter decreases (*p>0.05*) were observed in all the treated groups, except in EFPD (250 mg/kg)-treated group when compared to the control. Besides, remarkable (*p<0.05*) difference in Purkinje cell area was observed in EFPD (250 and 1,000 mg/kg)-treated groups, and in cell perimeter (AFPD 250 mg/kg-treated group) when compared to the mercury-treated group (**Table 4.3b**).

4.3.2.2 CA1 and CA3 Hippocampal Regions Histometric Characteristics

In category 'A' study, histometric analysis of CA1 pyramidal cells revealed increase in the cell area of all (especially (*p<0.05*) in AFPD 500 mg/kg) the treated groups, except in the

Table 4.3a: Effect of Aqueous Fruit Extract of *P. dactylifera* on Histometric Characteristics of Cells in the Cerebral and Cerebellar Cortices of Wistar Rats

Group/ treatment	Cerebral (LV) pyramidal cell area (*μm*)	Cerebral (LV) pyramidal cell perimeter (*μm*)	Cerebellar pyramidal (Purkinje) cell area (*μm*)	Cerebellar pyramidal (Purkinje) cell perimeter (*μm*)
Control (distilled H_2O 0.5 ml/kg)	478.78 ± 70.37	22.05 ± 1.48	664.09 ± 25.04	24.78 ± 0.41
$HgCl_2$ (5 mg/kg)	506.23 ± 67.25	22.67 ± 1.21	492.64 ± 51.51 **c**	21.09 ± 1.08
Vit C (100 mg/kg) + **$HgCl_2$** (5 mg/kg)	436.03 ± 43.18	22.60 ± 1.28	447.71 ± 21.97 **c**	22.88 ± 0.68
AFPD (250 mg/kg) + **$HgCl_2$** (5 mg/kg)	369.37 ± 51.53	20.97 ± 1.54	375.65 ± 26.96 **c***	19.52 ± 0.97
AFPD (500 mg/kg) + **$HgCl_2$** (5 mg/kg)	476.22 ± 33.95	22.96 ± 1.08	506.40 ± 21.37 **b**	22.85 ± 0.55
AFPD (1000 mg/kg) + **$HgCl_2$** (5 mg/kg)	426.81 ± 40.86	21.66 ± .67	437.44 ± 28.96 **c**	20.92 ± 0.75

n = 8; mean ± SEM; One way ANOVA LSD *post hoc test* **a/*=***p<0.05;* **b/**=***p<0.01;* **c/***=***p<0.001** *when compared with the control/ $HgCl_2$.* **AFPD=** *Aqueous fruit extract of Phoenix dactylifera;* **Vit C**= *Vitamin C;* **LV**= *layer V of cerebral cortex.*

Table 4.3b: Effect of Ethanol Fruit Extract of *P. dactylifera* on Histometric Characteristics of Cells in the Cerebral and Cerebellar Cortices of Wistar Rats

Group/ treatment	Cerebral (LV) pyramidal cell area (*μm*)	Cerebral (LV) pyramidal cell perimeter (*μm*)	Cerebellar pyramidal (Purkinje) cell area (*μm*)	Cerebellar pyramidal (Purkinje) cell perimeter (*μm*)
Control (distilled H_2O 0.5 ml/kg)	478.78 ± 70.37	22.05 ± 1.48	664.09 ± 25.04	24.78 ± 0.41
$HgCl_2$ (5 mg/kg)	506.23 ± 67.25	22.67 ± 1.21	492.64 ± 51.51 [c]	21.09 ± 1.08
Vit C (100 mg/kg) + **$HgCl_2$** (5 mg/kg)	436.03 ± 43.18	22.60 ± 1.28	447.71 ± 21.97 [c]	22.88 ± 0.68
EFPD (250 mg/kg) + **$HgCl_2$** (5 mg/kg)	454.36 ± 54.61	22.47 ± 1.34	622.43 ± 31.39**	39.41 ± 15.37*
EFPD (500 mg/kg) + **$HgCl_2$** (5 mg/kg)	565.03 ± 67.25	23.80 ± 1.29	523.20 ± 36.49 [b]	22.31 ± 0.93
EFPD (1000 mg/kg) + **$HgCl_2$** (5 mg/kg)	368.15 ± 39.81	21.39 ± 1.18	633.99 ± 16.55**	23.90 ± 0.30

n = 8; mean ± SEM; One way ANOVA *LSD post hoc test* **a/*=***p<0.05;* **b/**=***p<0.01;* **c/***=***p<0.001** *when compared with the control/ $HgCl_2$.* **EFPD=** *Ethanol fruit extract of Phoenix dactylifera;* **Vit C**= *Vitamin C;* **LV**= *layer V of cerebral cortex.*

mercury-treated group. Cell perimeter decrease was observed in all the treated groups, except the vitamin C- and AFPD (500 mg/kg)-treated groups. These differences, however, were not statistically significant ($p>0.05$) when compared to the control. Comparison with the mercury-treated group revealed remarkable ($p<0.01$) difference in cell area and perimeter with the vitamin C- and AFPD (500 mg/kg)-treated groups.

CA3 pyramidal cells showed decrease in the cell area of all the treated groups, except the AFPD (1,000 mg/kg)-treated group. However, differences were not striking ($p>0.05$) when compared to the control. Decreases in cell perimeter were observed in all the treated groups, especially ($p<0.05$) in AFPD (500 mg/kg)-treated group, when compared to the control (**Table 4.4a**).

Histometric analysis of CA1 pyramidal cells, in category 'B' study, revealed increase in the cell area of all the treated groups (especially ($p<0.05$) in EFPD 500 mg/kg), except the mercury-treated group. Decrease in cell perimeter was observed in all the treated groups, except the vitamin C- and EFPD (500 mg/kg)-treated groups. Difference was striking ($p<0.05$) in EFPD (500 mg/kg)-treated group only, when compared to the control. Comparison with the mercury-treated group revealed remarkable ($p<0.05$) difference in cell area and perimeter with EFPD (500 mg/kg)-treated group only.

Cell area of CA3 pyramidal cells revealed decrease in all the treated groups, except the EFPD (250 and 1,000 mg/kg)-treated groups. However, differences were not striking ($p>0.05$) when compared to the control. Cell perimeter decrease was observed in all the treated groups, except the EFPD (500 and 1,000 mg/kg)-treated groups. These differences, however, were not striking ($p>0.05$) when compared to the control. Remarkable ($p<0.05$)

Table 4.4a: Effect of Aqueous Fruit Extract of *P. dactylifera* on Histometric Characteristics of Pyramidal Cells in the CA1 and CA3 Hippocampal Regions of Wistar Rats

Group/ treatment	CA1 pyramidal cell area (*μm*)	CA1 pyramidal cell perimeter (*μm*)	CA3 pyramidal cell area (*μm*)	CA3 pyramidal cell perimeter (*μm*)
Control (distilled H_2O 0.5 ml/kg)	407.46 ± 36.01	21.42 ± 0.75	649.00 ± 36.25	28.57 ± 0.83
$HgCl_2$ (5 mg/kg)	353.88 ± 31.50	20.95 ± 1.22	543.09 ± 63.22	25.45 ± 1.15
Vit C (100 mg/kg) **+ $HgCl_2$** (5 mg/kg)	501.03 ± 31.56**	22.39 ± 0.83	624.69 ± 33.60	26.75 ± 1.08
AFPD (250 mg/kg) **+ $HgCl_2$** (5 mg/kg)	433.68 ± 35.31	21.40 ± 0.35	636.55 ± 58.07	26.54 ± 1.13
AFPD (500 mg/kg) **+ $HgCl_2$** (5 mg/kg)	515.75 ± 45.26 [a]**	22.47 ± 1.27	555.62 ± 46.56	24.20 ± 0.81[a]
AFPD (1000 mg/kg) **+ $HgCl_2$** (5 mg/kg)	414.90 ± 31.61	20.40 ± 0.65	677.17 ± 58.19	27.04 ± 1.33

n = 8; mean ± SEM; One way ANOVA LSD *post hoc test* **a/*=***p<0.05;* **b/**=***p<0.01;* **c/***=***p<0.001** *when compared with the control/ $HgCl_2$.* **AFPD=** *Aqueous fruit extract of Phoenix dactylifera;* **Vit C**= *Vitamin C;* **CA=** *Cornu Amonis*

difference in cell perimeter was observed with EFPD (500 and 1,000 mg/kg)-treated groups when compared to the mercury-treated group (**Table 4.4b**).

4.4 Histological and Histochemical Studies

Histological sections of Wistar rats' brain regions (cerebral cortex–layer V, cerebellar cortex and hippocampus) stained with routine (Haematoxylin and Eosin, H&E) histological and histochemical (Cresly Fast Violet and Silver–*Golgi*) stains and examined under the light microscope revealed the following:

4.4.1 Histological and Histochemical Features of the Cerebral Cortex

4.4.1.1 Histological and Histochemical Features of the Cerebral Cortex of Control (Untreated) Wistar Rat

The cerebral 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 layers differing in neurone morphology, size and population density. Particularly, the ganglionic layer (*layer V*) revealed large pyramidal (ganglion/ Betz) cells of the motor cortex and stellate cells. Histochemical (cresly fast violet, CFV) staining for Nissl substances revealed normal appearance of distinctly stained large pyramidal neurones and Golgi staining for neurites revealed normal appearance of large pyramidal neurone axonal and dendritic processes (**Plates 4.1 - 4.3**).

Table 4.4b: Effect of Ethanol Fruit Extract of *P. dactylifera* on Histometric Characteristics of Pyramidal Cells in the CA1 and CA3 Hippocampal Regions of Wistar Rats

Group/ treatment	CA1 pyramidal cell area (*μm*)	CA1 pyramidal cell perimeter (*μm*)	CA3 pyramidal cell area (*μm*)	CA3 pyramidal cell perimeter (*μm*)
Control (distilled H_2O 0.5 ml/kg)	407.46 ± 36.01	21.42 ± 0.75	649.00 ± 36.25	28.57 ± 0.83
$HgCl_2$ (5 mg/kg)	353.88 ± 31.50	20.95 ± 1.22	543.09 ± 63.22	25.45 ± 1.15
Vit C (100 mg/kg) **+ $HgCl_2$** (5 mg/kg)	501.03 ± 31.56	22.39 ± 0.83	624.69 ± 33.60	26.75 ± 1.08
EFPD (250 mg/kg) **+ $HgCl_2$** (5 mg/kg)	425.86 ± 43.40	20.50 ± 1.07	655.46 ± 48.07	26.81 ± 1.72
EFPD (500 mg/kg) **+ $HgCl_2$** (5 mg/kg)	592.71 ± 41.40 [b]***	24.29 ± 1.05 [a]*	631.21 ± 79.44	28.99 ± 1.85*
EFPD (1000 mg/kg) **+ $HgCl_2$** (5 mg/kg)	445.83 ± 39.43	21.39 ± 0.84	666.60 ± 66.99	29.54 ± 0.81*

n = 8; mean ± SEM; One way ANOVA *LSD post hoc test* **a/*=***p<0.05;* **b/**=***p<0.01;* **c/***=***p<0.001** *when compared with the control/ $HgCl_2$.* **EFPD=** *Ethanol fruit extract of Phoenix dactylifera;* **Vit C**= *Vitamin C;* **CA=** *Cornu Amonis*

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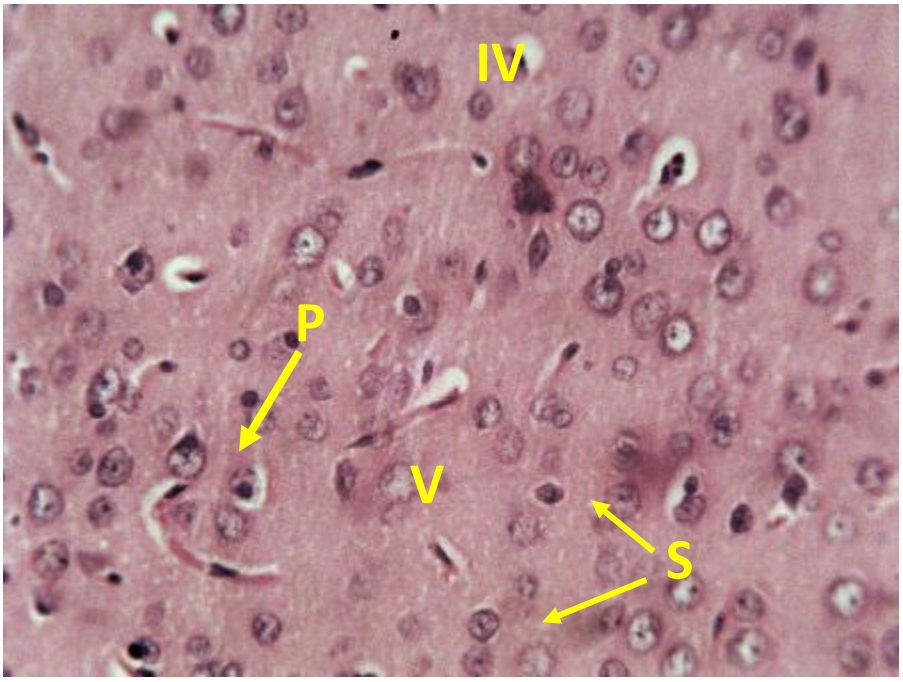


Plate 4.1: Section of Wistar rat cerebral cortex (layer V) of the control (untreated) group showing normal histology of the cerebral cortex: Pyramidal cell (P); Stellate cell (S); Layer V (V); Layer IV (IV) H and E stain (Mag x 250).

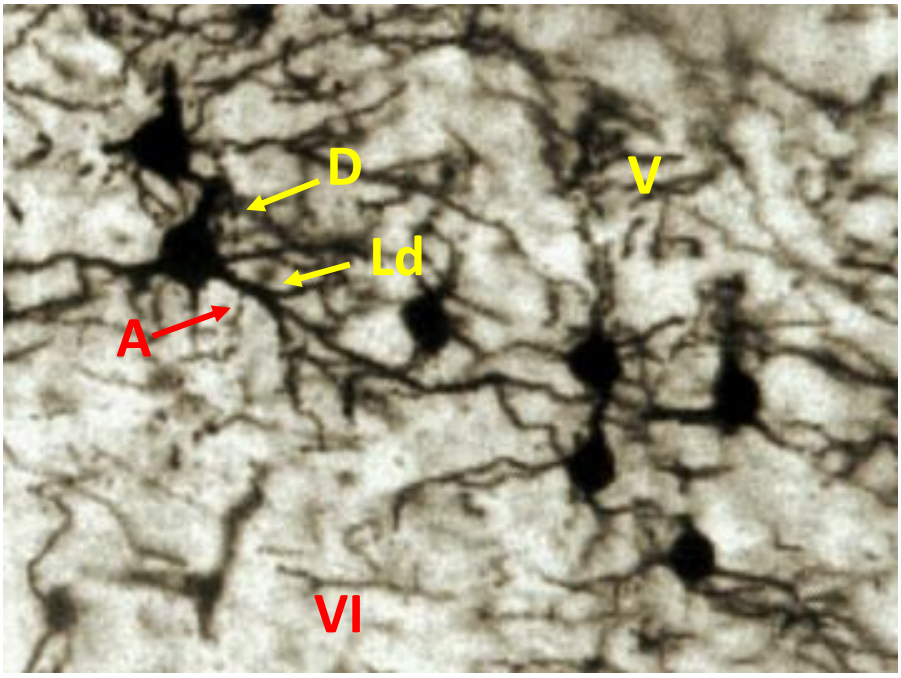


Plate 4.2: Section of Wistar rat cerebral cortex (layer V) of the control (untreated) group showing normal histology of the cerebral cortex: Axon (A); Dendrite (D); Lateral dendrite (Ld); Pyramidal cell (P); Stellate cell (S); Layer V (V); Layer IV (IV) Golgi stain (Mag x 250).

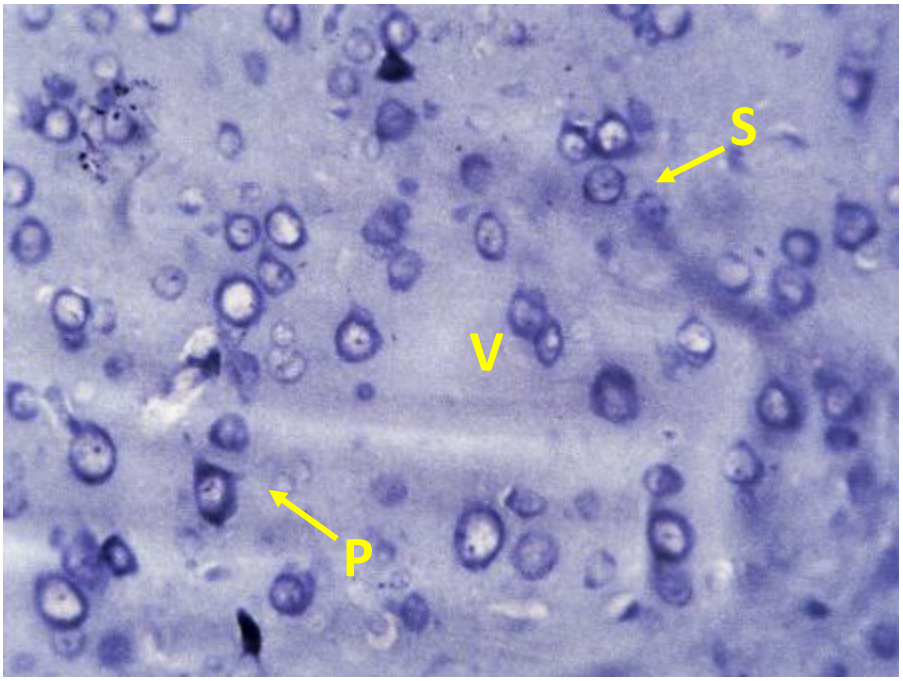


Plate 4.3: Section of Wistar rat cerebral cortex (layer V) of the control (untreated) group showing normal histology of the cerebral cortex: Pyramidal cell (P); Stellate cell (S); Layer V (V) CFV stain (Mag x 250).

4.4.1.2 Histological and Histochemical Features of the Cerebral Cortex of Treated Wistar Rats

The cerebral sections of Wistar rats treated with mercury revealed distortion in the histoarchitecture of the cerebral cortex (*layer V*); neuronal degenerative changes, such as neuronal shrinkage and perineuronal vacuolations, necrosis and chromatolysis. Histochemical (Golgi) staining revealed loss of pyramidal neurone processes and CFV staining revealed necrosis and chromatolysis (**Plates 4.4- 4.6**).

Histological and histochemical (Golgi- and CFV- stained) examinations of the cerebral sections of Wistar rats treated with vitamin C and extracts (AFPD and EFPD) followed by the administration of mercury revealed mild distortion of the histoarchitecture of the cerebral cortex (*layer V*). The histological features of the vitamin C- treated group were comparable with the control (**Plates 4.7- 4.9**).

In category 'A' study, histological features of the AFPD (250 mg/kg, 500 mg/kg and 1,000 mg/kg) - treated groups showed mild neurodegenerative changes, such as chromatolysis, pyknotic nuclei and satellitosis/ gliosis. Histoarchitectural preservation conferred by the administration of AFPD was dose dependent, except for 1,000 mg/kg AFPD - treated group compared to the control (**Plates 4.10- 4.17**).

In category 'B' study, histological features of the EFPD (250 mg/kg, 500 mg/kg and 1,000 mg/kg) - treated groups showed mild neurodegenerative changes, such as nuclear pkynosis, necrosis, satellitosis/ gliosis and indistinct neurones due to reduced staining intensity compared to the control. Treatment with EFPD conferred dose dependent histoarchi-

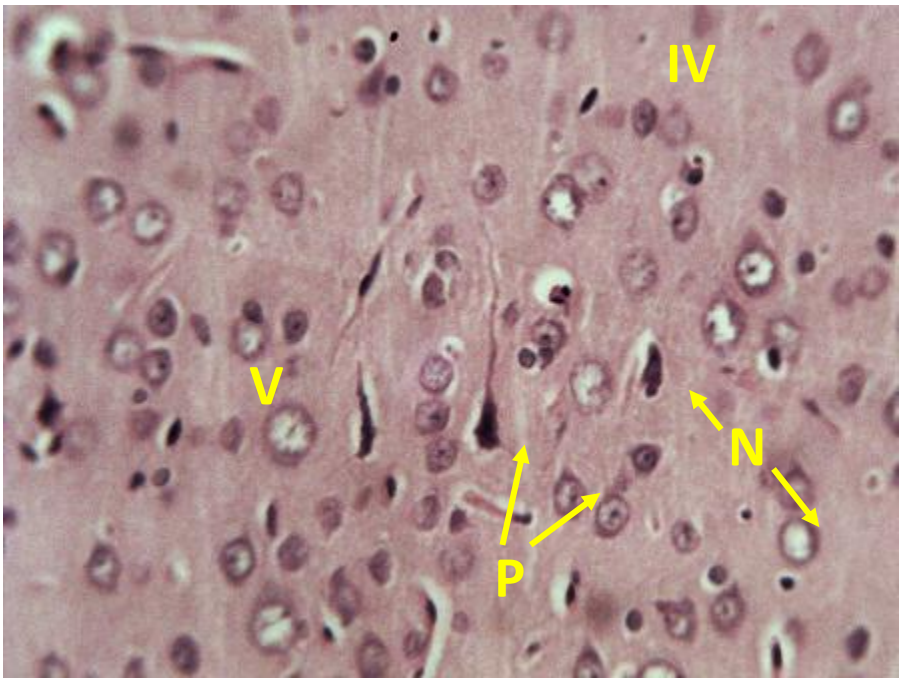


Plate 4.4: Section of Wistar rat cerebral cortex (layer V) of the group administered mercuric chloride (5 mg/kg) showing distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *necrosis and chromatolysis* **(N); Layer V (V); Layer IV (IV) H and E stain (Mag x 250).**

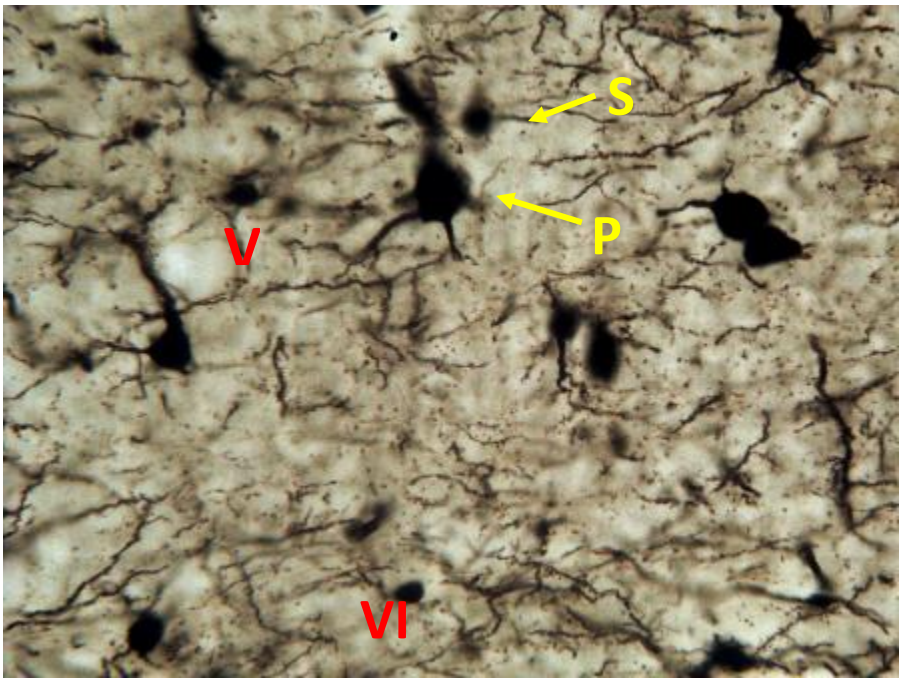


Plate 4.5: Section of Wistar rat cerebral cortex (layer V) of the group administered mercuric chloride (5 mg/kg) showing distortion in the histology of the cerebral cortex. Stellate cell (S); Pyramidal cell (P); Neuronal degeneration – *neuronal process loss* **(N); Layer V (V); Layer VI (VI) Golgi stain (Mag x 250).**

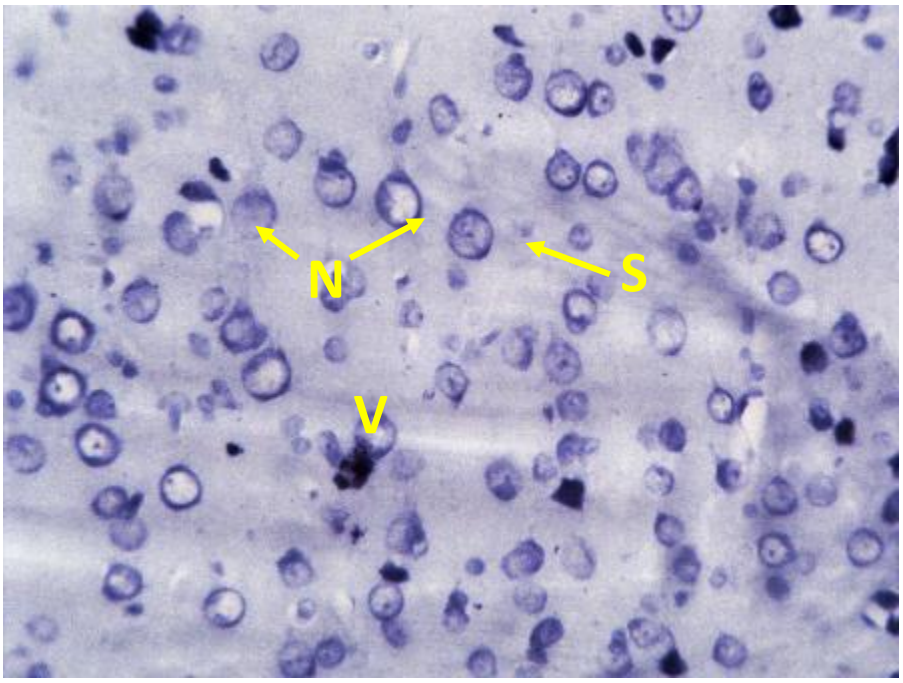


Plate 4.6: Section of Wistar rat cerebral cortex (layer V) of the group administered mercuric chloride (5 mg/kg) showing distortion in the histology of the cerebral cortex. Stellate cell (S); Pyramidal cell (P); Neuronal degeneration – *chromatolysis and pyknosis* **(N); Layer V (V) CFV stain (Mag x 250).**

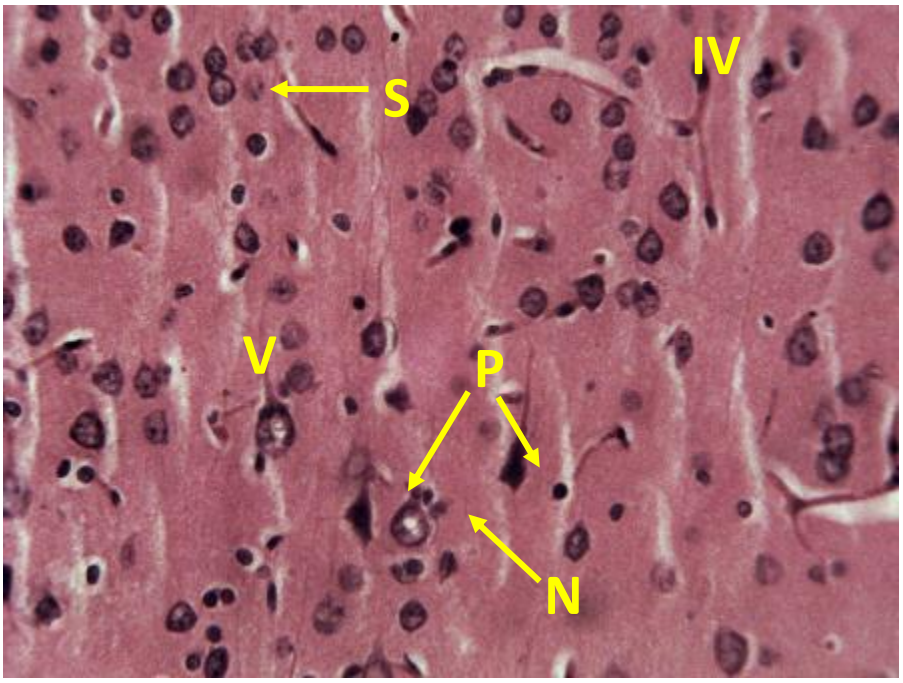


Plate 4.7: Section of Wistar rat cerebral cortex (layer V) of the group administered vitamin C (100 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Stellate cell (S); Pyramidal cell (P); Neuronal degeneration - ***satellitosis*** **(N); Layer V (V); Layer IV (IV) H and E stain (Mag x 250).**

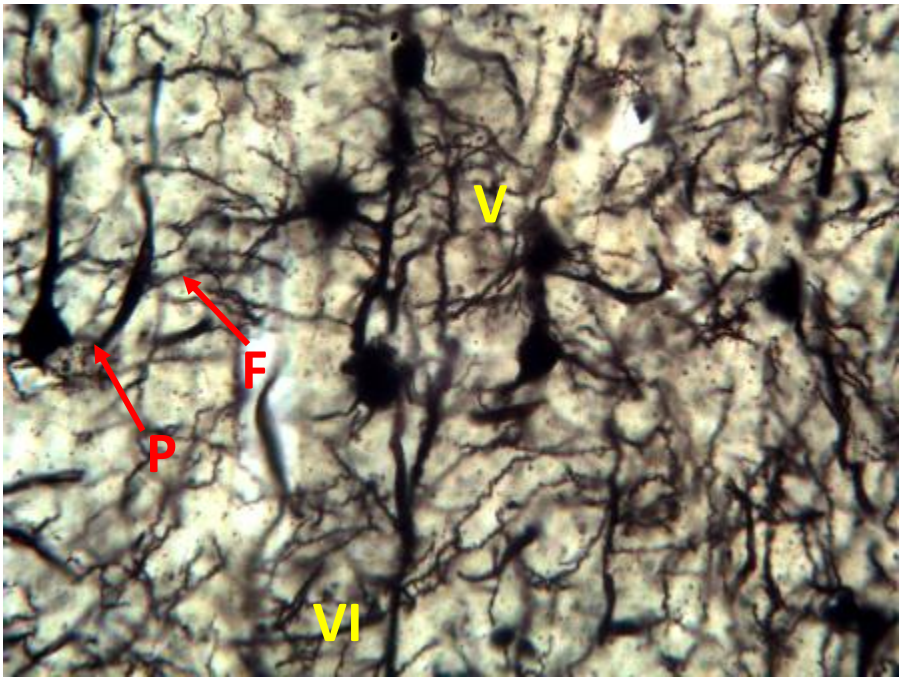


Plate 4.8: Section of Wistar rat cerebral cortex (layer V) of the group administered vitamin C (100 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Fusiform cell (F); Pyramidal cell (P); Layer V (V); Layer VI (VI) Golgi stain (Mag x 250).

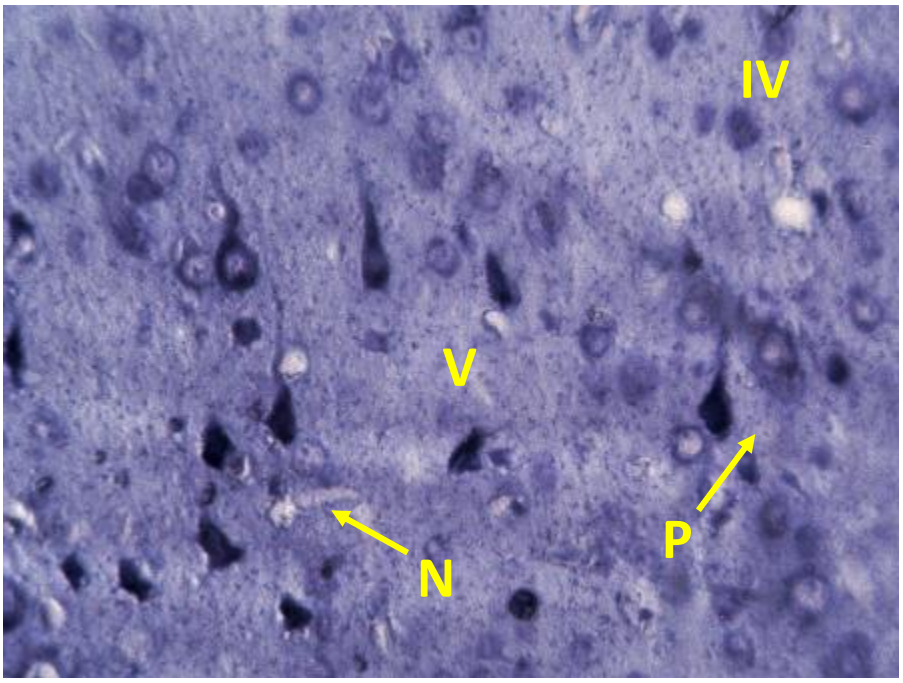


Plate 4.9: Section of Wistar rat cerebral cortex (layer V) of the group administered vitamin C (100 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *pyknosis* (N); Layer V (V); Layer IV (IV) CFV stain (Mag x 250).

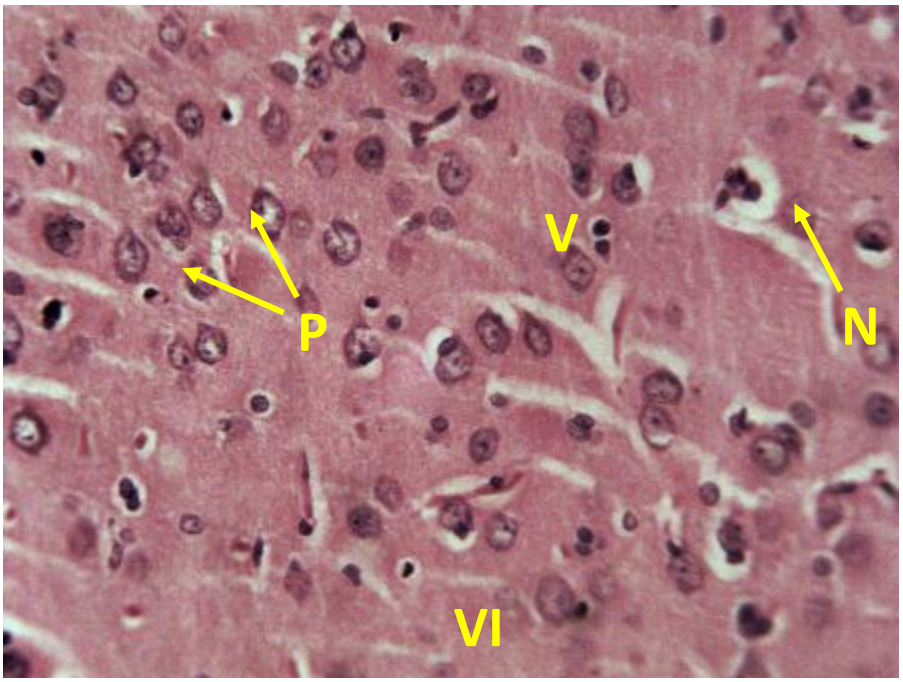


Plate 4.10: Section of Wistar rat cerebral cortex (layer V) of the group administered aqueous fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *gliosis/ satellitosis* **(N); Layer V (V); Layer IV (IV) H and E stain (Mag x 250).**

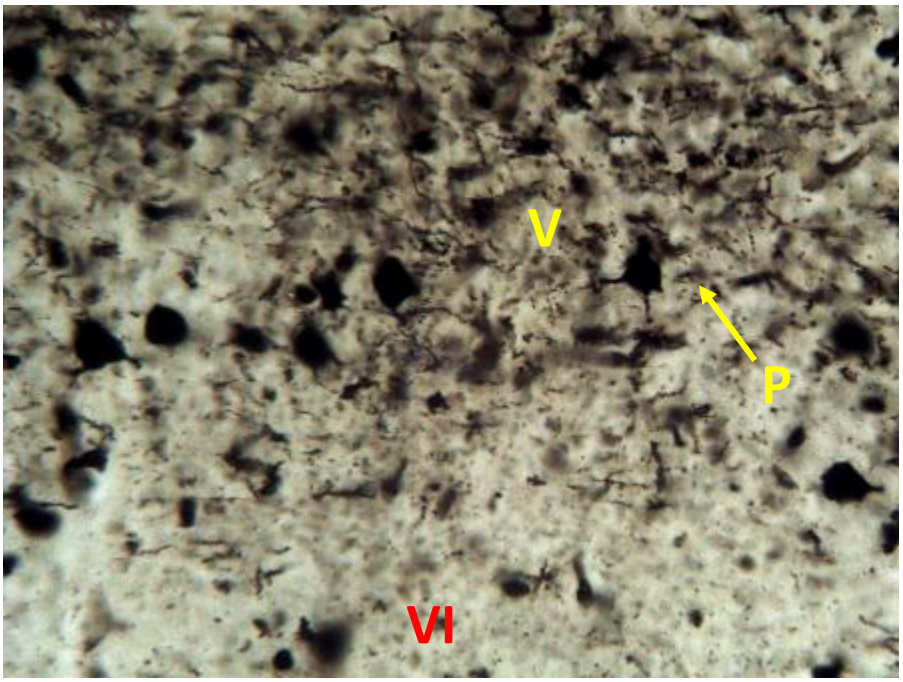


Plate 4.11: Section of Wistar rat cerebral cortex (layer V) of the group administered aqueous fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration– *neuronal process loss*; **Layer V (V); Layer VI (VI) Golgi stain (Mag x 250).**

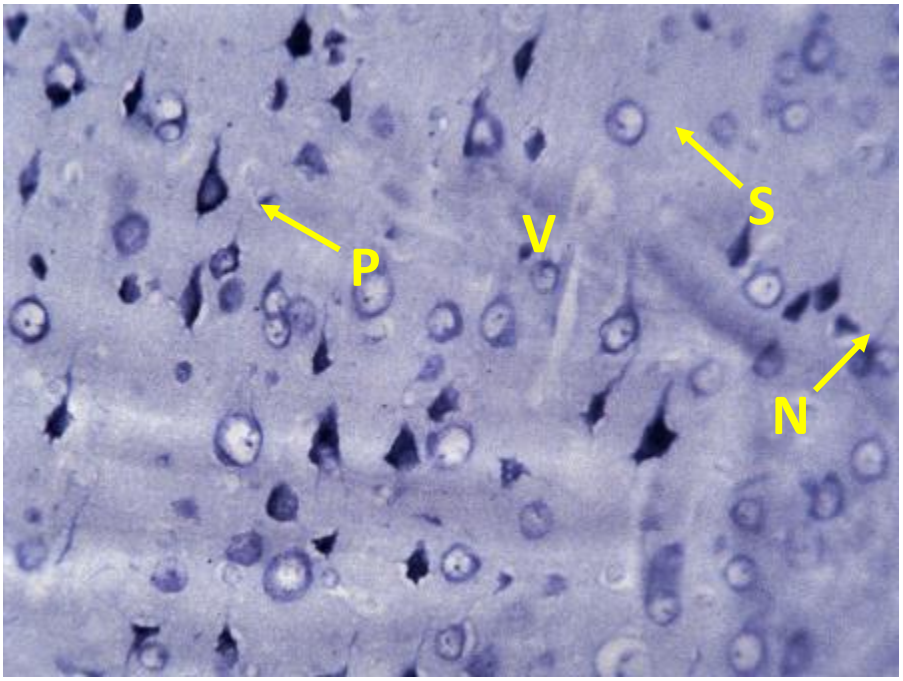


Plate 4.12: Section of Wistar rat cerebral cortex (layer V) of the group administered aqueous fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Stellate cell (S); Pyramidal cell (P); Neuronal degeneration - *pyynosis* **(N); Layer V (V); Layer IV (IV) CFV stain (Mag x 250).**

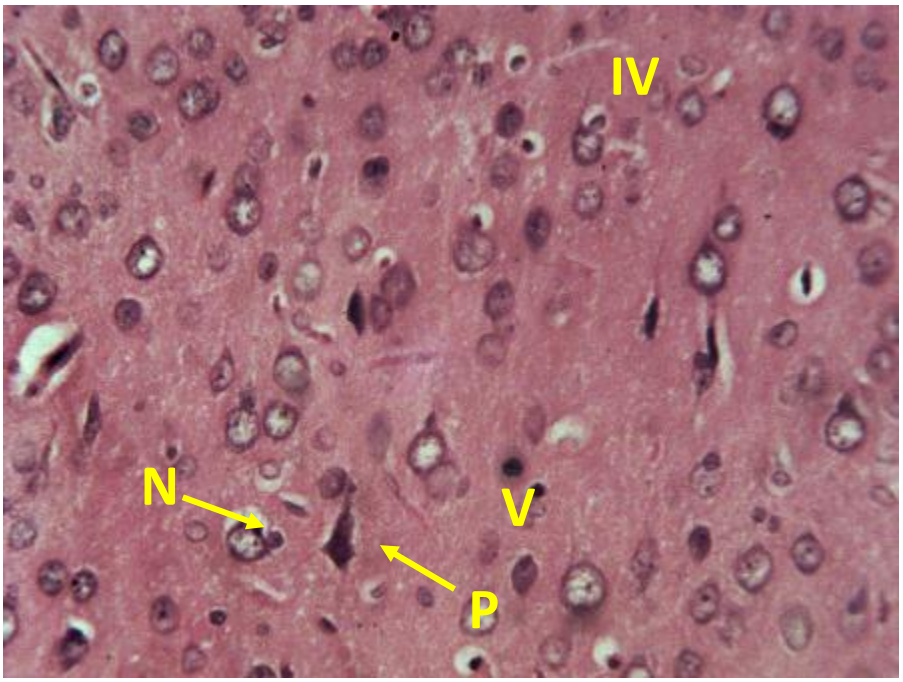


Plate 4.13: Section of Wistar rat cerebral cortex (layer V) of the group administered aqueous fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *pyknosis* **(N); Layer V (V); Layer IV (IV) H and E stain (Mag x 250).**

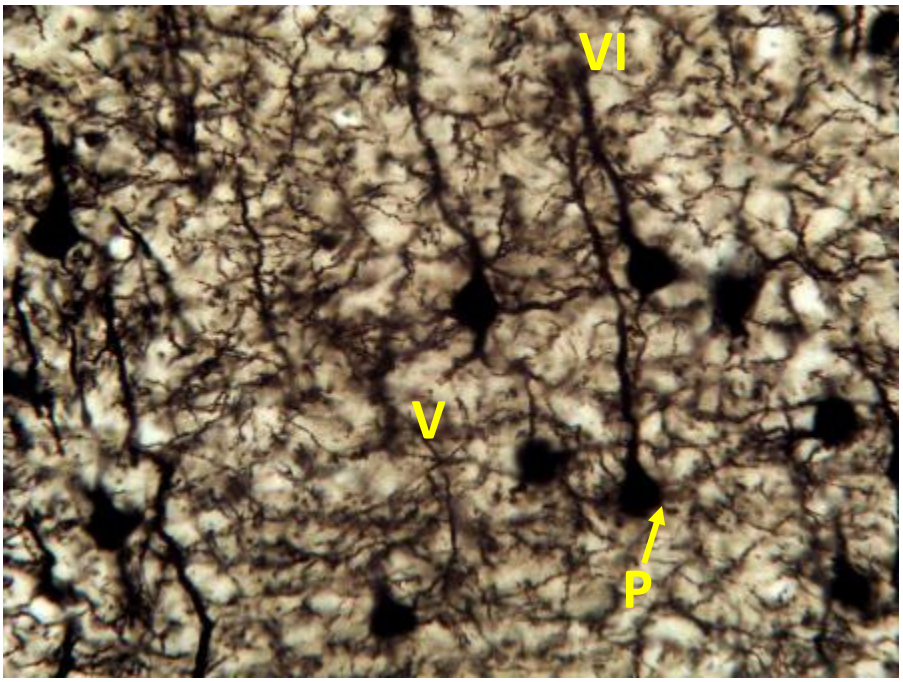


Plate 4.14: Section of Wistar rat cerebral cortex (layer V) of the group administered aqueous fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Layer V (V); Layer IV (IV) Golgi stain (Mag x 250).

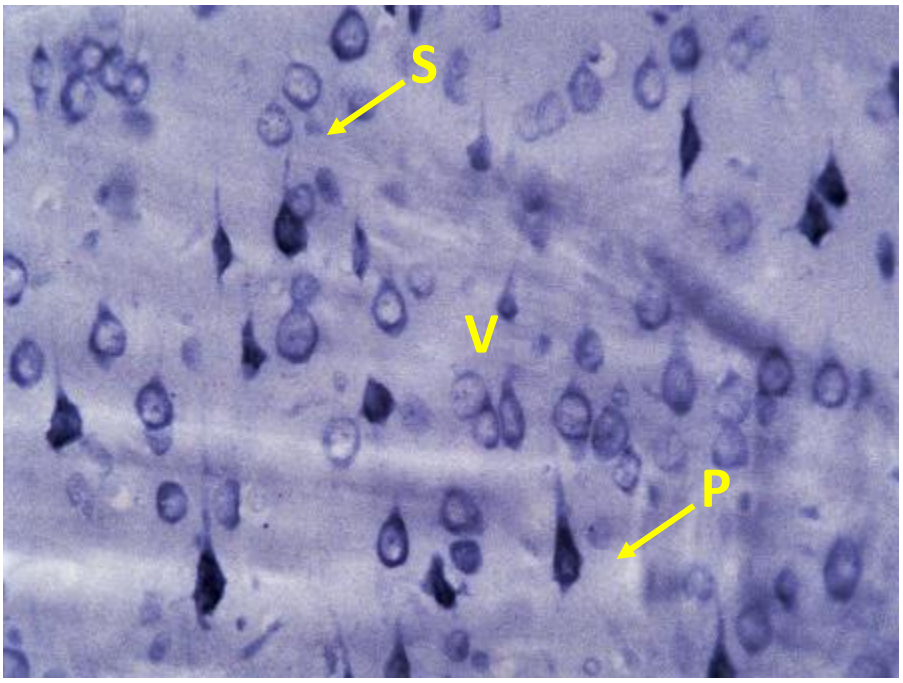


Plate 4.15: Section of Wistar rat cerebral cortex (layer V) of the group administered aqueous fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Stellate cell (S); Pyramidal cell (P); Layer V (V) CFV stain (Mag x 250).

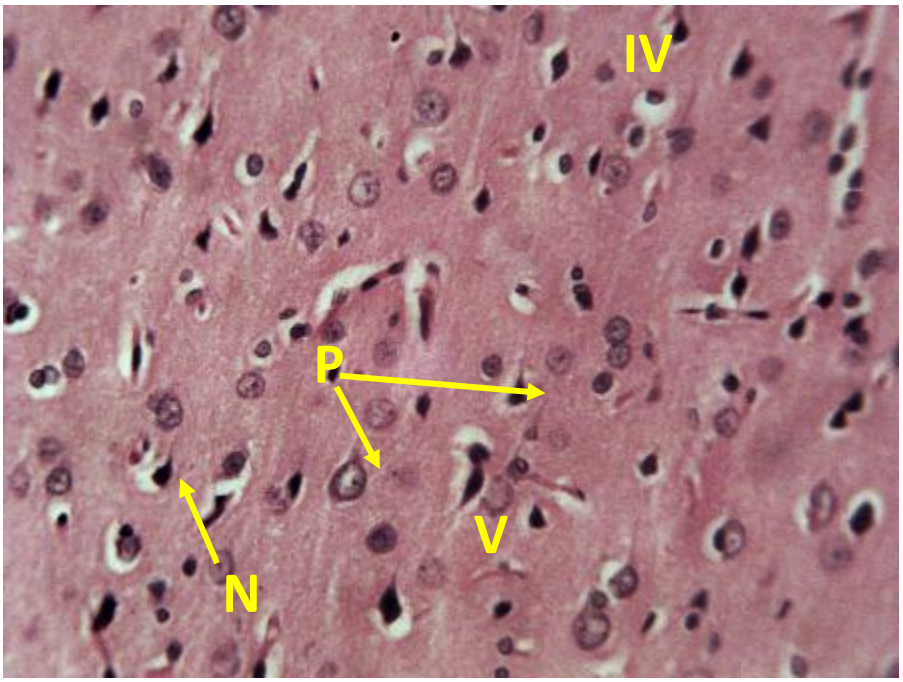


Plate 4.16: Section of Wistar rat cerebral cortex (layer V) of the group administered aqueous fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *pyknosis and gliosis* **(N); Layer V (V); Layer IV (IV) H and E stain (Mag x 250).**

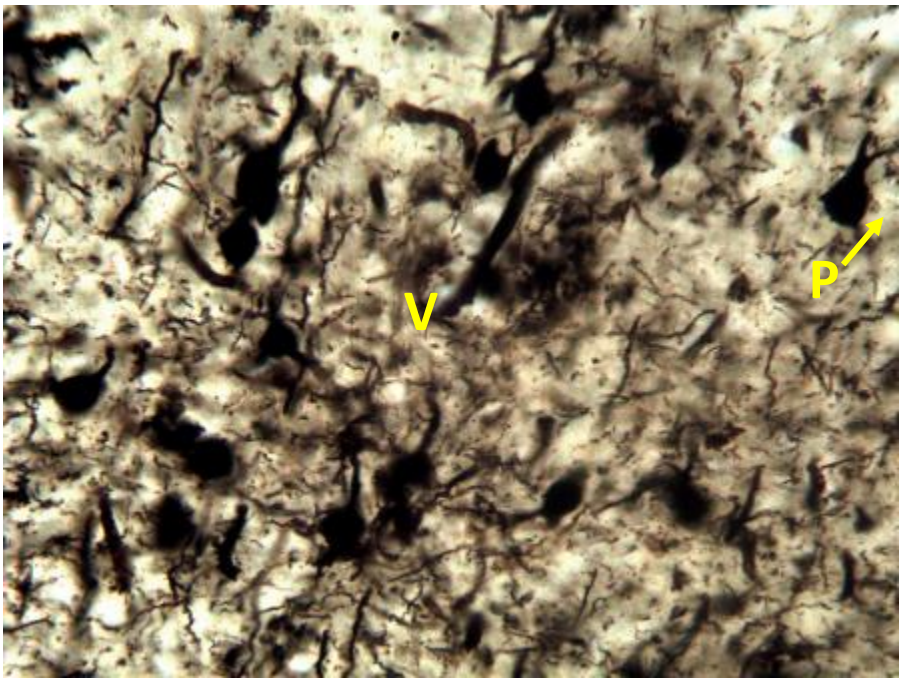


Plate 4.17: Section of Wistar rat cerebral cortex (layer V) of the group administered aqueous fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *lose of neural processes* **(N); Layer V (V) Golgi stain (Mag x 250).**

tectural preservation, except for 1,000 mg/kg EFPD - treated group compared to the control (**Plates 4.18- 4.27**).

4.4.2 Histological and Histochemical Features of the Cerebellar Cortex

4.4.2.1 Histological and Histochemical Features of the Cerebellar Cortex of Control (Untreated) Wistar Rat

The cerebellar sections of Wistar rats in the control group showed normal histoarchitecture of the cerebellar cortex; the characteristic appearance of the three cortical layers: an *outer molecular layer* with distinct neurones and an *inner granular layer*. Sandwiched between these layers is a monolayer of flask-shaped Purkinje cells, the *Purkinje cell layer*. Histochemical (CFV) staining for Nissl substances revealed normal appearance of distinct intensely stained cortical neurones (**Plates 4.28 and 4.29**).

4.4.2.2 Histological and Histochemical Features of the Cerebellar Cortex of Treated Wistar Rats

The cerebellar sections of mercury-treated Wistar rats revealed histoarchitectural distortion of the cerebellar cortex; neurodegenerative changes, such as perineuronal vacuolations, neuronal cytoplasmic shrinkage and gliosis. Histochemical (CFV) staining revealed Pukinje cell and internal granule cell necrosis, and indistinct neurones due to reduced staining intensity (**Plates 4.30 and 4.31**).

Histological and histochemical (CFV stained) examinations of the cerebellar sections of vitamin C- and extracts (AFPD and EFPD)- treated Wistar rats, followed by mercury treatment, revealed mild cerebellar cortex histoarchitectural distortions compared to the severe distortions of the mercury-treated. The histological features of the vitamin C-

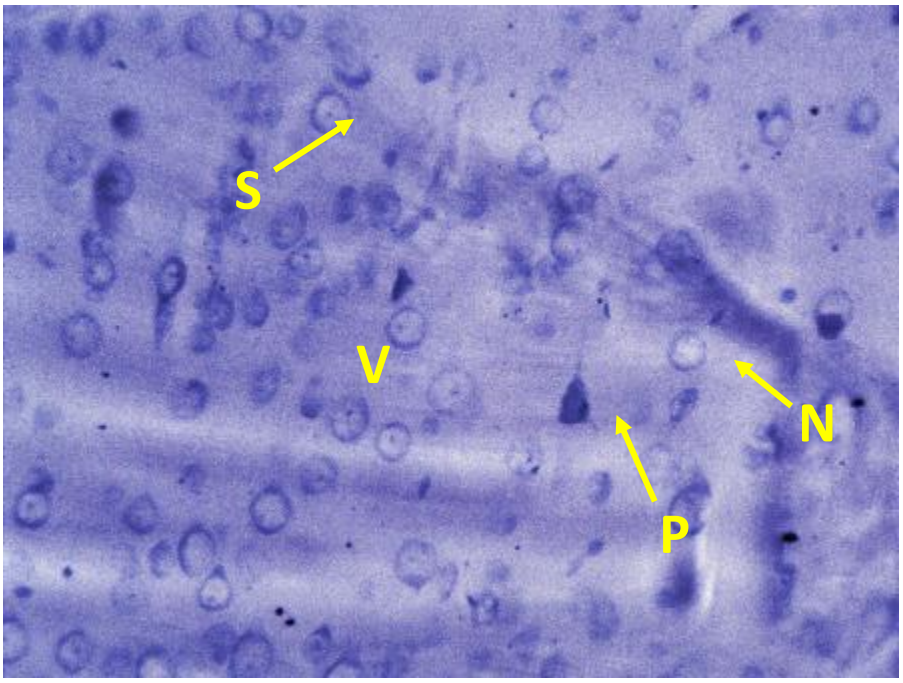


Plate 4.18: Section of Wistar rat cerebral cortex (layer V) of the group administered aqueous fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *chromatolysis* **(N); Layer V (V) CFV stain (Mag x 250).**

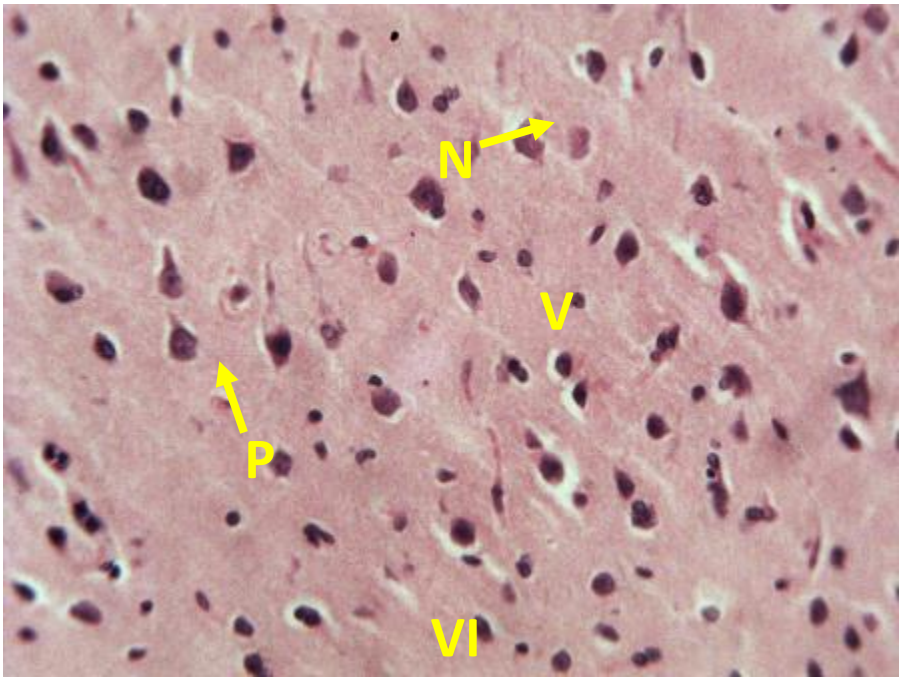


Plate 4.19: Section of Wistar rat cerebral cortex (layer V) of the group administered ethanol fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *pyknosis and satellitosis* **(N); Layer V (V); Layer VI (VI) H and E stain (Mag x 250).**

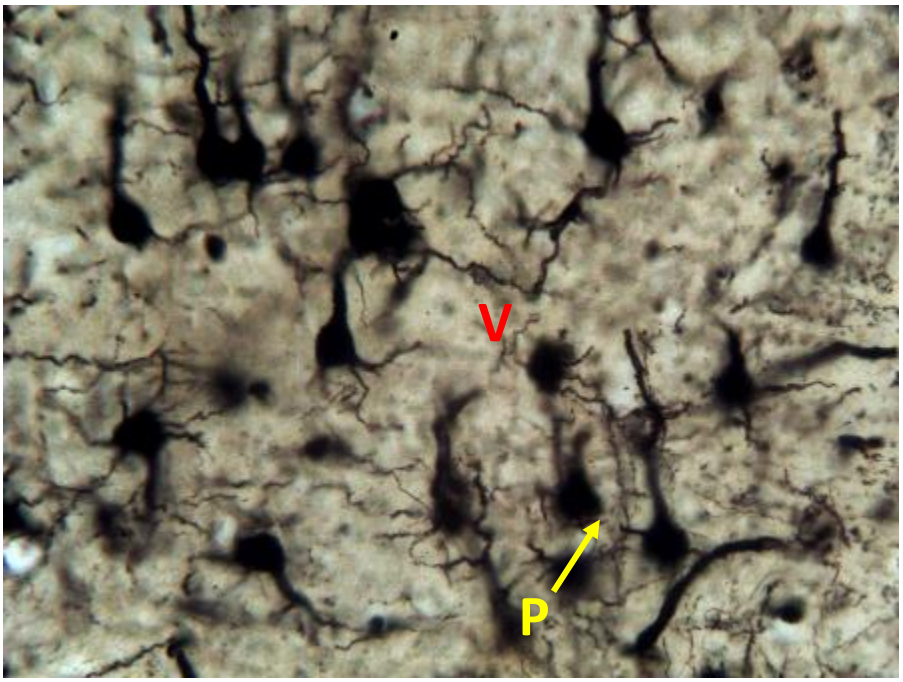


Plate 4.20: Section of Wistar rat cerebral cortex (layer V) of the group administered ethanol fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration– *neuronal process loss* **(N); Layer V (V) Golgi stain (Mag x 250).**

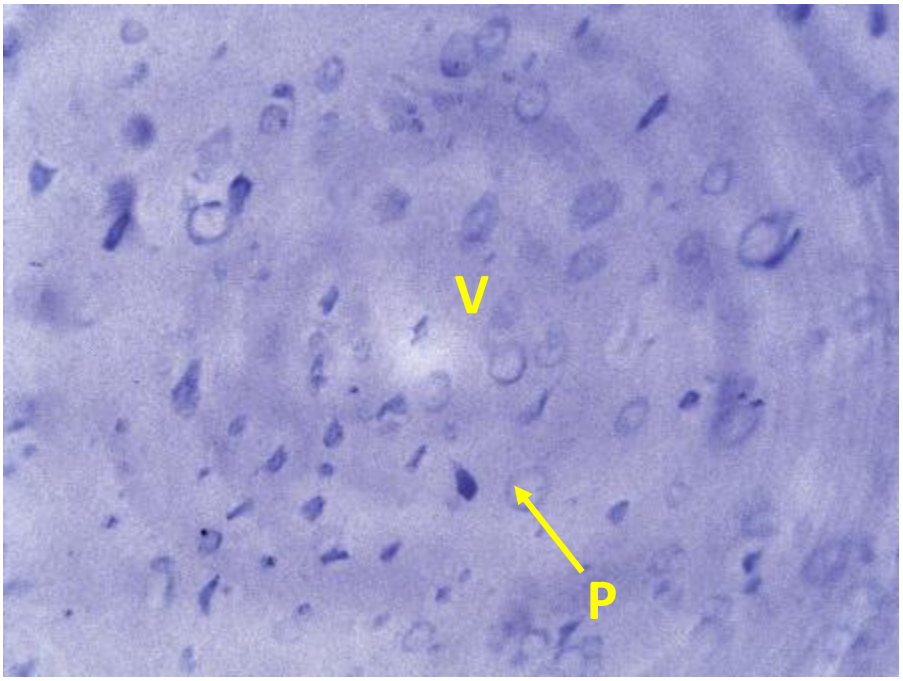


Plate 4.21: Section of Wistar rat cerebral cortex (layer V) of the group administered ethanol fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *reduced staining intensity* **(N); Layer V (V) CFV stain (Mag x 250).**

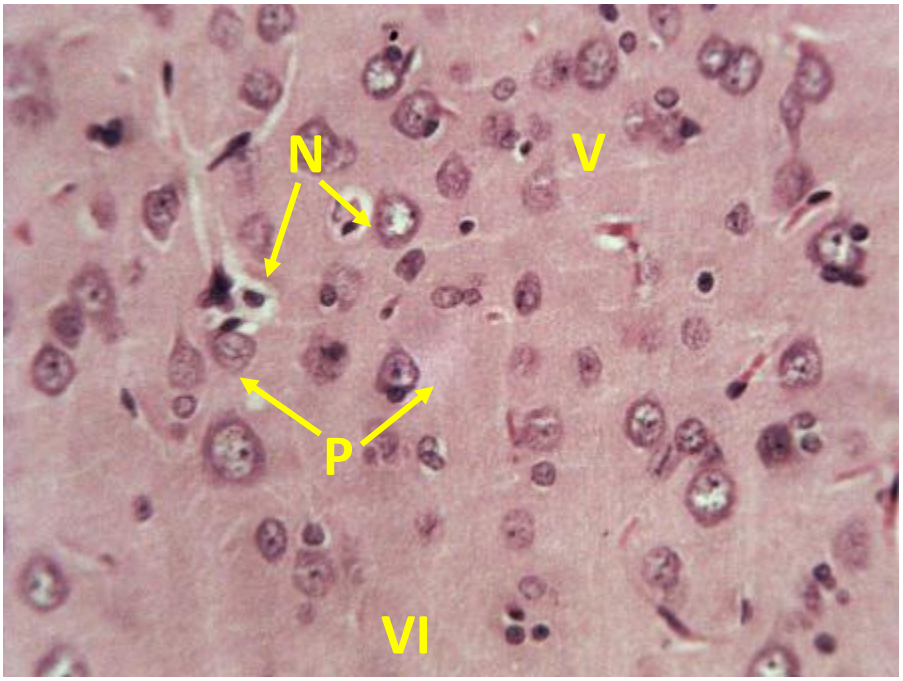


Plate 4.22: Section of Wistar rat cerebral cortex (layer V) of the group administered ethanol fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration – *pyknosis and gliosis* **(N); Layer V (V); Layer VI (VI) H and E stain (Mag x 250).**

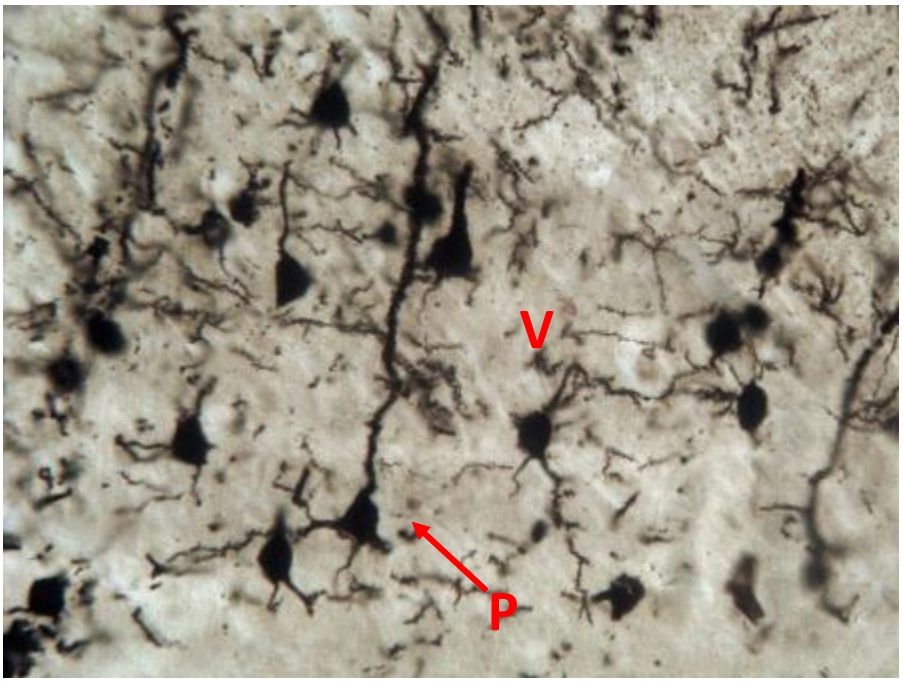


Plate 4.23: Section of Wistar rat cerebral cortex (layer V) of the group administered ethanol fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Layer V (V) Golgi stain (Mag x 250).

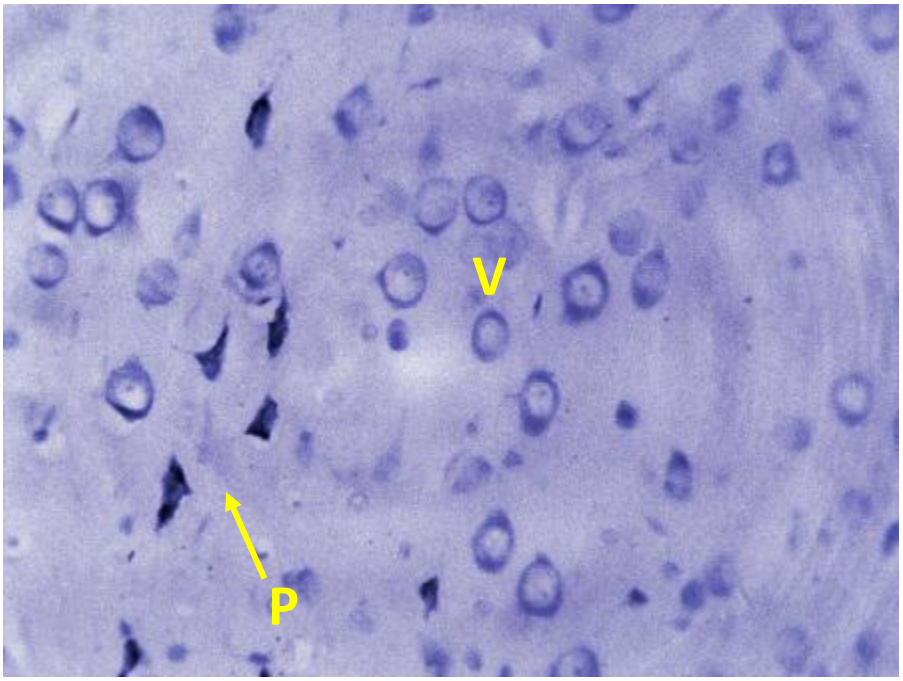


Plate 4.24: Section of Wistar rat cerebral cortex (layer V) of the group administered ethanol fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Layer V (V) CFV stain (Mag x 250).

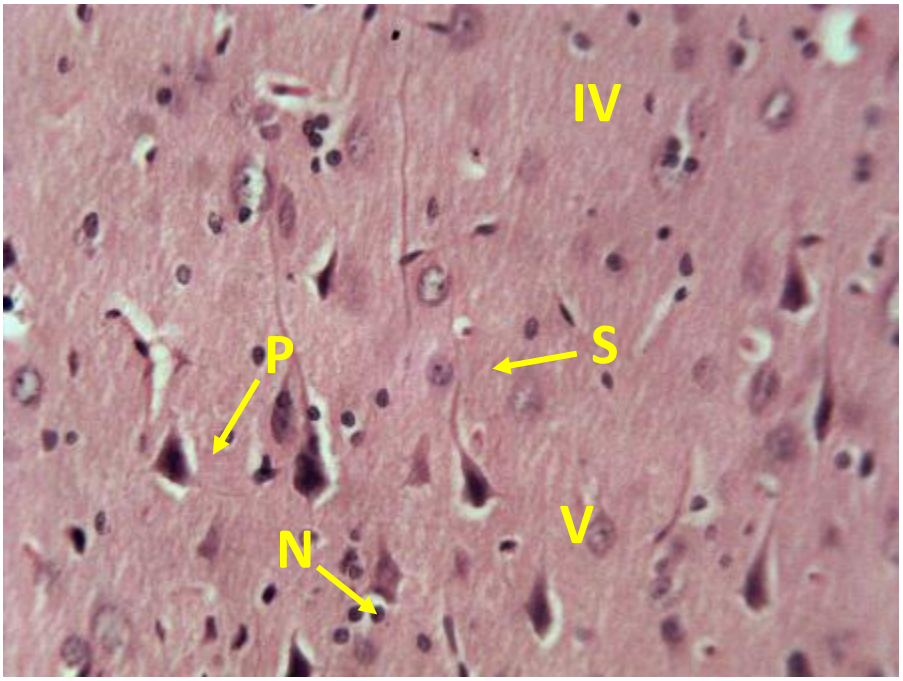


Plate 4.25: Section of Wistar rat cerebral cortex (layer V) of the group administered ethanol fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Stellate cell (S); Pyramidal cell (P); Neuronal degeneration – *satellitosis/ gliosis* (N); Layer V (V) H and E stain (Mag x 250).

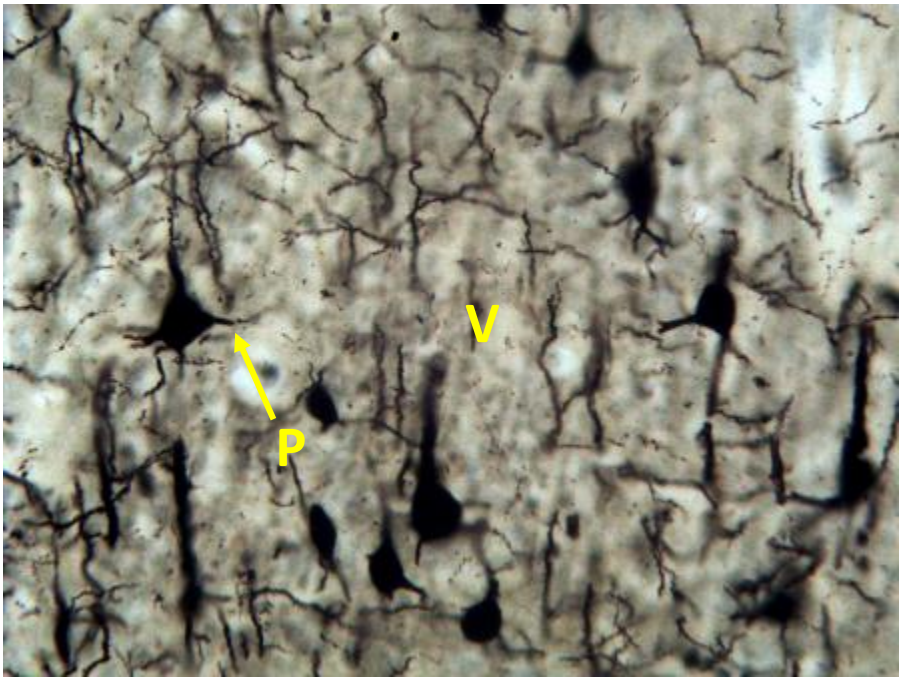


Plate 4.26: Section of Wistar rat cerebral cortex (layer V) of the group administered ethanol fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration– *loss of neuronal processes*; **Layer V (V) Golgi stain (Mag x 250).**

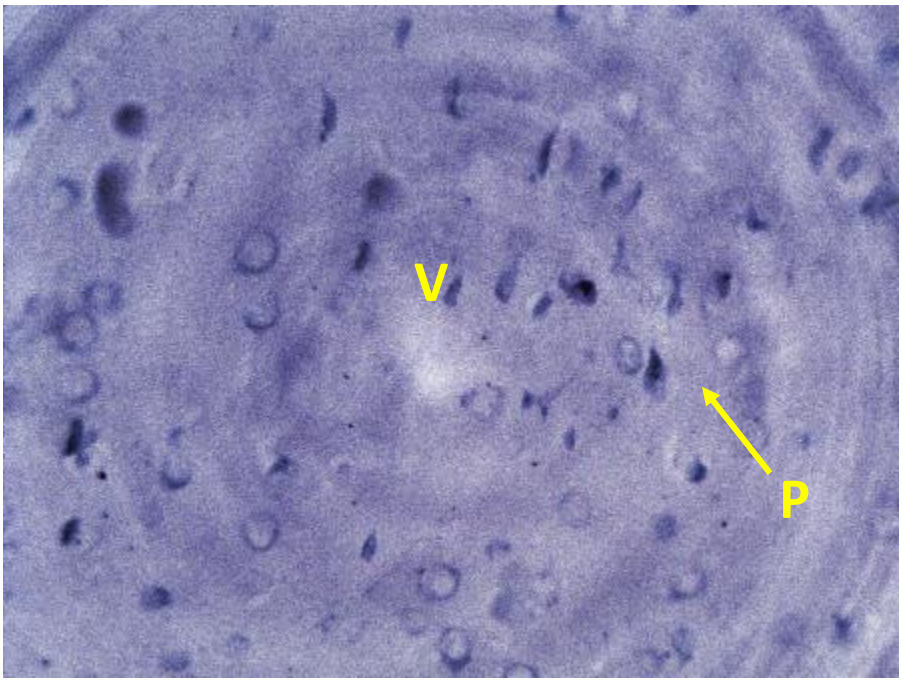


Plate 4.27: Section of Wistar rat cerebral cortex (layer V) of the group administered ethanol fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebral cortex. Stellate cell (S); Pyramidal cell (P); Neuronal degeneration – *reduced staining intensity***; Layer V (V) CFV stain (Mag x 250).**

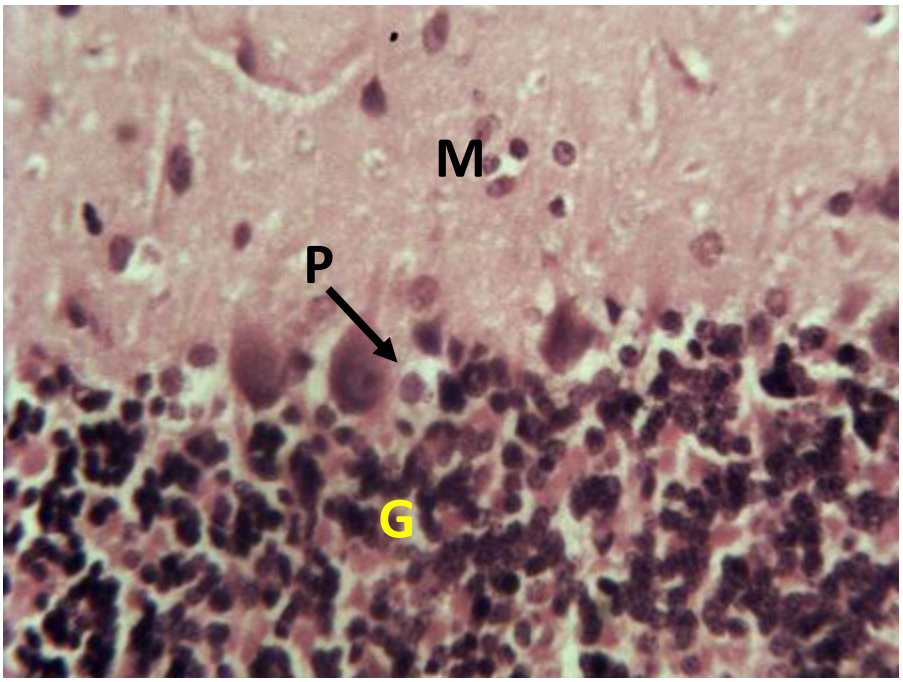


Plate 4.28: Section of Wistar rat cerebellar cortex of the control (untreated) group showing normal histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M) H and E stain (Mag x 400).



Plate 4.29: Section of Wistar rat cerebellar cortex of the control (untreated) group showing normal histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M) CFV stain (Mag x 400).

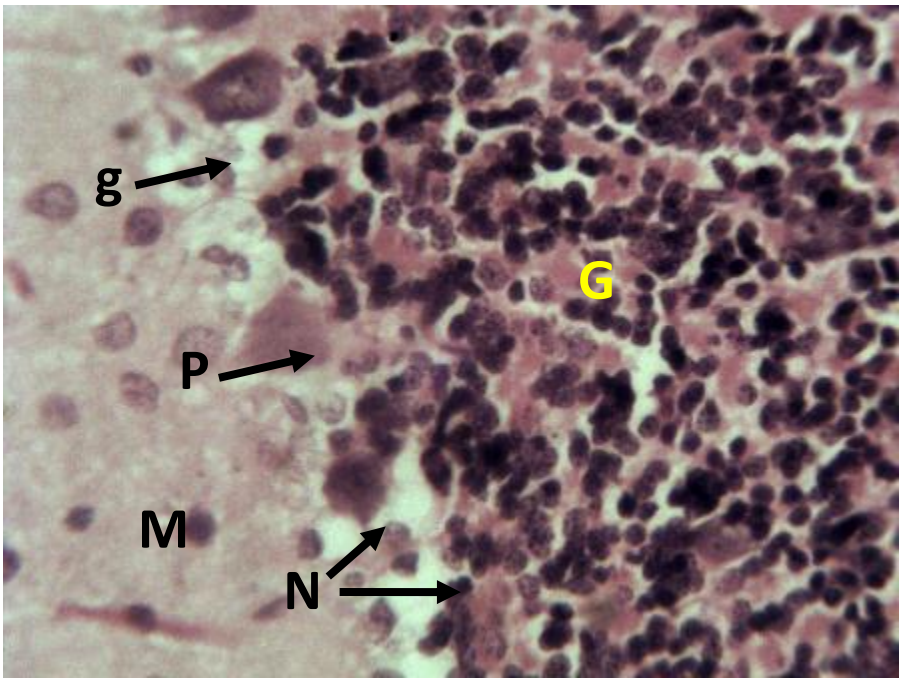


Plate 4.30: Section of Wistar rat cerebellar cortex of the group administered mercuric chloride (5 mg/kg) showing distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *perineuronal vacuolations* **(N); Gliosis (g) H and E stain (Mag x 400).**

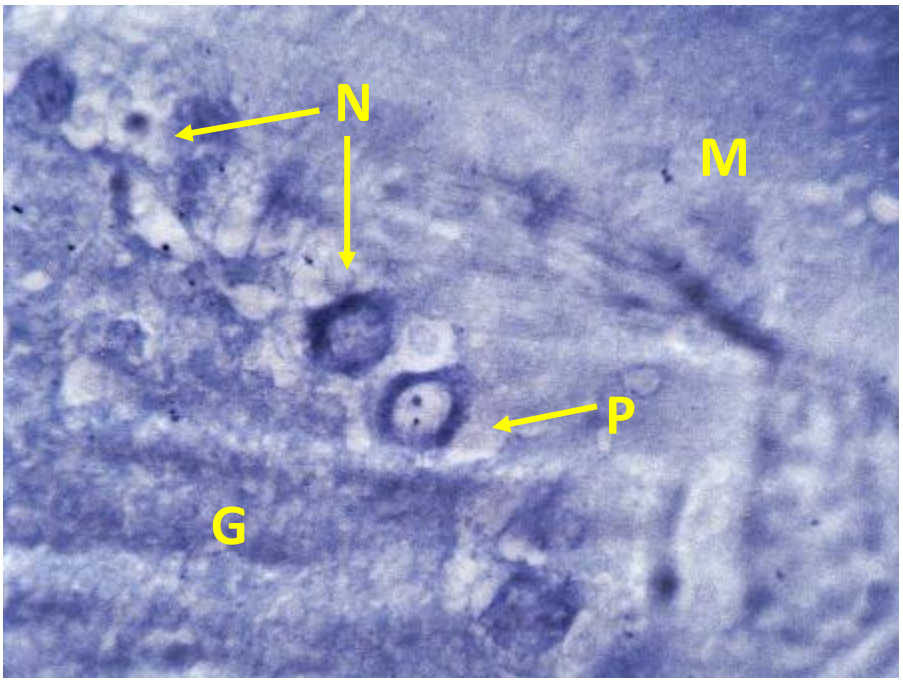


Plate 4.31: Section of Wistar rat cerebellar cortex of the group administered mercuric chloride (5 mg/kg) showing distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *perineuronal vacuolations and necrosis* **(N); Gliosis (g) CFV stain (Mag x 400).**

treated group showed perineuronal vacuolations, satellitosis and internal granule cell necrosis (**Plates 4.32 and 4.33**).

Histological features of the AFPD (250 mg/kg, 500 mg/kg and 1,000 mg/kg) - treated groups (category 'A' study) revealed mild neurodegenerative changes, such as perineuronal vacuolations, satellitosis, outer molecular cell and internal granule cell necrosis. AFPD administration conferred preservation of the histoarchitecture dose-dependently compared to the control (**Plates 4.34- 4.39**).

In category 'B' study, histological features of the EFPD (250 mg/kg, 500 mg/kg and 1,000 mg/kg) - treated groups showed mild neurodegenerative changes; perineuronal vacuolations, satellitosis, outer molecular cell and internal granule cell necrosis. Treatment with EFPD conferred dose dependent histoarchitectural preservation, except for 1,000 mg/kg EFPD - treated group compared to the control (**Plates 4.40- 4.45**).

4.4.3 Histological and Histochemical Features of the Hippocampus

The Wistar rat hippocampus (*hippocampus proprius*) is subdivided into four regions (CA1 - CA4, CA standing for *cornu ammonis*) according to density, size and branching of axons and dentrites of the pyramidal cells. Each of these regions consists of three layers: *stratum molecular* (molecular layer), *stratum pyramidale* (pyramidal layer) which contains bodies of the pyramidal cells *and stratum multiforme* (multiform layer). The continuation of CA3 in the concavity of dendate gyrus (*fascia dentata*) is the CA4. CA1 is characterized by densely packed medium sized cells; CA3 is the region with large less densely packed cells, while CA2 is a transition field between CA3 and CA1.

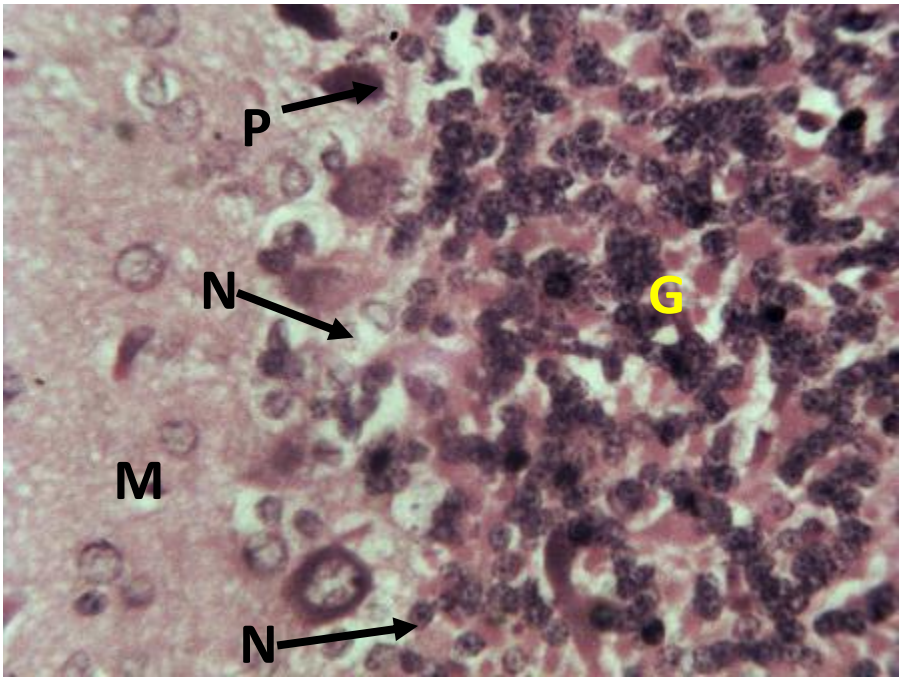


Plate 4.32: Section of Wistar rat cerebellar cortex of the group administered vitamin C (100 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *perineuronal vacuolations, satellitosis and necrosis* **(N) H and E stain (Mag x 400).**

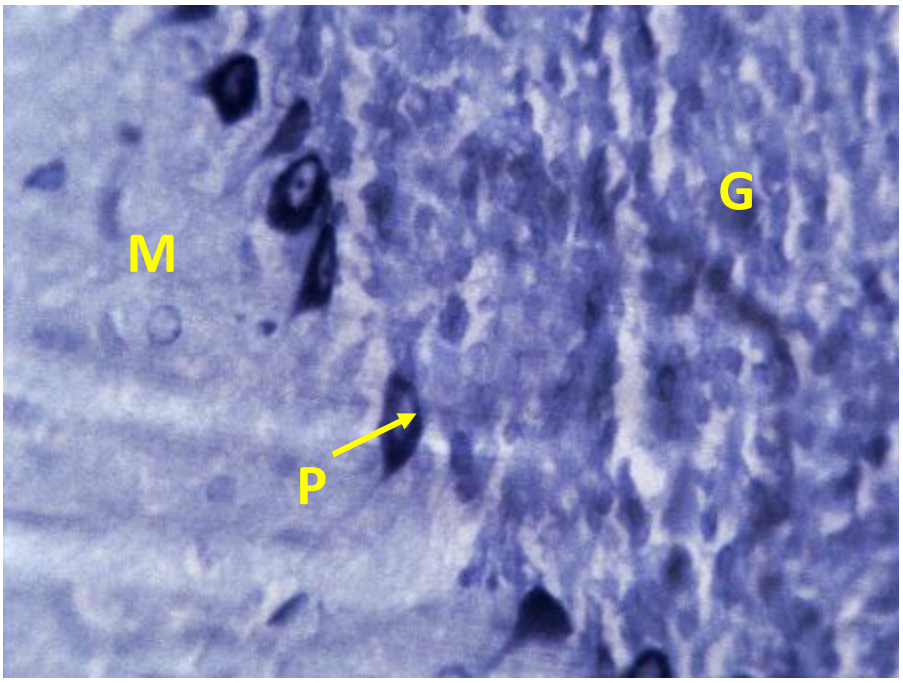


Plate 4.33: Section of Wistar rat cerebellar cortex of the group administered vitamin C (100 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration — *internal granule cell* **(N) CFV stain (Mag x 400).**

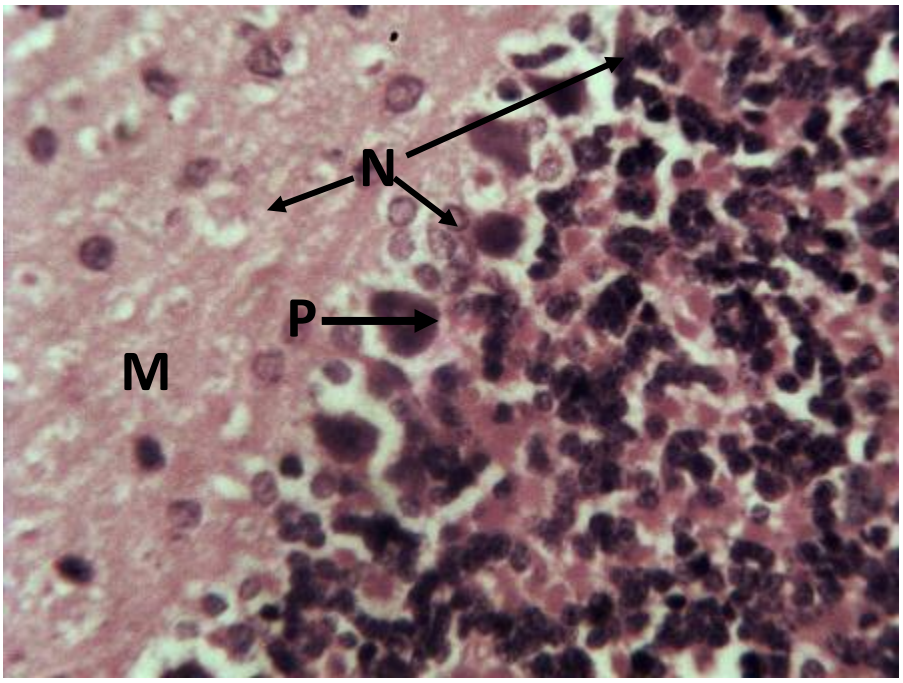


Plate 4.34: Section of Wistar rat cerebellar cortex of the group administered aqueous fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *perineuronal vacuolations, satellitosis and necrosis* **(N) H and E stain (Mag x 400).**

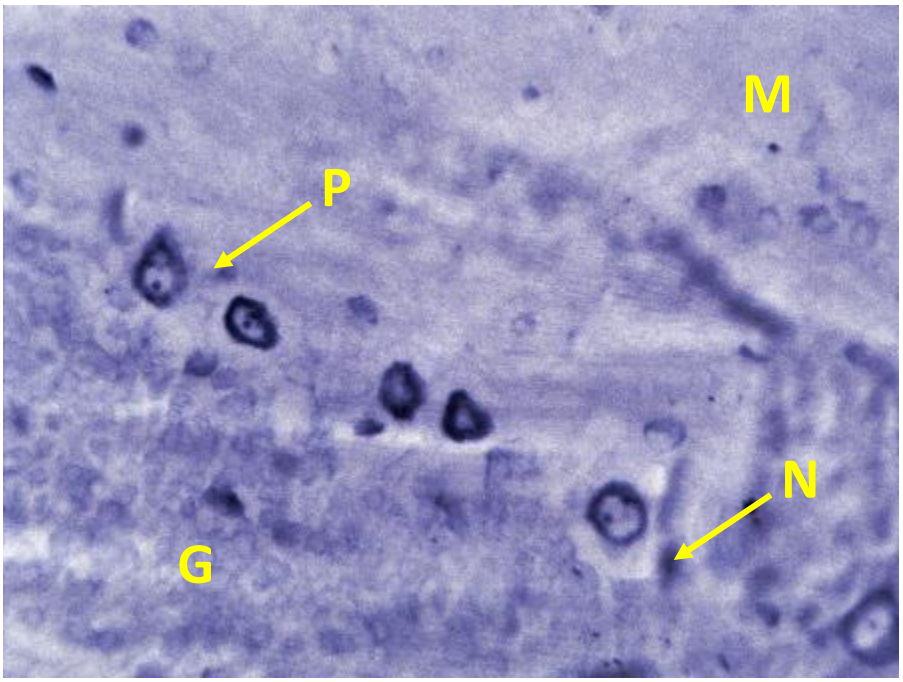


Plate 4.35: Section of Wistar rat cerebellar cortex of the group administered aqueous fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *perineuronal vacuolations* **(N) CFV stain (Mag x 400).**

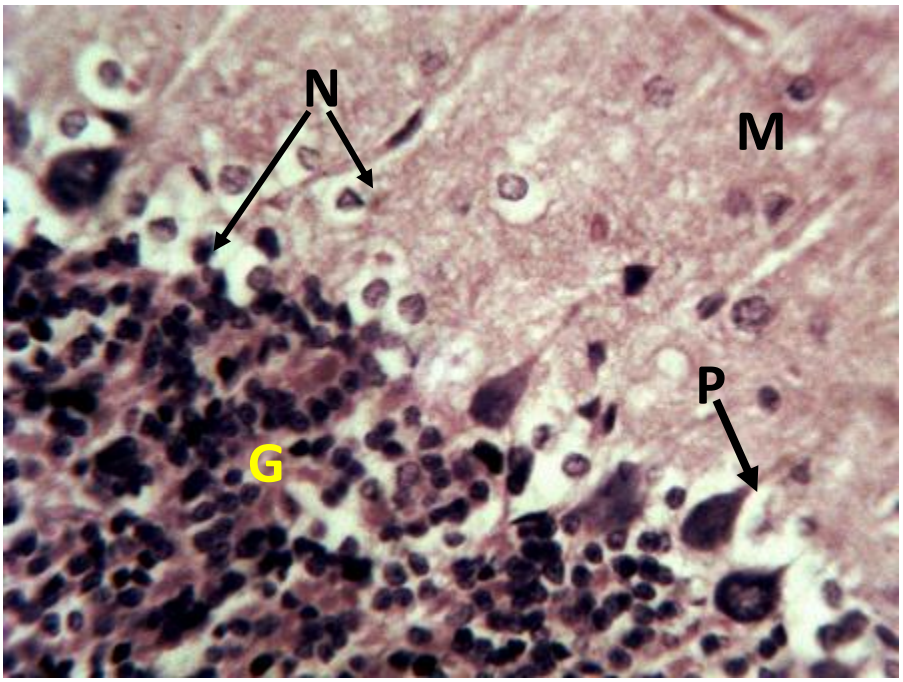


Plate 4.36: Section of Wistar rat cerebellar cortex of the group administered aqueous fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *perineuronal vacuolations and necrosis* **(N) H and E stain (Mag x 400).**

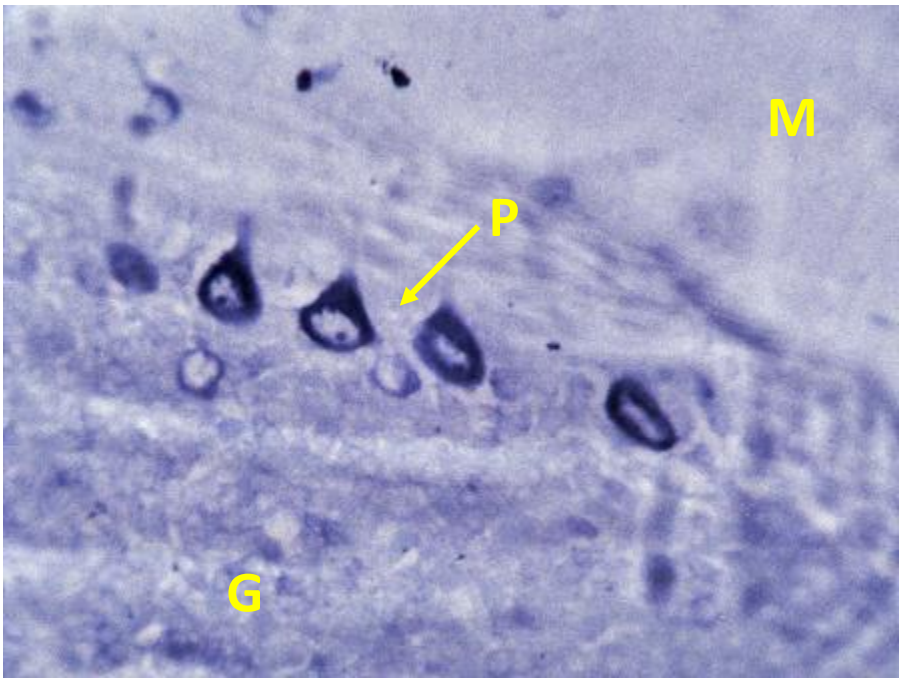


Plate 4.37: Section of Wistar rat cerebellar cortex of the group administered aqueous fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration– *internal granule cell* **(N) CFV stain (Mag x 400).**

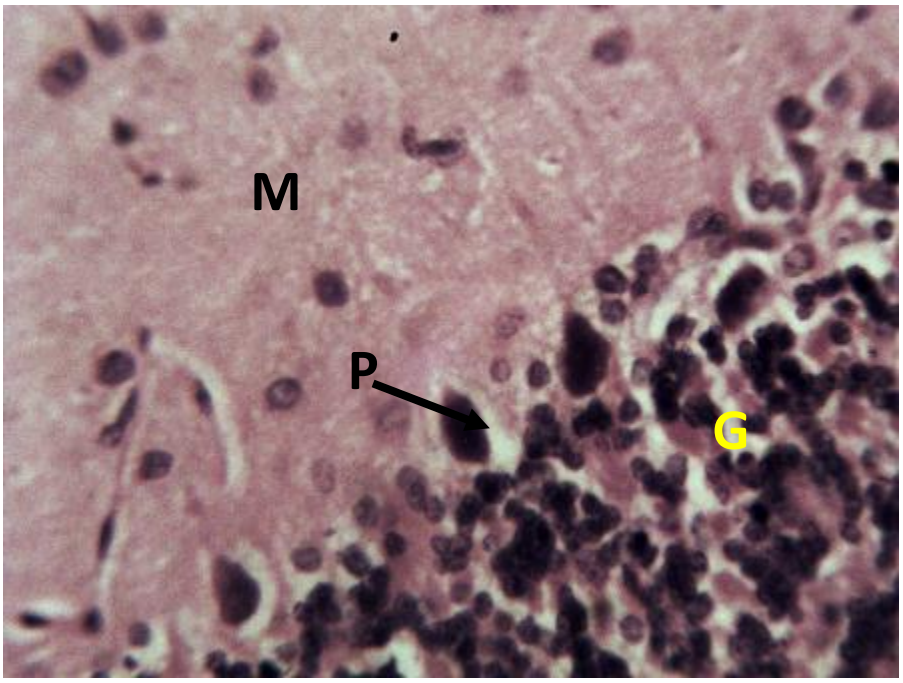


Plate 4.38: Section of Wistar rat cerebellar cortex of the group administered aqueous fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *internal granule cell* **(N) H and E stain (Mag x 400).**

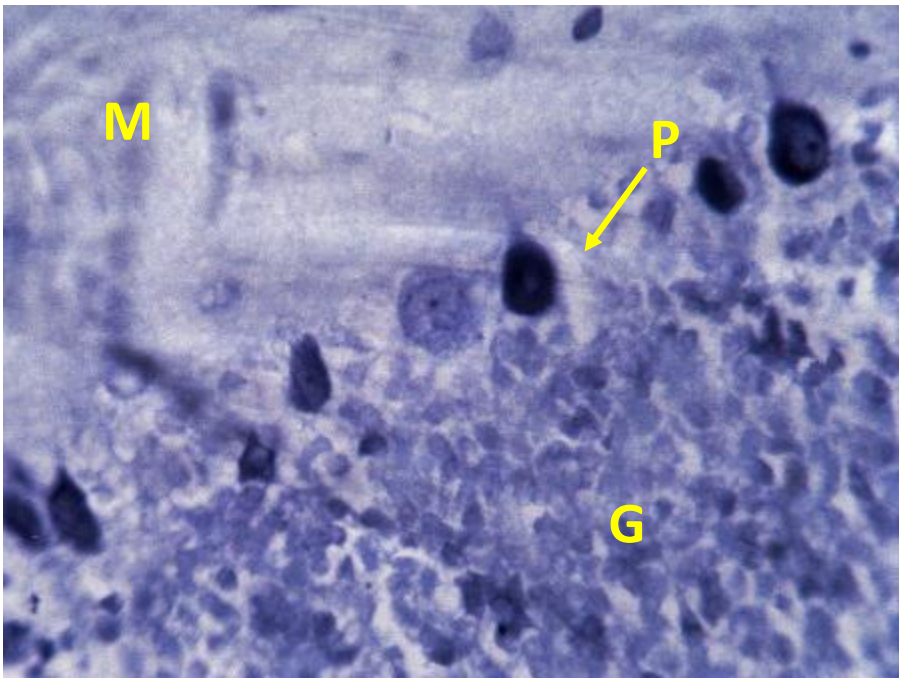


Plate 4.39: Section of Wistar rat cerebellar cortex of the group administered aqueous fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration– *internal granule cell* **(N) CFV stain (Mag x 400).**

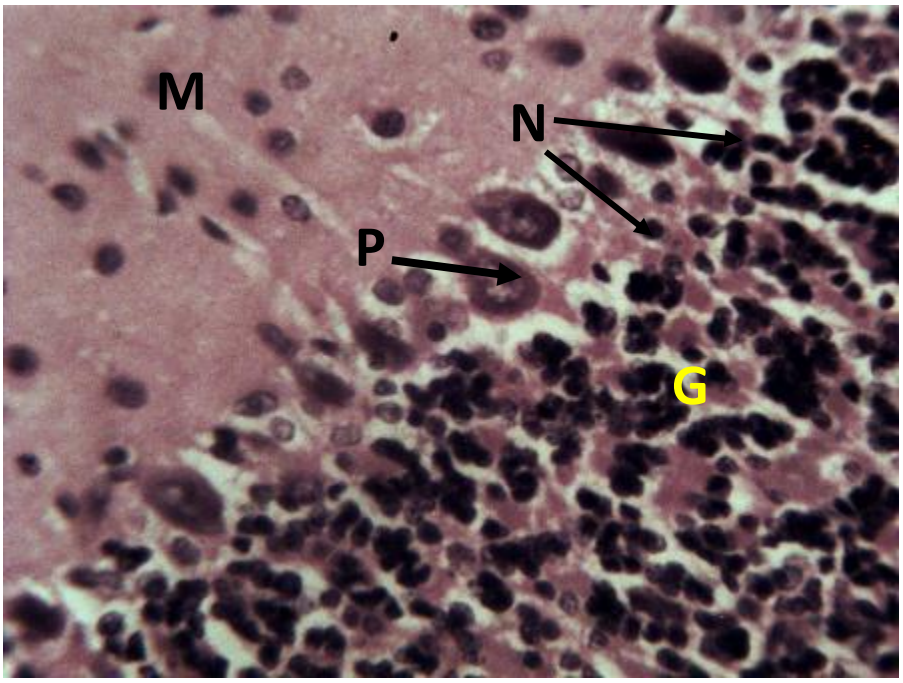


Plate 4.40: Section of Wistar rat cerebellar cortex of the group administered ethanol fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *perineuronal vacuolations* **(N) H and E stain (Mag x 400).**

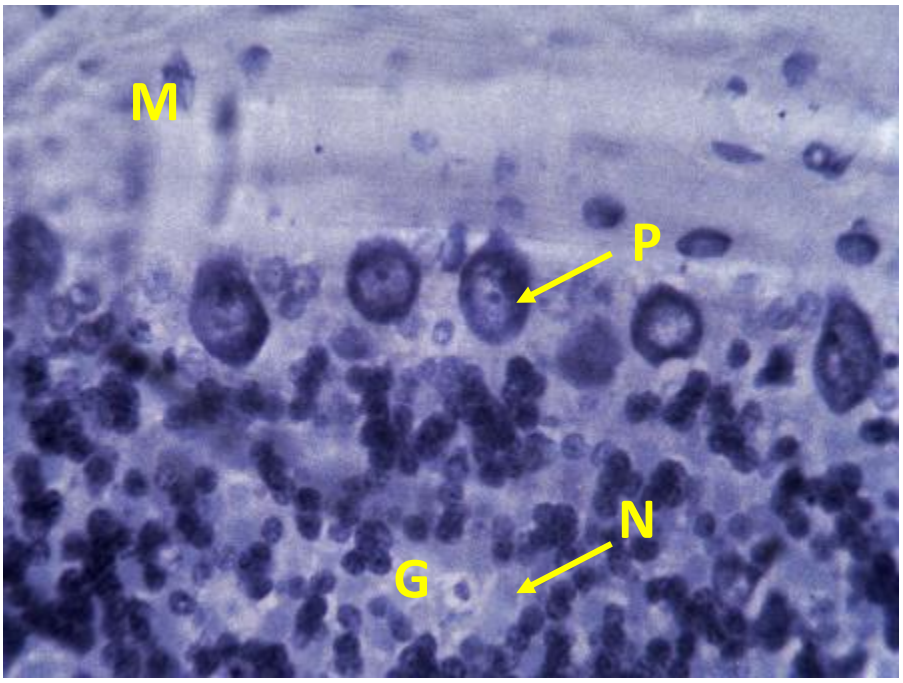


Plate 4.41: Section of Wistar rat cerebellar cortex of the group administered ethanol fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *satellitosis and internal granule cell necrosis* **(N) CFV stain (Mag x 400).**

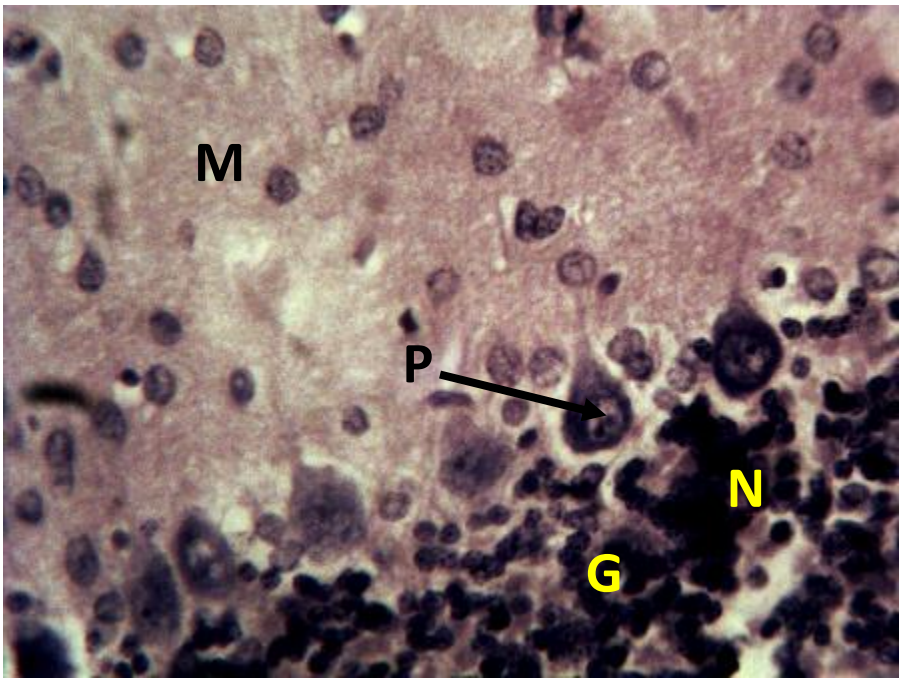


Plate 4.42: Section of Wistar rat cerebellar cortex of the group administered ethanol fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration– *internal granule cell* **(N) H and E stain (Mag x 400).**

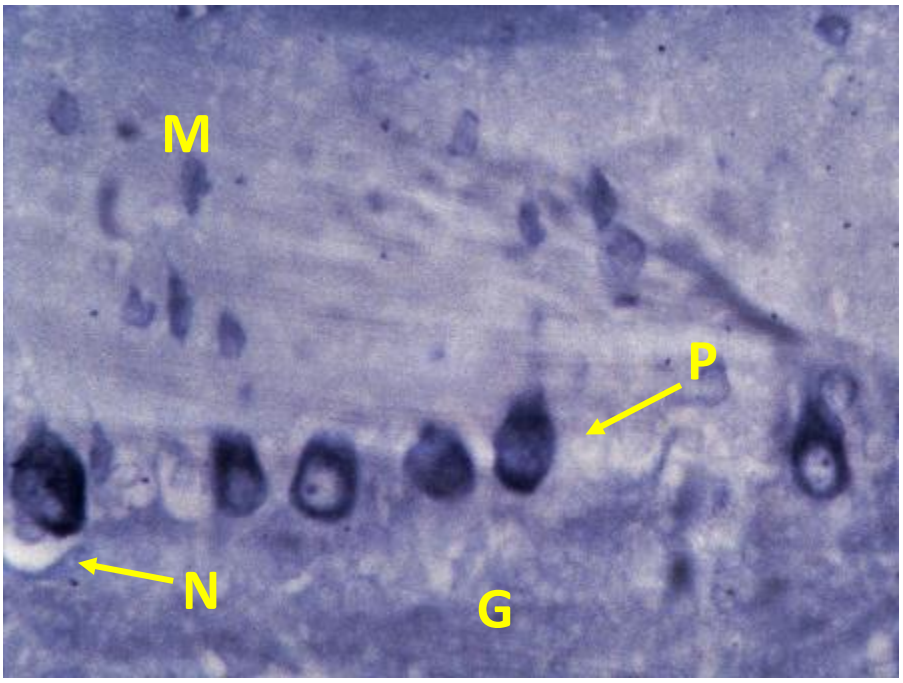


Plate 4.43: Section of Wistar rat cerebellar cortex of the group administered ethanol fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *perineuronal vacuolations* **(N) CFV stain (Mag x 400).**

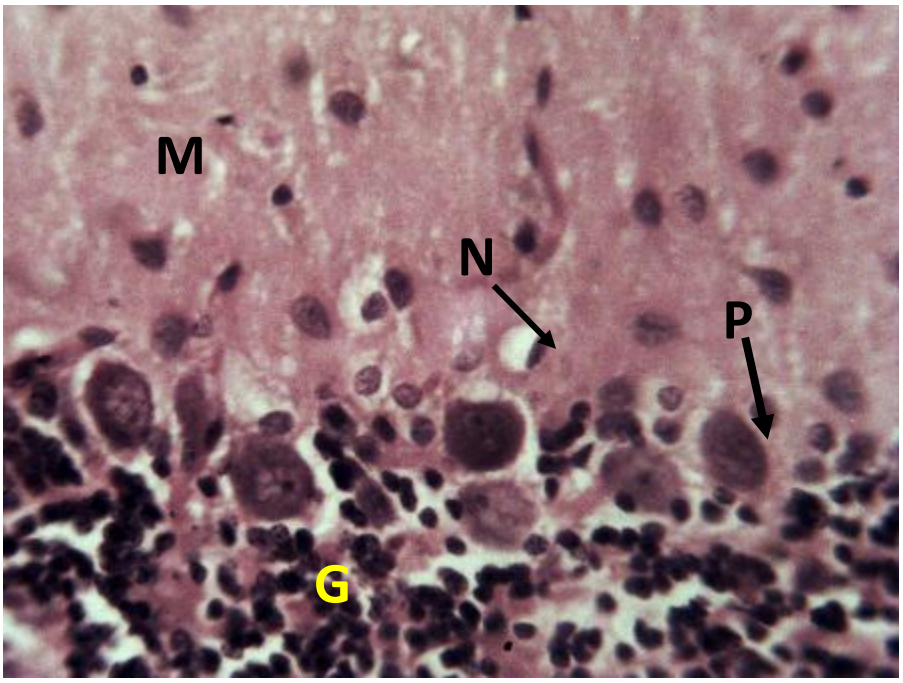


Plate 4.44: Section of Wistar rat cerebellar cortex of the group administered ethanol fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *pkynotic nuclei and internal granule cell vacuolations* **(N) H and E stain (Mag x 400).**

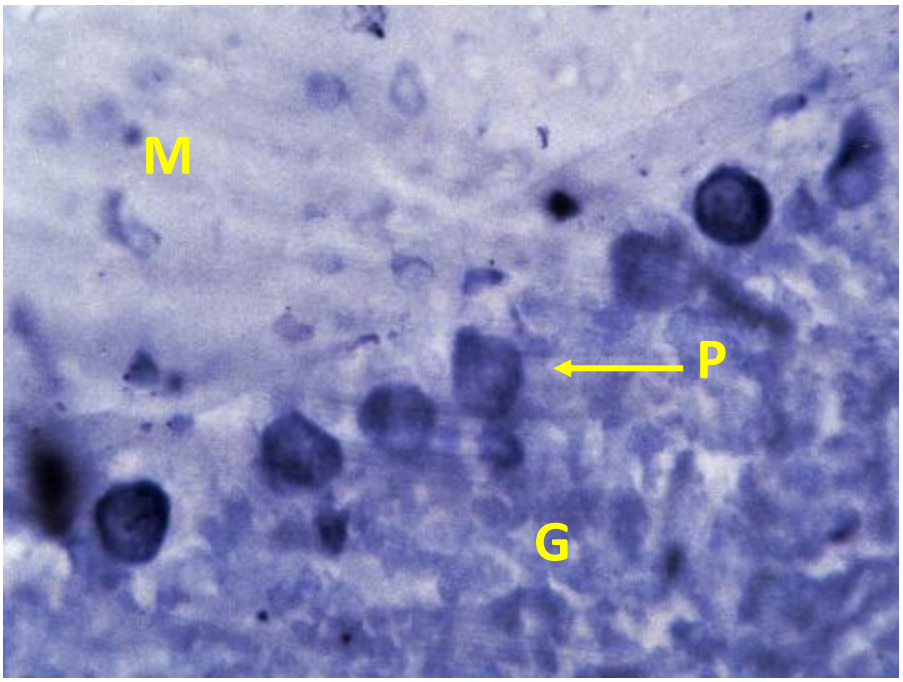


Plate 4.45: Section of Wistar rat cerebellar cortex of the group administered ethanol fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the cerebellar cortex. Purkinje cell (P); Granular layer (G); Molecular layer (M); Neuronal degeneration – *internal granule cell vacuolations* **(N) CFV stain (Mag x 400).**

4.4.3.1 Histological and Histochemical Features of the Hippocampus of Control (Untreated) Wistar Rat

Histological examination of Wistar rats' hippocampi, particularly CA1 and CA3 regions, in the control group showed normal histoarchitecture of these regions: the basic pattern of an ordered sheet of large neurones (pyramidal and granule cells) whose cell bodies are all packed together. The large neurones are the giant pyramids of CA3 and the smaller pyramids of CA1. Histochemical (CFV) staining for Nissl substances revealed normal appearance of hippocampal neurones and other interneurones, such as, stellate, fusiform and basket cells of Cajal, which differ from the pyramidal and granule cells, observed most clearly in CA3 (**Plates 4.46 and 4.47**).

4.4.3.2 Histological and Histochemical Features of the Hippocampus of Treated Wistar Rats

Histological and histochemical (CFV stained) examinations of Wistar rats' hippocampi treated with mercury revealed histoarchitectural distortion of the hippocampal regions (CA1 and CA3); neurodegenerative changes, such as, irregular arrangement of CA1 hippocampal neurones, pyknosis and basophilic necrosis (**Plates 4.48 and 4.49**).

Histological and histochemical (CFV stained) examinations of the hippocampi of vitamin C- and extracts (AFPD and EFPD)- treated Wistar rats, followed by mercury treatment, revealed mild histoarchitectural distortions of hippocampal regions (CA1 and CA3). The histological features of the vitamin C- treated group showed chromatolysis and karyorrhexis (**Plates 4.50 and 4.51**).

In category 'A' study, histological features of the AFPD (250 mg/kg, 500 mg/kg and 1,000 mg/kg) - treated groups showed mild neurodegenerative changes: irregular arrangement of

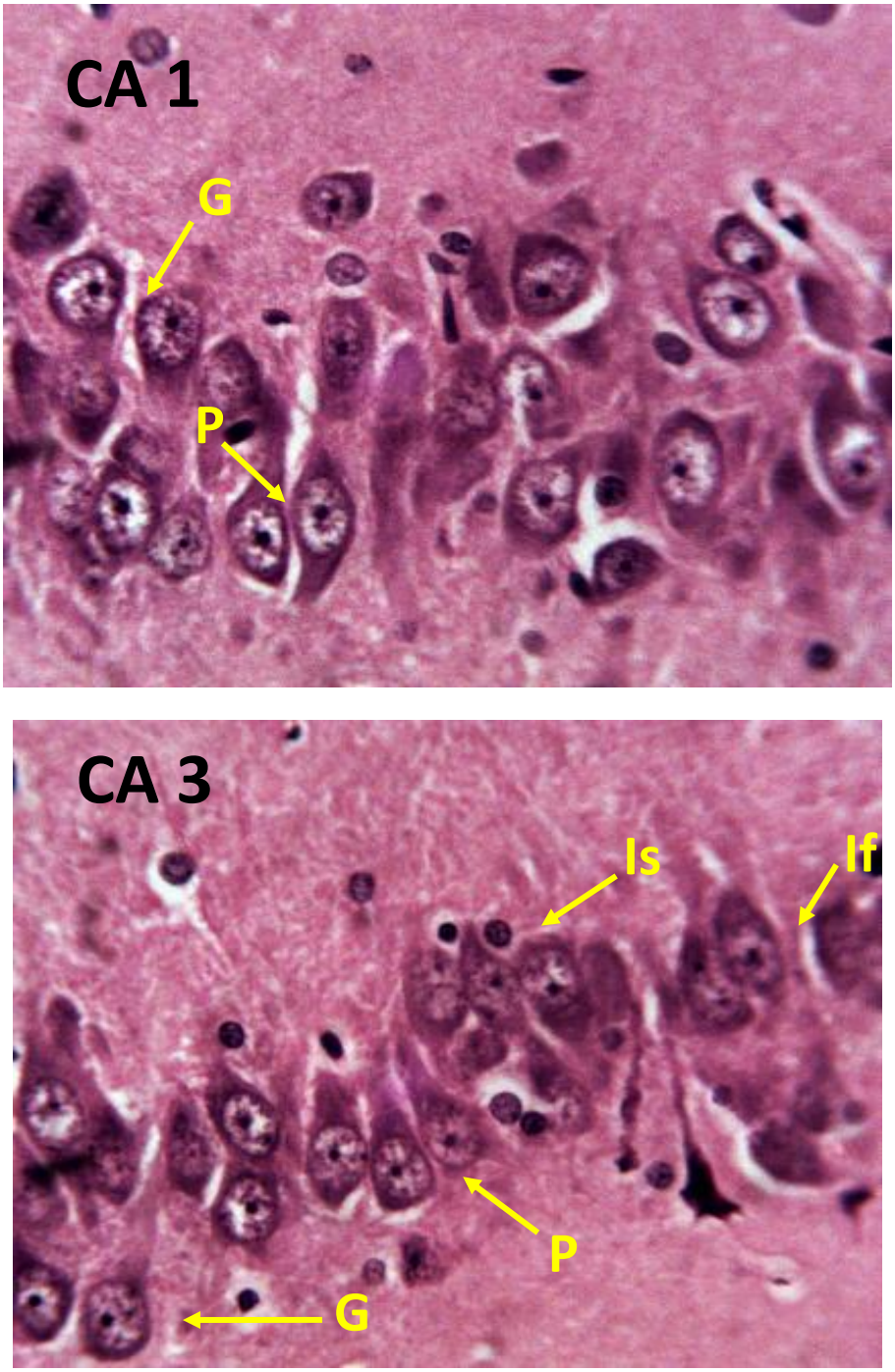


Plate 4.46: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the control (untreated) group showing normal histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Interneuron – *fusiform cell* (If); Interneuron – *stellate cell* (Is) H and E stain (Mag x 400).

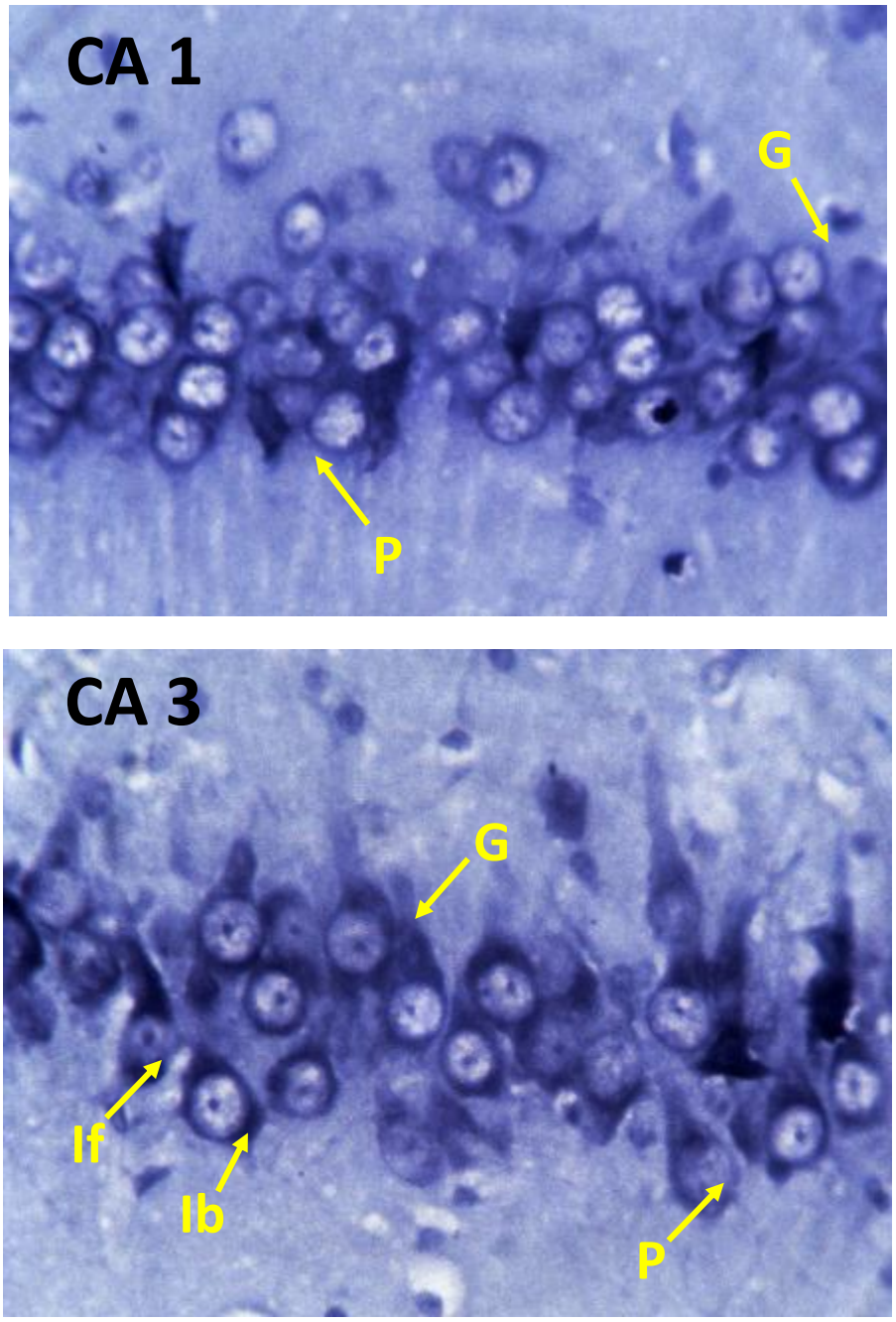


Plate 4.47: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the control (untreated) group showing normal histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Interneuron – *fusiform cell* (If); Interneuron – *basket cell* (Ib) CFV stain (Mag x 400).

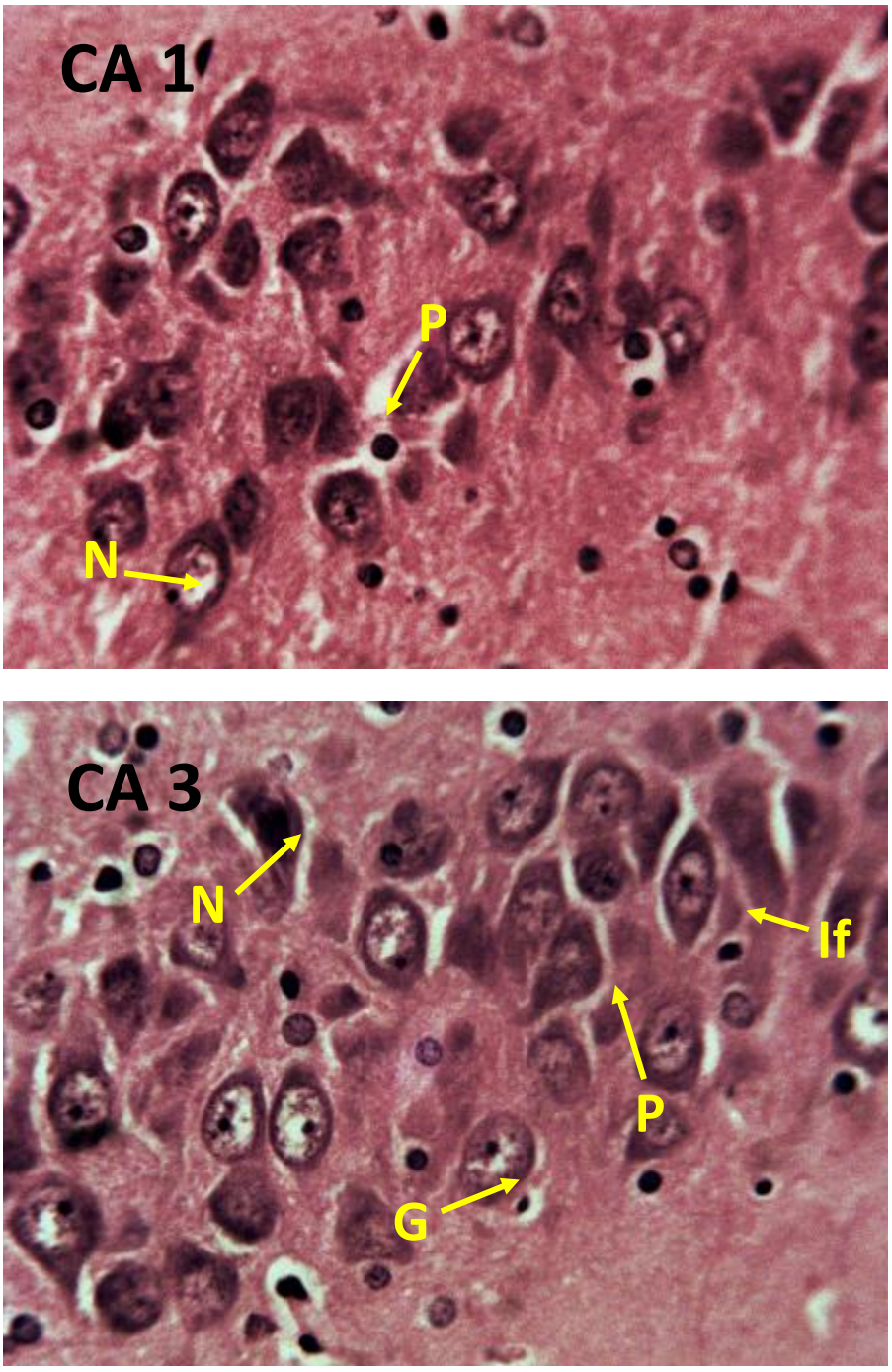


Plate 4.48: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered mercuric chloride (5 mg/kg) showing distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Interneuron – *fusiform cell* (If); Neuronal degeneration – *pyknosis and basophilic necrosis* **(N) H and E stain (Mag x 400).**

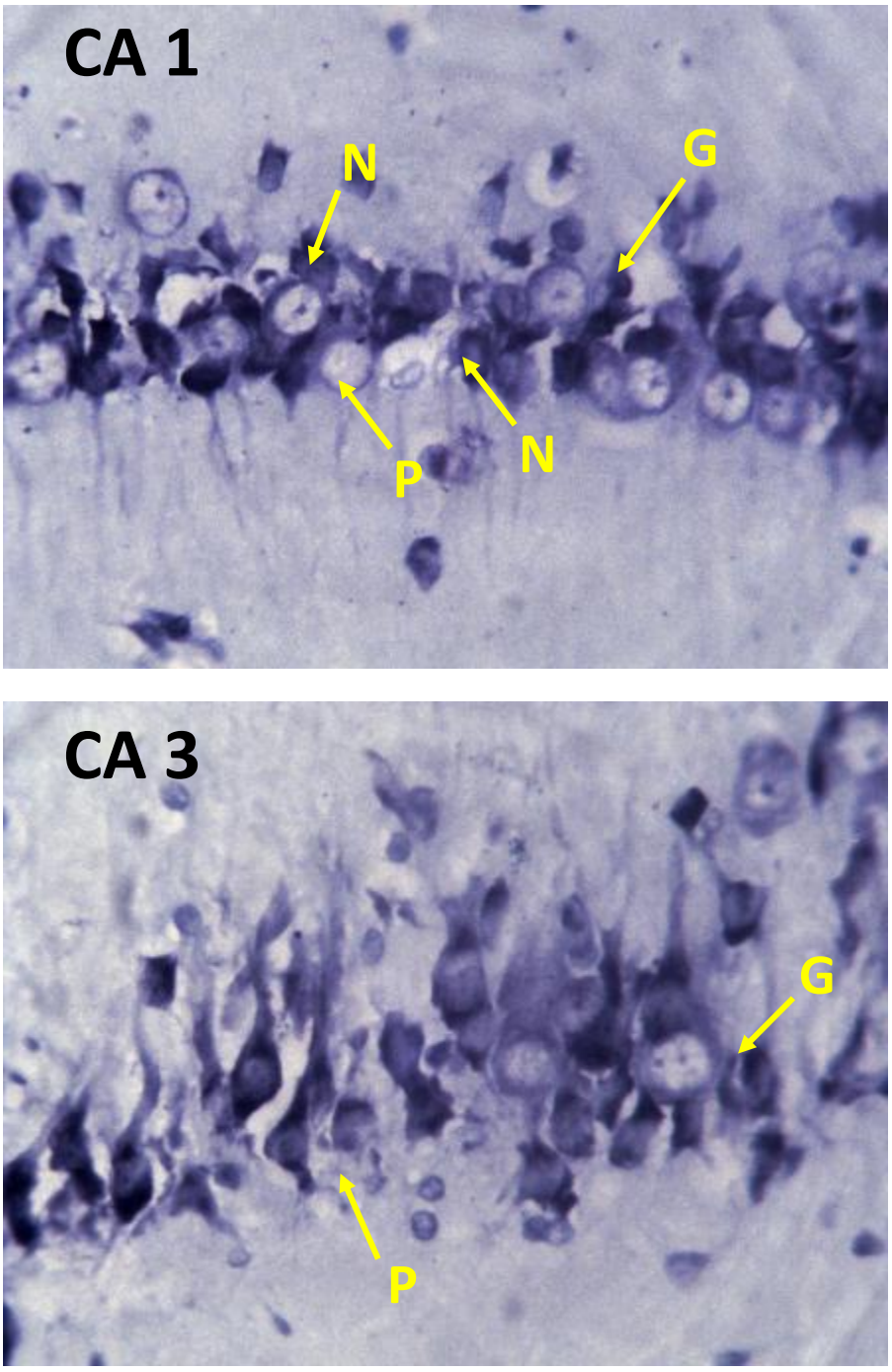


Plate 4.49: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered mercuric chloride (5 mg/kg) showing distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration – *pkynotic necrosis* **(N) CFV stain (Mag x 400).**

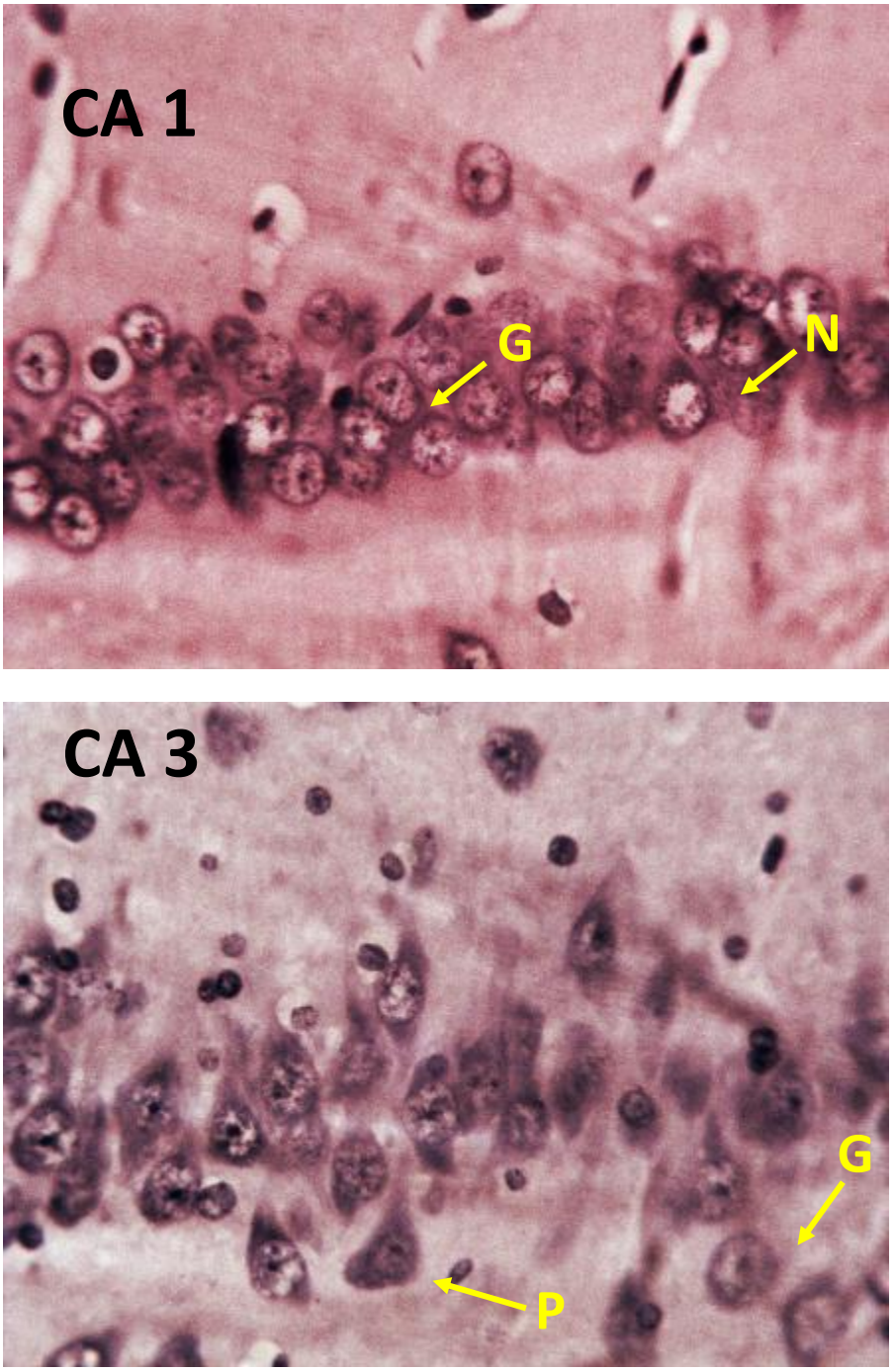


Plate 4.50: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered vitamin C (100 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration– *chromatolysis* (N) H and E stain (Mag x 400).

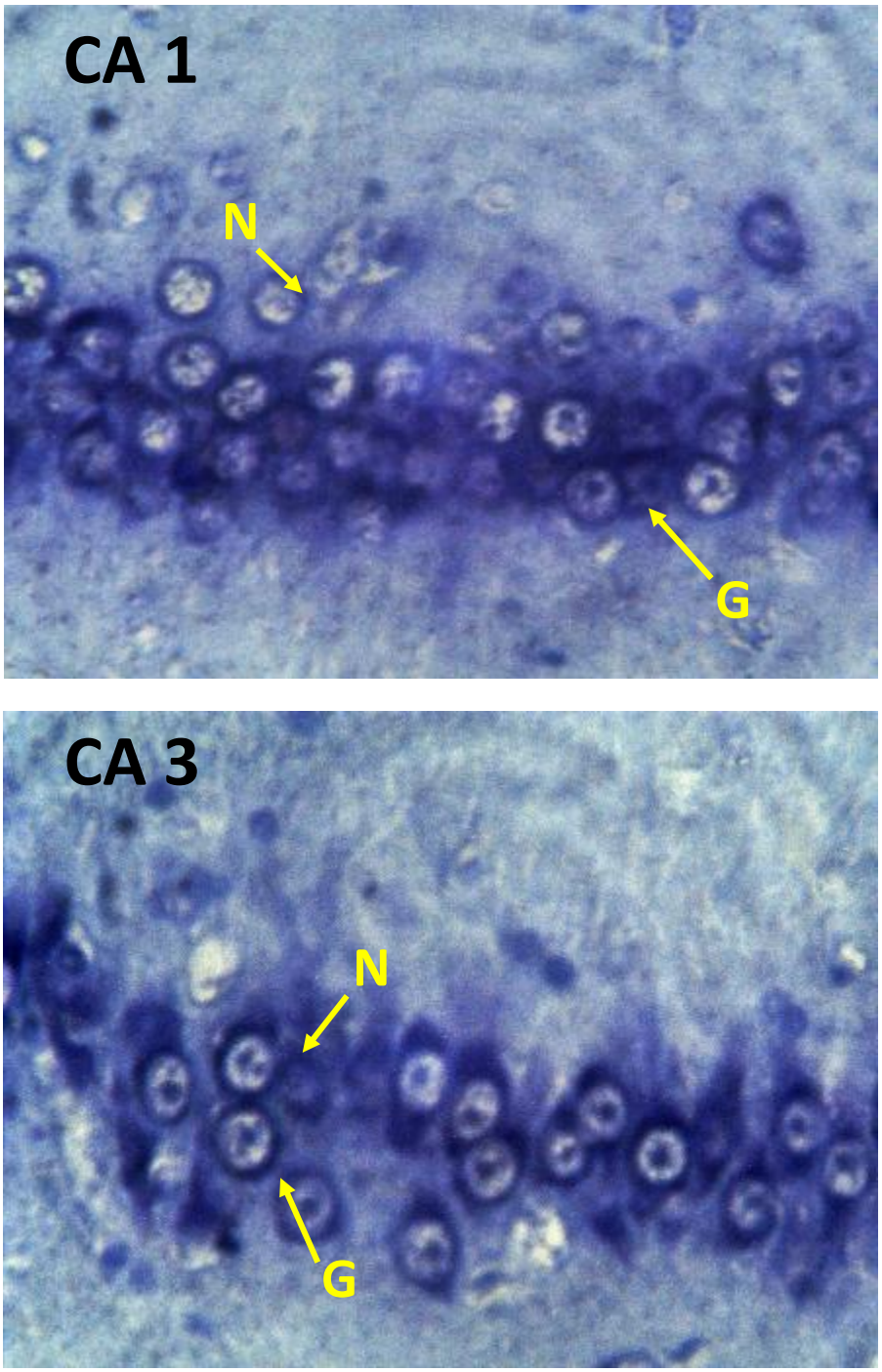


Plate 4.51: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered vitamin C (100 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration – *chromatolysis and karyorrhexis* **(N) CFV stain (Mag x 400).**

CA1 hippocampal neurones, karyorrhexis, pyknotic necrosis and satellitosis. AFPD administration conferred preservation of the histoarchitecture in a dose dependent manner compared to the control (**Plates 4.52- 4.57**).

Histological features of the EFPD (250 mg/kg, 500 mg/kg and 1,000 mg/kg) - treated groups (category 'B' study) showed mild neurodegenerative changes: irregular arrangement of CA1 hippocampal neurones, karyorrhexis, indistinct neurones (reduced staining intensity), eosinophilic necrosis, perineuronal vacuolations and pyknotic necrosis. Treatment with EFPD conferred histoarchitectural preservation, especially with 500 mg/kg EFPD - treated group compared to the control (**Plates 4.58- 4.63**).

4.5 Neurobehavioural Studies

A battery of neurobehavioural test was conducted to assess a variety of endpoints, such as cognition, sensory-motor activity and, motor coordination and balance in the Wistar rats. Elevated plus maze (EPM) was used to assess anxiety-related behaviour; transfer latency (TL) on EPM open arm to evaluate learning and memory; Morris water maze (MWM) for spatial learning and memory; forelimb grip strength (FGS) test to assess muscular strength in the forelimbs and beam walking test (BWT) to monitor motor coordination and balance of Wistar rats.

4.5.1 EPM Studies

In the elevated plus maze test, specific variables (time spent in the open/ closed arm, frequency of entries to the open/ closed arm, grooming, rearing and head dipping) were used to characterize the level of anxiety in Wistar rats.

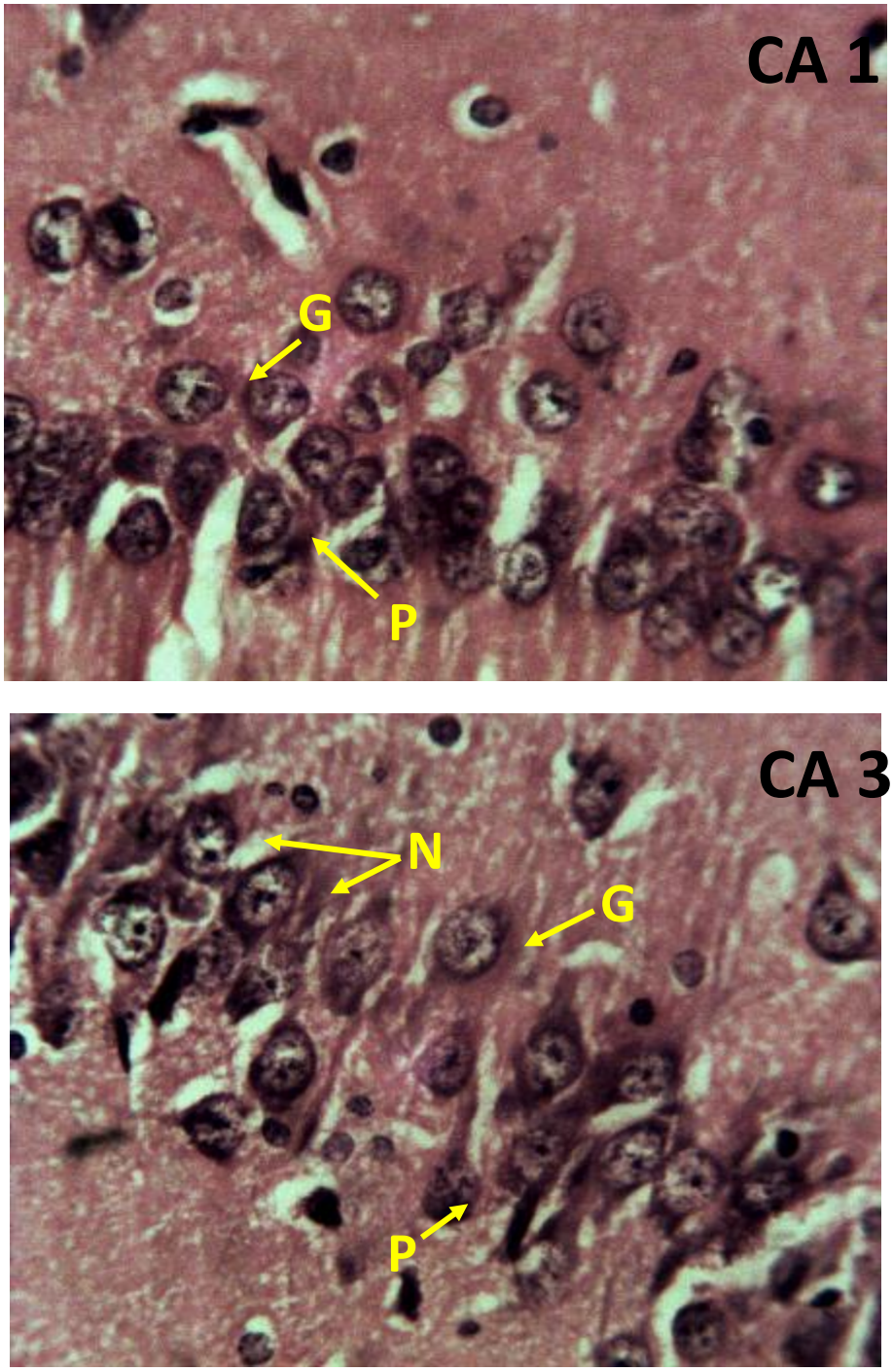


Plate 4.52: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered aqueous fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration– *karyorrhexis* **(N) H and E stain (Mag x 400).**

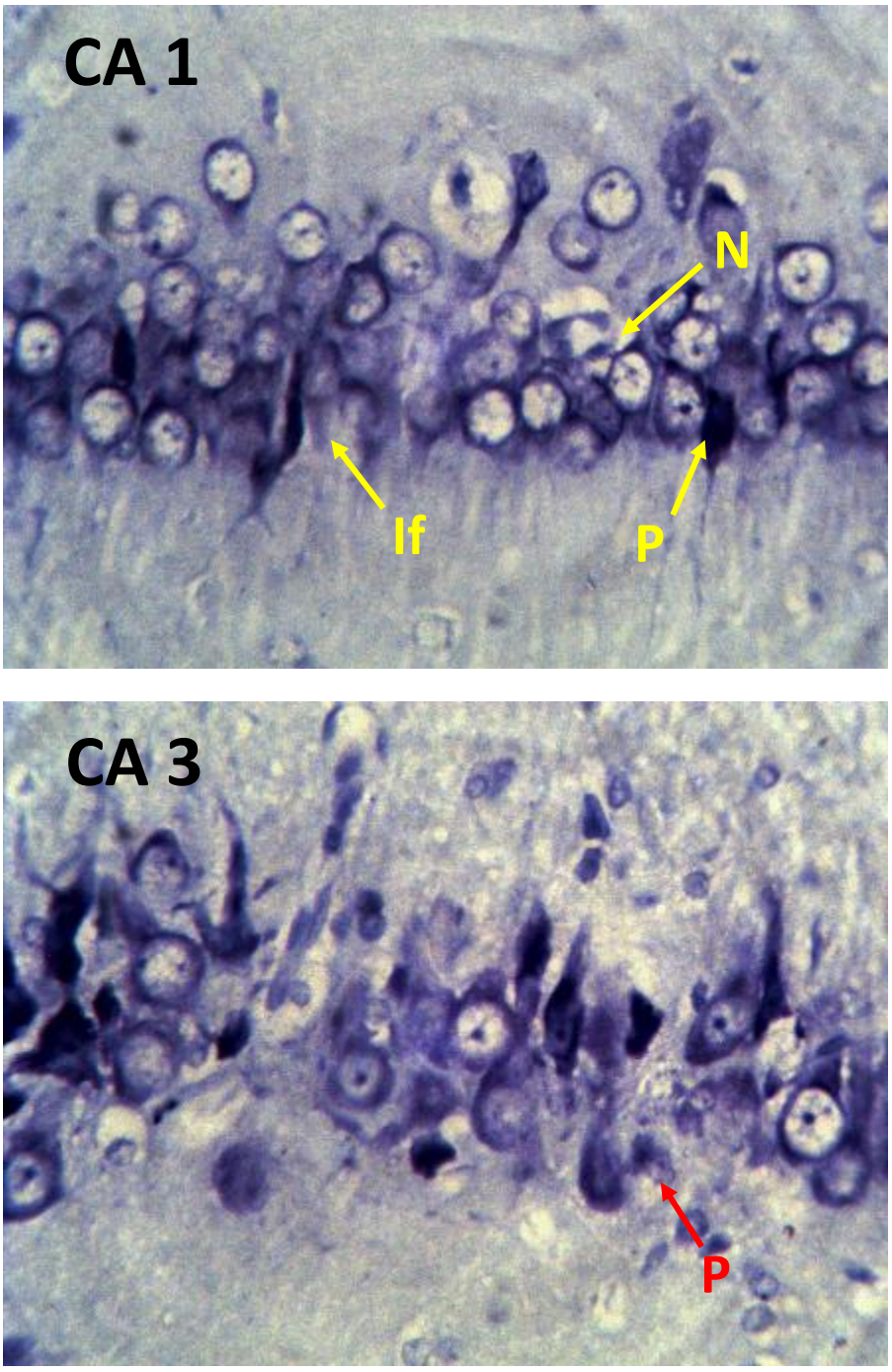


Plate 4.53: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered aqueous fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Interneuron – *fusiform cell* (If); Neuronal degeneration – *pkynotic nuclei* **(N) CFV stain (Mag x 400).**

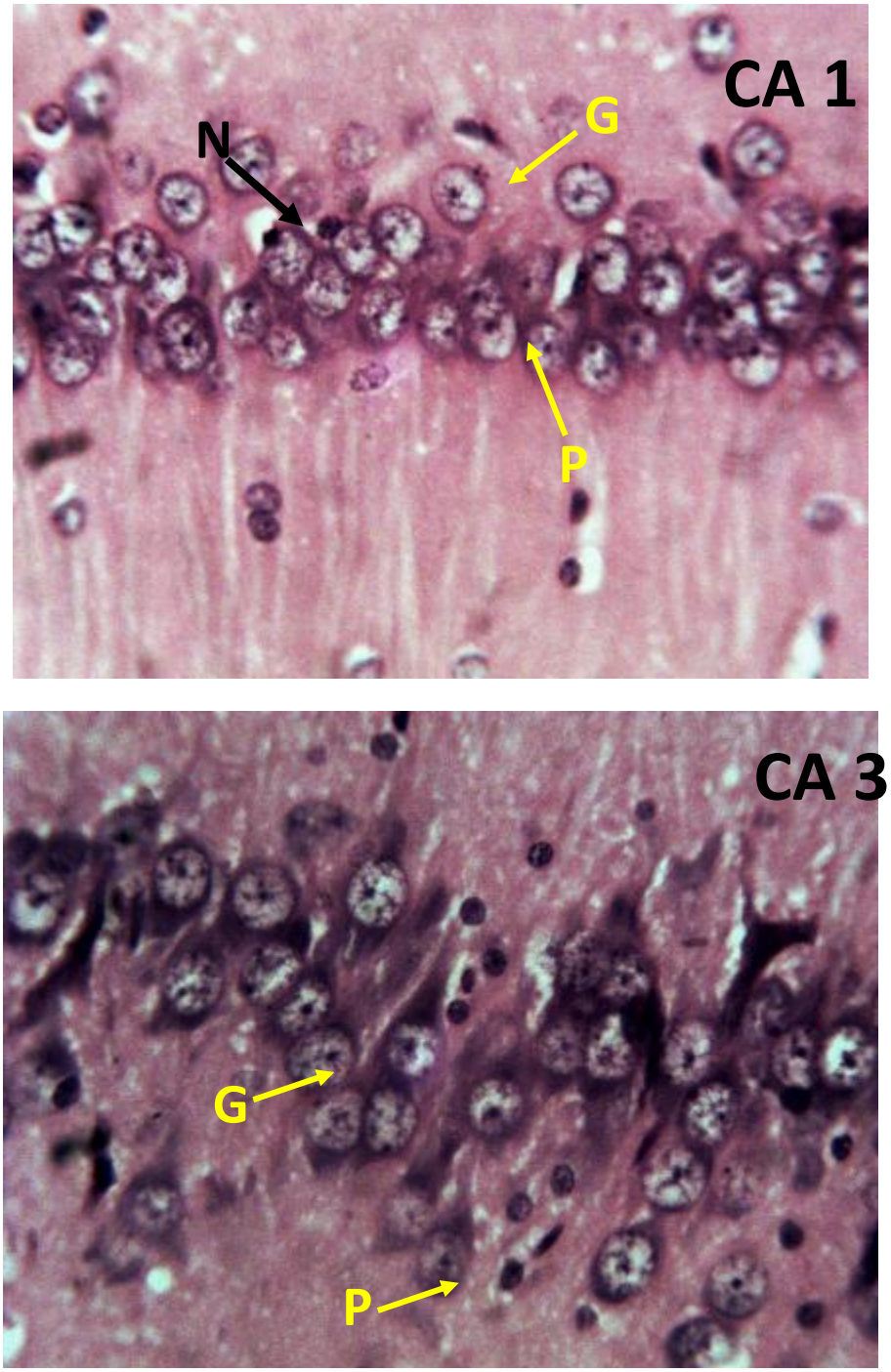


Plate 4.54: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered aqueous fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration – *pyknosis* (N) H and E stain (Mag x 400).

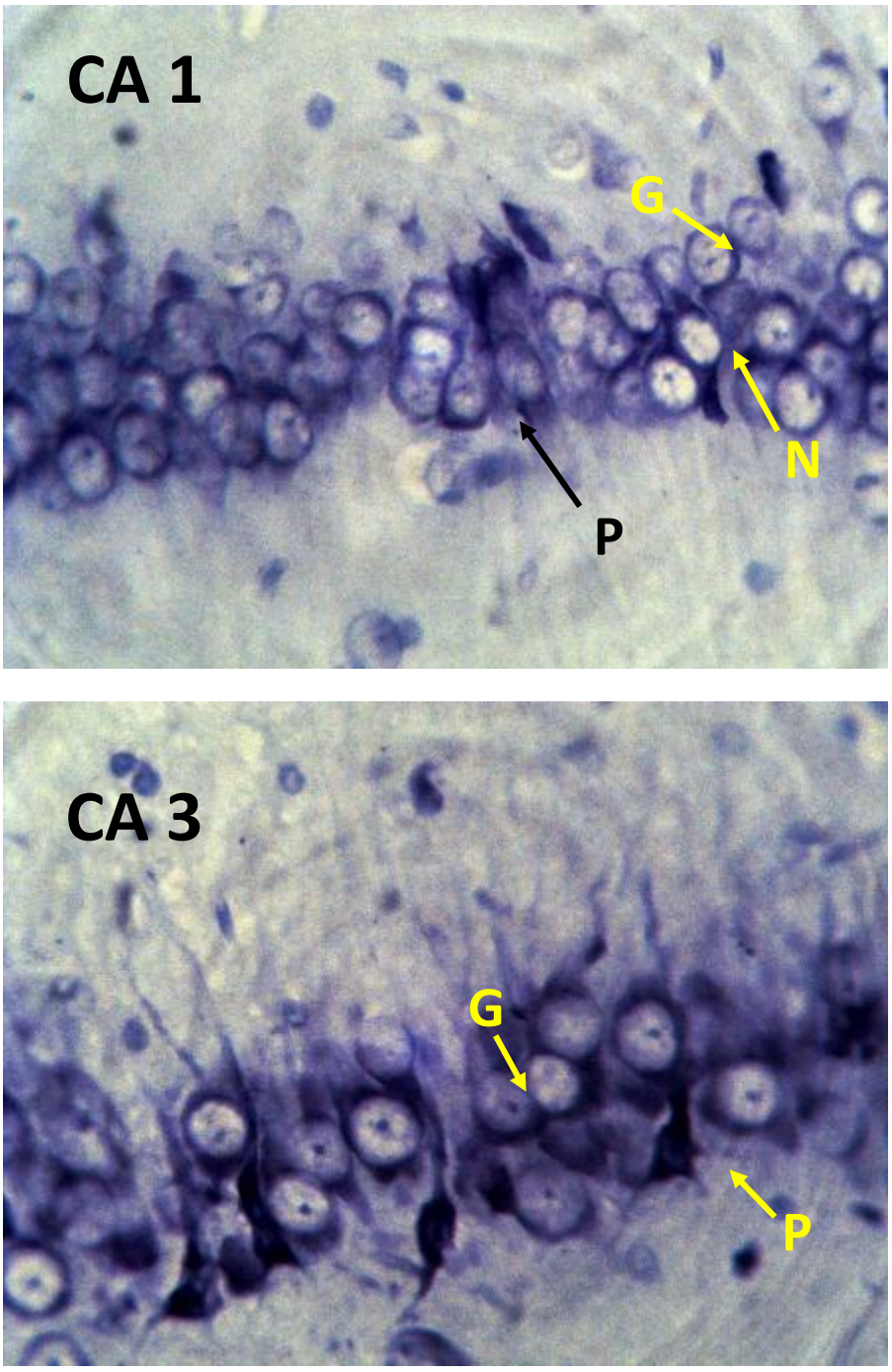


Plate 4.55: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered aqueous fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration (N) CFV stain (Mag x 400).

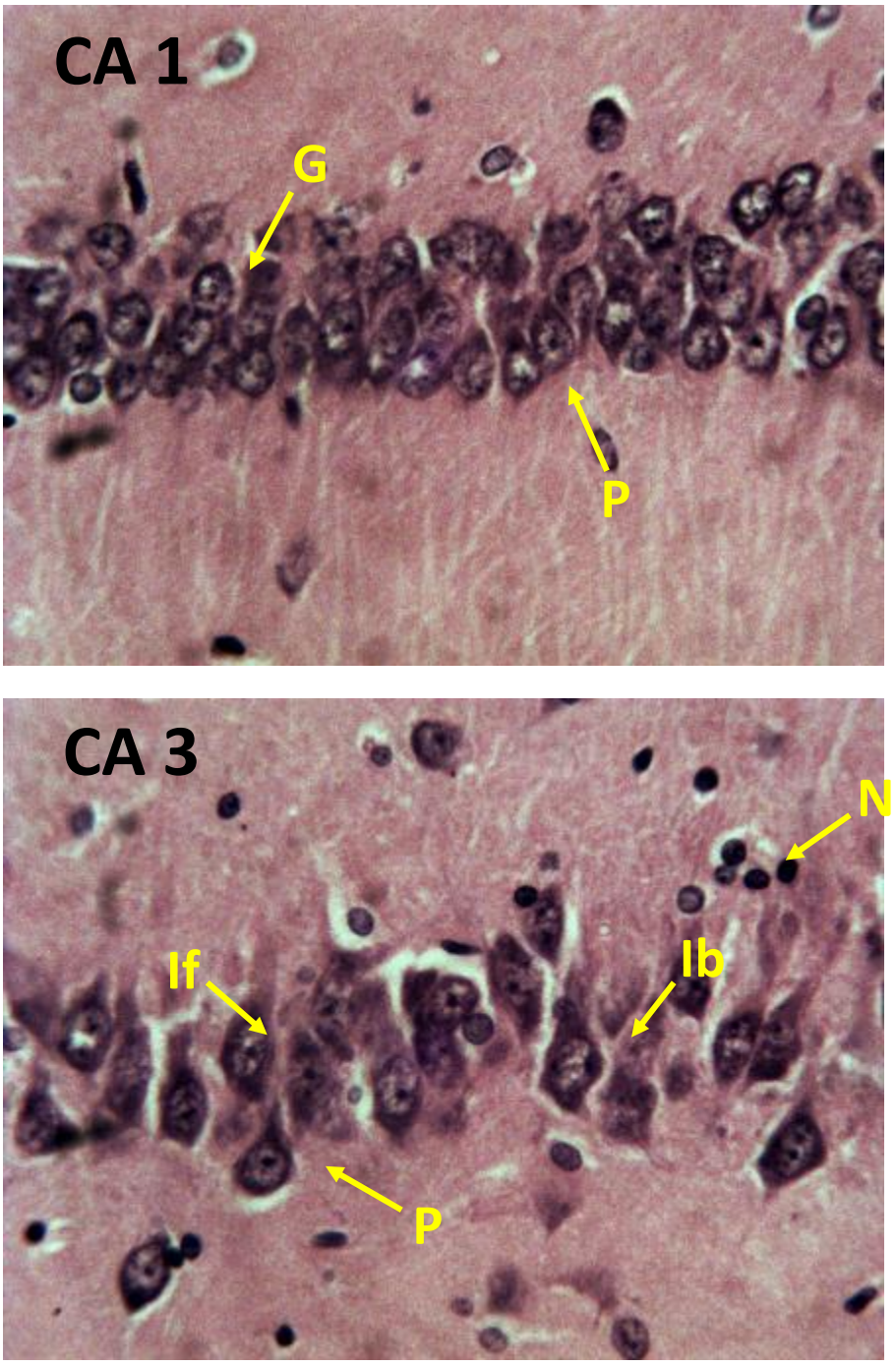


Plate 4.56: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered aqueous fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Interneuron – *fusiform cell* (If); Interneuron – *basket cell* (Ib) Neuronal degeneration– *satellitosis* **(N) H and E stain (Mag x 400).**

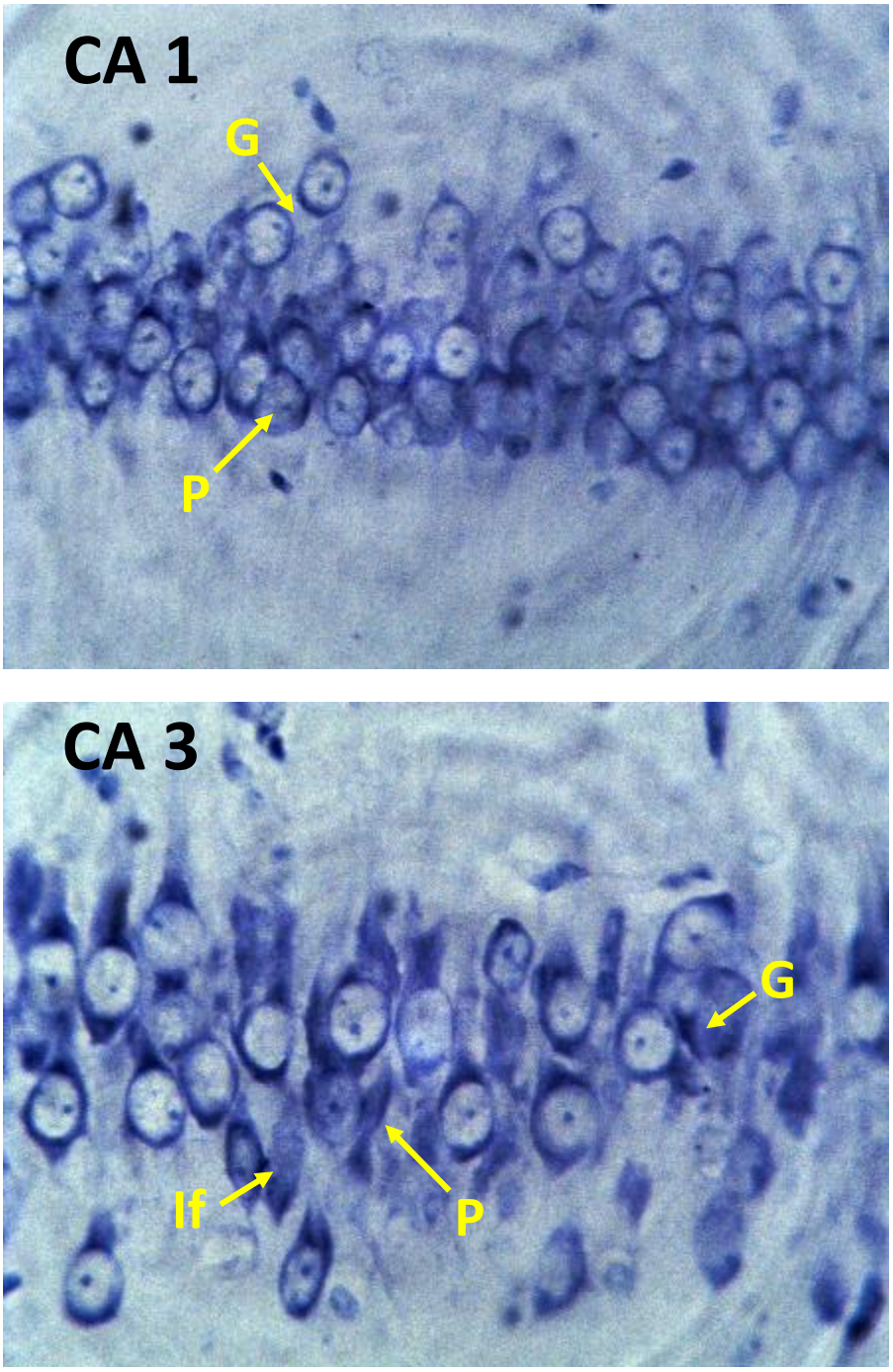


Plate 4.57: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered aqueous fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Interneuron– *fusiform cell* (If); Neuronal degeneration (N) CFV stain (Mag x 400).

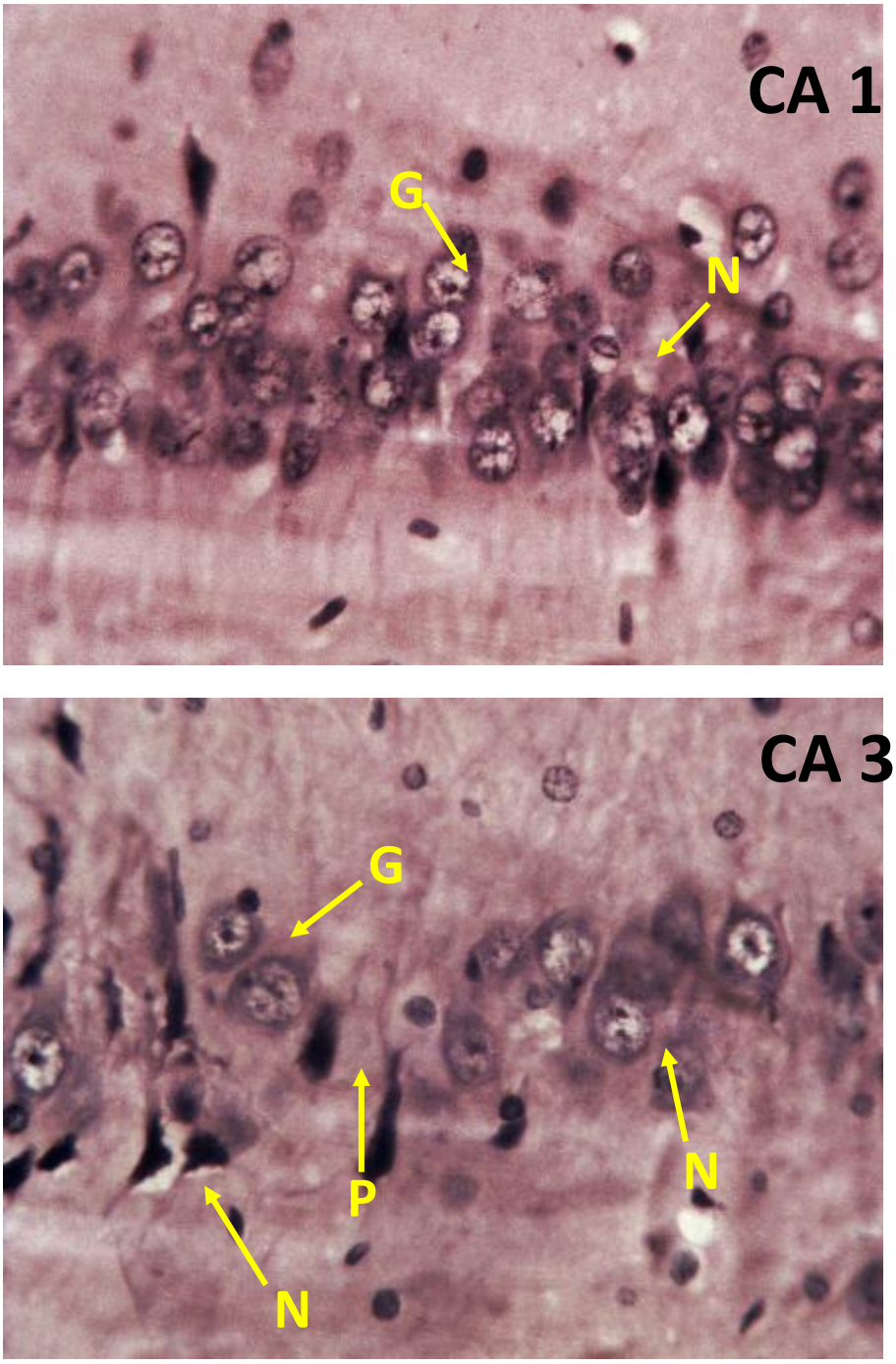


Plate 4.58: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered ethanol fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration – *pkynotic nuclei and karyorrhexis* **(N) H and E stain (Mag x 400).**

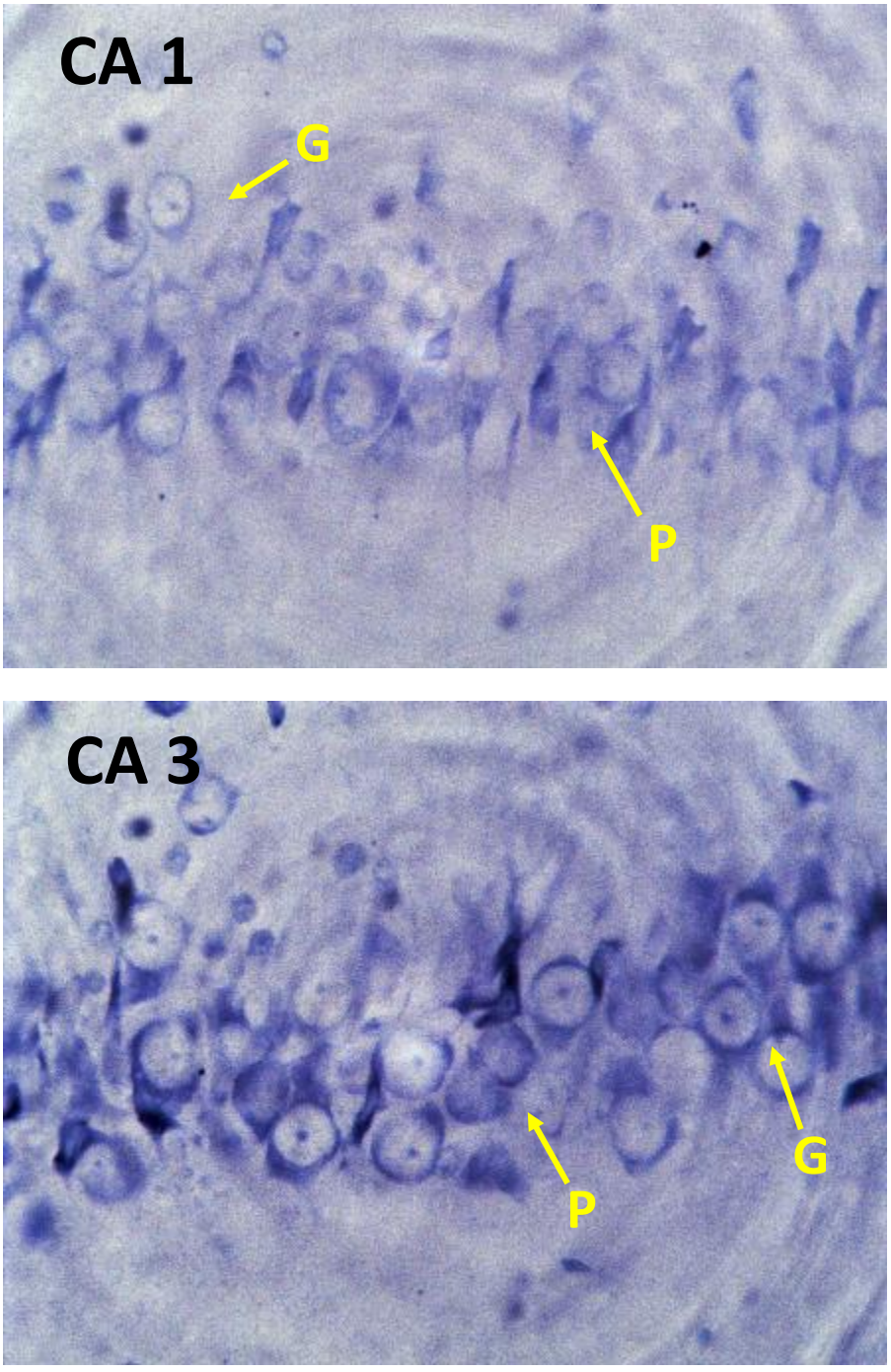


Plate 4.59: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered ethanol fruit extract *of P. dactylifera* (250 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); CFV stain (Mag x 400).

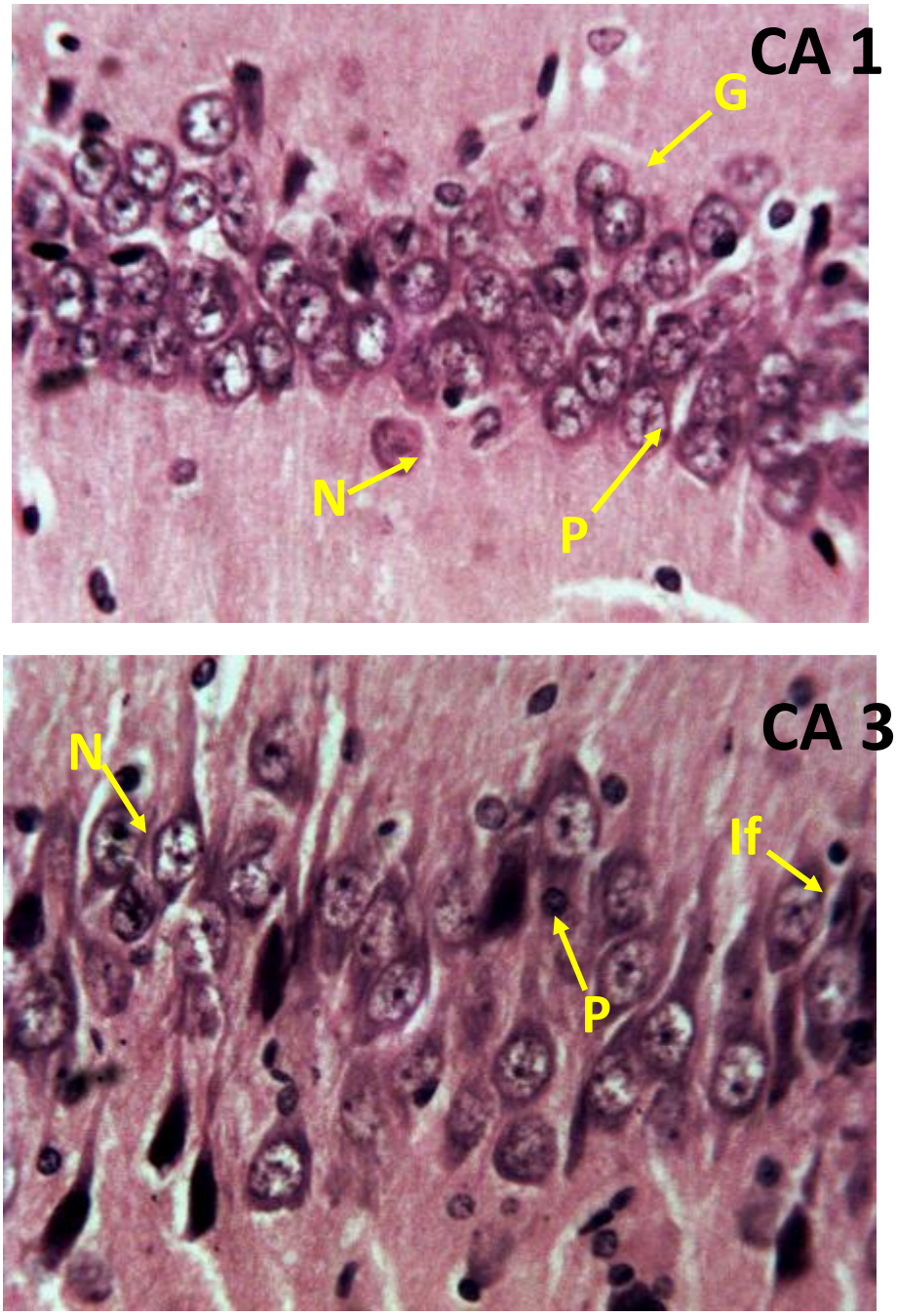


Plate 4.60: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered ethanol fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration – *eosinophilic necrosis and karyorrhexis* **(N) H and E stain (Mag x 400).**

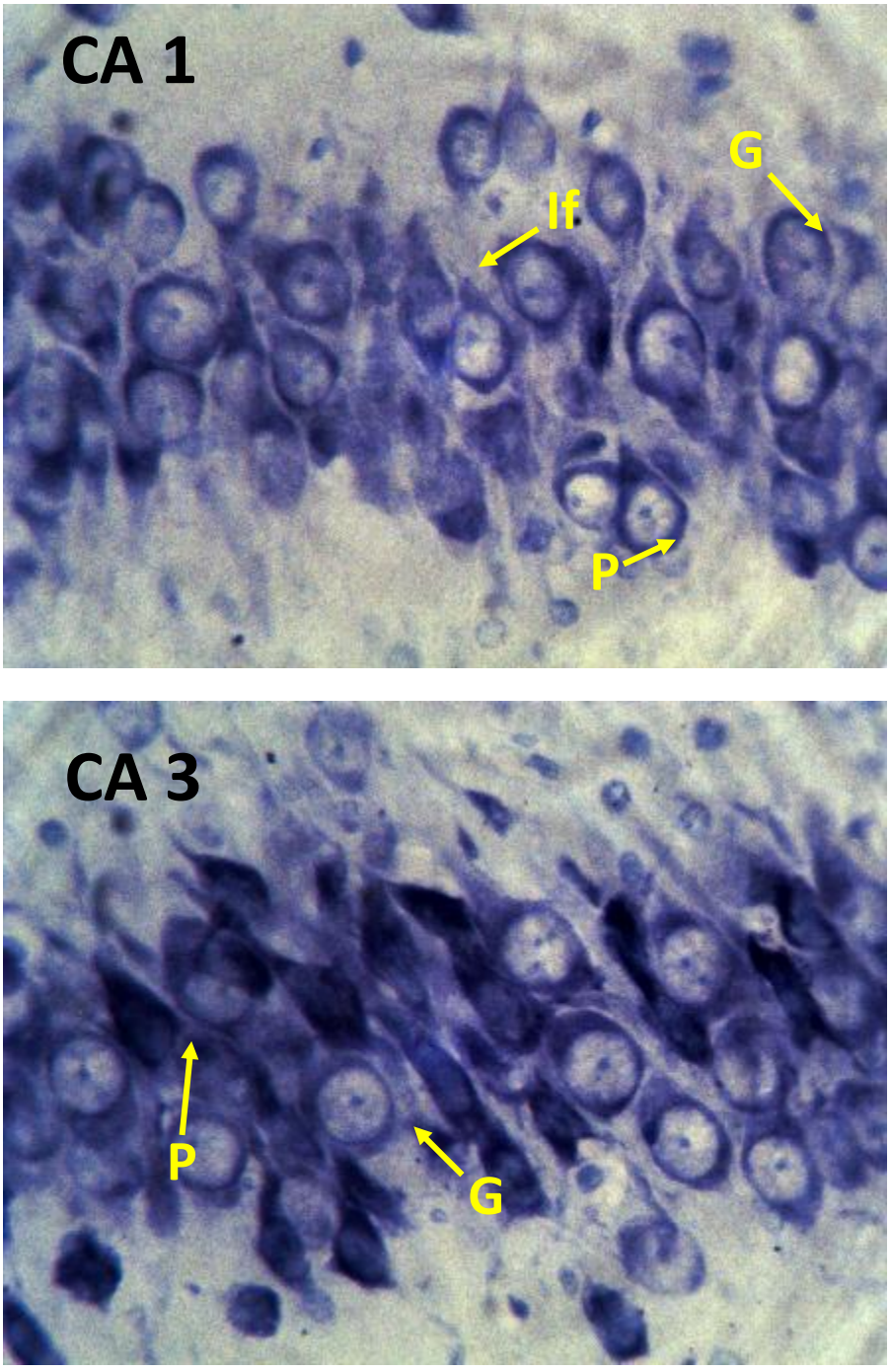


Plate 4.61: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered ethanol fruit extract *of P. dactylifera* (500 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Interneuron – *fusiform cell* (If) CFV stain (Mag x 400).

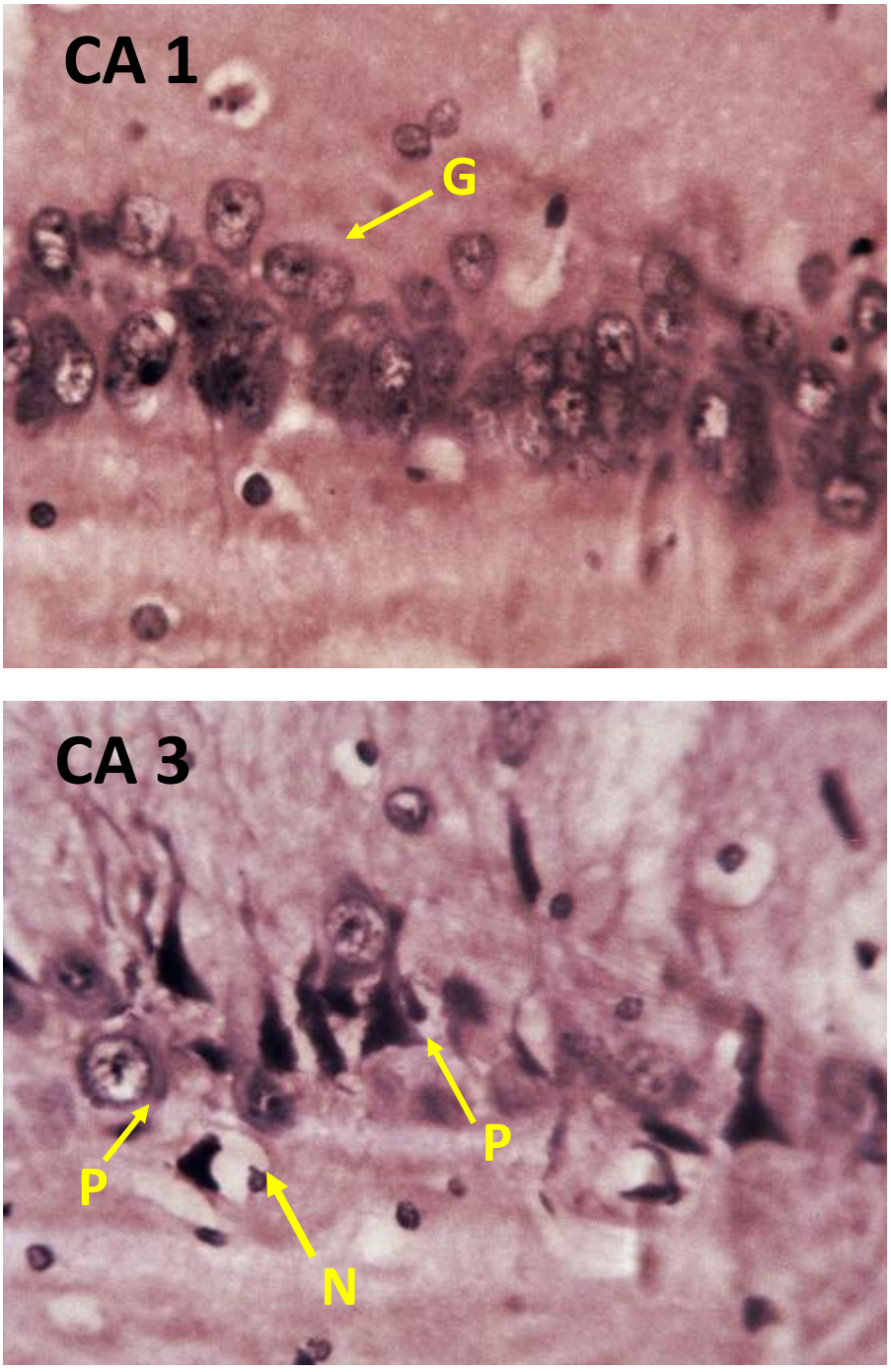


Plate 4.62: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered ethanol fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration– *perineuronal vacuolations and pkynotic nuclei* **(N) H and E stain (Mag x 400).**

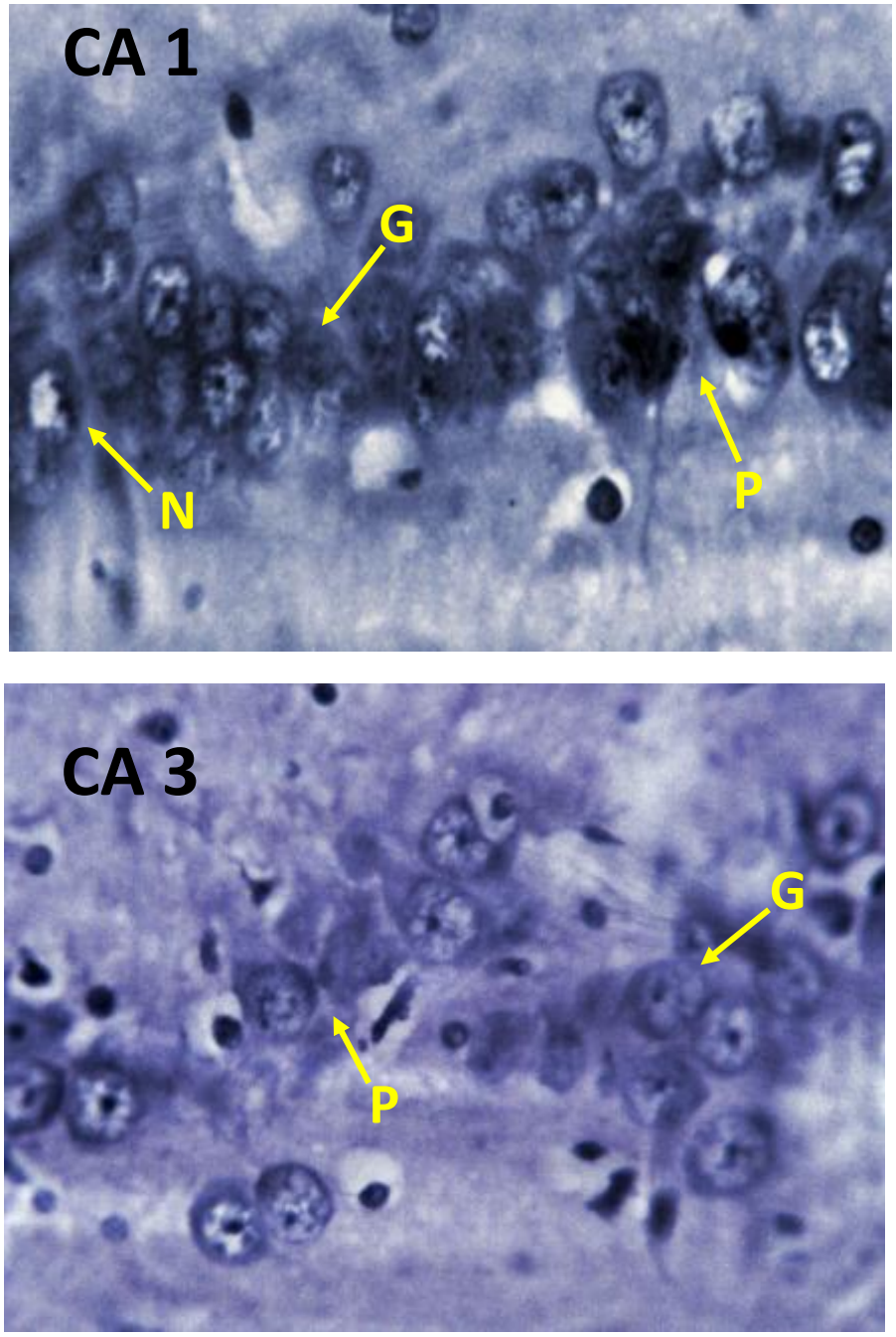


Plate 4.63: Sections of Wistar rat hippocampal regions (CA 1 and CA 3) of the group administered ethanol fruit extract *of P. dactylifera* (1000 mg/kg) and mercuric chloride (5 mg/kg) showing mild distortion in the histology of the hippocampus. Pyramidal cell (P); Granule cell (G); Neuronal degeneration– *pkynotic nuclei* **(N) H and E stain (Mag x 400).**

Across the period of study (pre-treatment through day-14 of treatment), the control group Wistar rats demonstrated remarkable ($p<0.01$) decreased anxiety levels by the increased time (duration) spent in the open arm and decreased duration in the closed arm. Mercury-treated group showed an irregular pattern in anxiety levels across the period of study; decrease and increase at day-7 and day-14 treatment, respectively. Vitamin C- treated group revealed decreased anxiety levels across the period of study, however, not remarkable ($p>0.05$).

In category 'A' study, all the AFPD-treated groups showed an irregular pattern in anxiety levels across the period of study. No striking difference was observed in the time spent in the open/ closed arm at days -7 and 14 treatment when compared to control (**Table 4.5a**).

The EFPD-treated groups, in category 'B' study, showed decreased anxiety levels across the period of study by the increased time spent in the open arm, except EFPD (500 mg/kg)-treated group. No striking difference was observed in the time spent in the open/ closed arm at days -7 and 14 treatment when compared to control (**Table 4.5b**).

Relative to the frequency of entries to the open/ closed arm, control group Wistar rats revealed striking ($p<0.05$) decreased level of anxiety across the period of study (pre-treatment through day-14 of treatment) by reduced frequency of entry into the closed arm. Mercury-treated group showed increased anxiety levels by increased entry frequency into the closed arm (from day-7 to day-14 treatment). Vitamin C- treated group revealed remarkable ($p<0.01$) decreased anxiety levels across the period of study. AFPD-treated groups showed reduced entry frequency into the closed arm across the period of study, except AFPD (1,000 mg/kg)-treated group. Comparison between the AFPD-treated groups

Table 4.5a: Effect of Aqueous Fruit Extract of *P. dactylifera* on Anxiety-like Behaviour (OAD and CAD) of Wistar Rats in Elevated Plus Maze

Group/ treatment	OAD Pre-treatment (s)	OAD day-7 (s)	OAD day-14 (s)	CAD Pre-treatment (s)	CAD day-7 (s)	CAD day-14 (s)
Control (H_2O 0.5ml)	40.20±1.75	98.40±5.70††	146.85±51.19††	259.80±1.75	201.60±5.70††	153.15±51.19††
$HgCl_2$ (5mg/kg)	43.85±5.80	145.20±51.66	139.95±53.35	256.15±5.80	154.80±51.66	160.05±53.35
Vit C (100mg/kg) **+ $HgCl_2$** (5mg/kg)	58.20±4.76	141.90±52.71	142.95±52.38	241.80±4.76	158.10±52.71†	157.05±52.38
AFPD (250mg/kg**) + $HgCl_2$** (5mg/kg)	51.10±6.81	100.05±4.43†	93.60±4.60†	248.90±6.81	199.95±4.43†	206.40±4.60†
AFPD (500mg/kg**) + $HgCl_2$** (5mg/kg)	49.60±5.46	200.55±57.76†	197.85±58.99	250.40±5.46	99.45±57.76†	102.15±58.99
AFPD (1000mg/kg**)+ $HgCl_2$** (5mg/kg)	43.05±5.36	197.25±59.33†	195.00±60.63	256.95±5.36	102.75±59.33†	105.00±60.63

n = 4; Mean ± SEM; Paired sample *t*- test; †=p<0.05; ††=p<0.01; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test* p>0.05 *when compared with the control.* **AFPD=** *Aqueous fruit extract of Phoenix dactylifera*; **OAD**=Open arm duration; **CAD**=Closed arm duration.

Table 4.5b: Effect of Ethanol Fruit Extract of *P. dactylifera* on Anxiety-like Behaviour (OAD and CAD) of Wistar Rats in Elevated Plus Maze

Group/ treatment	OAD Pre-treatment (s)	OAD day-7 (s)	OAD day-14 (s)	CAD Pre-treatment (s)	CAD day-7 (s)	CAD day-14 (s)
Control (H_2O 0.5ml)	40.20±1.75	98.40±5.70††	146.85±51.19††	259.80±1.75	201.60±5.70††	153.15±51.19††
$HgCl_2$ (5mg/kg)	43.85±5.80	145.20±51.66	139.95±53.35	256.15±5.80	154.80±51.66	160.05±53.35
Vit C (100mg/kg) + **$HgCl_2$** (5mg/kg)	58.20±4.76	141.90±52.71	142.95±52.38	241.80±4.76	158.10±52.71	157.05±52.38
EFPD (250mg/kg) + **$HgCl_2$** (5mg/kg)	49.65±8.45	195.75±60.22†	200.85±57.31	250.35±8.45	104.25±60.22†	99.15±57.31
EFPD (500mg/kg **)+ $HgCl_2$** (5mg/kg)	47.23±4.61	201.75±56.78†	197.10±59.54	252.78±4.61	98.25±56.78†	102.90±59.54
EFPD (1000mg/kg**) + $HgCl_2$** (5mg/kg)	45.48±8.51	195.75±60.22	199.80±57.90	254.53±8.51	104.25±60.22	100.20±57.90

n = 4; Mean ± SEM; Paired sample *t*- test; †=p<0.05; ††=p<0.01; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test* p>0.05 *when compared with the control*. **EFPD**= *Ethanol fruit extract of Phoenix dactylifera*; **OAD**=Open arm duration; **CAD**=Closed arm duration.

and control relative to entry frequency into the closed arm across the period of study showed striking difference, especially at day -7 treatment (**Table 4.5c**).

EFPD-treated groups showed reduced entry frequency into the closed arm across the period of study, except EFPD (500 mg/kg)-treated group. Closed arm entry frequency compared between the control and EFPD-treated groups, across the period of study, showed striking difference (**Table 4.5d**).

Relative to the frequency of grooming and rearing in the closed arm, and head dipping in open arm, control group Wistar rats revealed decreased level of anxiety across the period of study by reduced grooming and head dipping frequencies. Mercury-treated group showed increased anxiety levels by increased grooming, rearing and head dipping frequencies (from day-7 to day-14 treatment). Vitamin C- treated group revealed decreased anxiety levels across the period of study, remarkable ($p<0.05$) in rearing and head dipping frequencies. AFPD-treated groups showed decreased anxiety levels across the period of study, except AFPD (500 mg/kg)-treated group. Besides, striking differences were observed, especially in grooming and rearing, when AFPD-treated groups were compared with the mercury-treated group (**Table 4.5e**).

EFPD-treated groups, relative to rearing and head dipping frequencies, showed decreased anxiety levels across the period of study, except EFPD (1,000 mg/kg)-treated group. Besides, comparison with mercury-treated group revealed remarkable ($p<0.05$) differences in grooming and rearing frequencies, especially at day-14 treatment (**Table 4.5f**).

Table 4.5c: Effect of Aqueous Fruit Extract of *P. dactylifera* on Anxiety-like Behaviour (OAF and CAF) of Wistar Rats in Elevated Plus Maze

Group/ treatment	OAF Pre-treatment	OAF day-7	OAF day-14	CAF Pre-treatment	CAF day-7	CAF day-14
Control (H_2O 0.5ml)	1.46±0.04	0.75±0.25†	0.75±0.25†	3.04±0.34	1.75±0.25†	1.00±0.41†
$HgCl_2$ (5mg/kg)	1.63±0.13	0.50±0.29†	0.75±0.25†	2.50±0.10	0.75±0.25^{a}	1.25±0.48
Vit C (100mg/kg) **+ $HgCl_2$** (5mg/kg)	2.13±0.38	1.75±0.75	1.75±0.75	3.34±0.30	0.75±0.25^{a††}	0.50±0.29††
AFPD (250mg/kg) **+ $HgCl_2$** (5mg/kg)	2.13±0.31	1.50±0.29	0.50±0.29††	3.34±0.93	1.00±0.00	1.00±0.00
AFPD (500mg/kg **)+ $HgCl_2$** (5mg/kg)	2.00±0.35	0.75±0.25†	0.75±0.25†	2.42±0.52	0.50±0.29^{b}	0.50±0.29
AFPD (1000mg/kg**) + $HgCl_2$** (5mg/kg)	1.25±0.14	1.25±0.75	0.50±0.29	2.58±0.53	0.50±0.29^{b†}	0.75±0.25

n = 4; Mean ± SEM; Paired sample *t*- test; †=p<0.05; ††=p<0.01; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test* **a=** p<0.05; **b=** p<0.01 *when compared with the control.* **AFPD=** *Aqueous fruit extract of Phoenix dactylifera*; **OAF**=Open arm frequency; **CAF**=Closed arm frequency.

Table 4.5d: Effect of Ethanol Fruit Extract of *P. dactylifera* on Anxiety-like Behaviour (OAF and CAF) of Wistar Rats in Elevated Plus Maze

Group/ treatment	OAF Pre-treatment	OAF day-7	OAF day-14	CAF Pre-treatment	CAF day-7	CAF day-14
Control (H_2O 0.5ml)	1.46±0.04	0.75±0.25†	0.75±0.25†	3.04±0.34	1.75±0.25†	1.00±0.41†
$HgCl_2$ (5mg/kg)	1.63±0.13	0.50±0.29†	0.75±0.25†	2.50±0.10	0.75±0.25 ^{a}	1.25±0.48
Vit C (100mg/kg) **+ $HgCl_2$** (5mg/kg)	2.13±0.38	1.75±0.75	1.75±0.75	3.34±0.30	0.75±0.25 ^{a††}	0.50±0.29††
EFPD (250mg/kg) **+ $HgCl_2$** (5mg/kg)	2.25±0.48	1.50±0.87	0.75±0.48	3.17±0.35	1.00±0.58	0.50±0.29†
EFPD (500mg/kg **)+ $HgCl_2$** (5mg/kg)	2.29±0.29 ^{a}	0.75±0.48	1.00±0.58	3.33±0.24	0.50±0.29 ^{b††}	1.00±0.58†
EFPD (1000mg/kg**) + $HgCl_2$** (5mg/kg)	1.21±0.12	1.25±0.25	1.00±0.00	2.42±0.50	0.50±0.29 ^{b†}	0.50±0.29†

n = 4; Mean ± SEM; Paired sample *t*- test; †=p<0.05; ††=p<0.01; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test* **a=** p<0.05; **b=** p<0.01 *when compared with the control*. **EFPD**= *Ethanol fruit extract of Phoenix dactylifera*; **OAF**=Open arm frequency; **CAF**=Closed arm frequency.

Table 4.5e: Effect of Aqueous Fruit Extract of *P. dactylifera* on Anxiety-like Behaviour (GR, RR and HD) of Wistar Rats in Elevated Plus Maze

Group/ treatment	GR Pre-treatment	GR day-7	GR day-14	RR Pre-treatment	RR day-7	RR day-14	HD Pre-treatment	HD day-7	HD day-14
Control (H_2O 0.5ml)	4.58±1.11	2.25±0.75	1.50±0.65	6.67±1.13	1.75±0.25†	2.00±0.00††	3.00±0.49	1.75±1.11	1.75±1.11
$HgCl_2$ (5mg/kg)	4.42±1.08	1.00±0.00	3.50±0.87^{a}	5.75±0.90	1.75±0.25	2.25±0.25	1.84±0.35	0.50±0.29	0.75±0.25
Vit C (100mg/kg) + **$HgCl_2$** (5mg/kg)	4.17±0.87	2.75±1.03	1.50±0.50*	8.00±0.95	2.25±0.85††	0.75±0.48*††	2.83±0.44	0.50±0.29†	0.50±0.29†
AFPD (250mg/kg) + **$HgCl_2$** (5mg/kg)	3.92±0.42	1.75±0.48†	1.00±0.00*††	6.67±1.17	1.50±0.50†	1.75±0.48††	3.34±1.50	0.75±0.25	0.75±0.25
AFPD (500mg/kg **)+** **$HgCl_2$** (5mg/kg)	5.75±0.37	1.25±0.75††	2.00±1.22†	7.29±0.99	1.50±0.96†	1.75±1.03†	3.21±0.41	0.75±0.25††	0.75±0.25††
AFPD (1000mg/kg**) +** **$HgCl_2$** (5mg/kg)	2.83±0.44	1.75±1.18	1.00±0.71*	5.92±1.50	1.25±0.95††	0.75±0.48*†	2.50±0.68	0.75±0.48†	0.75±0.48

n = 4; Mean ± SEM; Paired sample *t*- test; †=p<0.05; ††=p<0.01; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test* **a/*=**p<0.05; **b=**p<0.01; **c=**p<0.001 *when compared with the control/ $HgCl_2$.* **AFPD=** *Aqueous fruit extract of Phoenix dactylifera*; **EFPD**= *Ethanol fruit extract of Phoenix dactylifera*; **GR**=Grooming; **RR**=Rearing; **HD**=Head dipping

Table 4.5f: Effect of Ethanol Fruit Extract of *P. dactylifera* on Anxiety-like Behaviour (GR, RR and HD) of Wistar Rats in Elevated Plus Maze

Group/ treatment	GR Pre-treatment	GR day-7	GR day-14	RR Pre-treatment	RR day-7	RR day-14	HD Pre-treatment	HD day-7	HD day-14
Control (H_2O 0.5ml)	4.58±1.11	2.25±0.75	1.50±0.65	6.67±1.13	1.75±0.25†	2.00±0.00††	3.00±0.49	1.75±1.11	1.75±1.11
$HgCl_2$ (5mg/kg)	4.42±1.08	1.00±0.00	3.50±0.87^{a}	5.75±0.90	1.75±0.25	2.25±0.25	1.84±0.35	0.50±0.29	0.75±0.25
Vit C (100mg/kg) + **$HgCl_2$** (5mg/kg)	4.17±.87	2.75±1.03	1.50±0.50*	8.00±0.95	2.25±0.85††	0.75±0.48*††	2.83±0.44	0.50±0.29†	0.50±0.29†
EFPD (250mg/kg) + **$HgCl_2$** (5mg/kg)	4.08±.22	0.75±0.48††	0.75±0.48**††	6.92±0.97	2.50±1.44	0.50±0.29 ^{a}*†	3.00±1.20	0.75±0.48	1.25±0.95
EFPD (500mg/kg **)+** **$HgCl_2$** (5mg/kg)	3.75±.75	1.50±0.87	0.75±0.48**†	5.96±0.94	0.50±0.29††	0.50±0.29 ^{a}*††	1.96±0.38	0.50±0.29††	2.00±1.15
EFPD (1000mg/kg**) +** **$HgCl_2$** (5mg/kg)	4.67±.83	0.50±0.29†	0.75±0.48**	4.59±0.85	1.25±0.75	0.50±0.29 ^{a}*	2.21±0.49	1.00±0.00†	1.50±0.29

n = 4; Mean ± SEM; Paired sample *t*- test; †=p<0.05; ††=p<0.01; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test* **a/*=**p<0.05; **b/**=**p<0.01; **c=**p<0.001 *when compared with the control/ $HgCl_2$.* **AFPD=** *Aqueous fruit extract of Phoenix dactylifera*; **EFPD**= *Ethanol fruit extract of Phoenix dactylifera*; **GR**=Grooming; **RR**=Rearing; **HD**=Head dipping

4.5.2 TL on EPM open arm

The elevated plus maze served as the exteroceptive behavioural model (wherein the stimulus existed outside the body) to evaluate learning and memory in Wistar rats. Learning behaviour of Wistar rats were reflected by transfer latency (TL) on the first day (pre-treatment) and retention of information or memory reflected by TL after 24 hours, day-7 and day-14 of treatments. Reduction in TL of 24 hours in all the Wistar rats (of both study categories), except AFPD (1,000 mg/kg)-treated group, indicated improvement on learning and memory. Across the period of study (24 hours through day-14 of treatment), varying levels of retention were observed in the control, mercury-treated and vitamin C-treated groups.

Administration of AFPD, especially at day-14 of treatment, revealed reduction ($p>0.05$) in TL when compared the mercury-treated group, and remarkable ($p<0.05$) improvement on learning and memory by reduction in TL compared to the control (**Figure 4.4a**).

Administration of EFPD, especially at day-14 of treatment, revealed reduction in TL, particularly ($p<0.05$) in EFPD (500 mg/kg)-treated group, when compared to the mercury-treated group. Comparison with the control revealed remarkable ($p<0.05$) improvement on learning and memory by reduction in TL (**Figure 4.4b**).

4.5.3 MWM Studies

Morris water maze, a well validated test for spatial learning and memory was used to assess learning and memory in experimental animals (Wistar rats). Performance in the training/ habituation sessions revealed reduced latency (*latency time*) to find the hidden platform from day-1 to day-3 of the training sessions (**Figure 4.5a**).

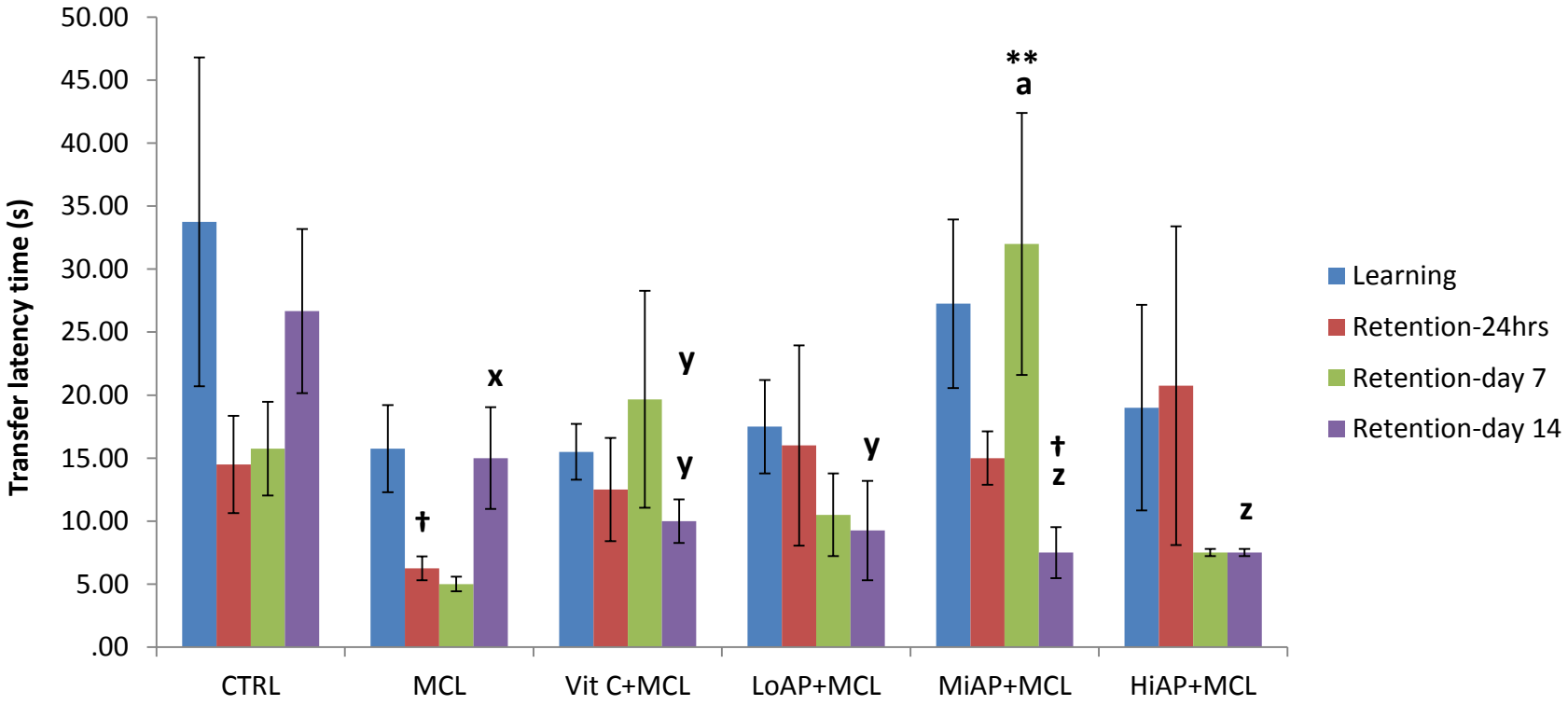


Figure 4.4a: Effect of aqueous fruit extract of *P. dactylifera* on transfer latency of Wistar rats in Elevated plus maze open arm

n = 4; mean ± SEM; Paired sample *t*- test; †=p<0.05 *when compared with the* learning; One way ANOVA *LSD post hoc test.* ***a/x****=p<0.05;* ***b/y*** *= p<0.01;* ***c/z*** *= p<0.001 when compared with the* control (day-7/ day-14); ****= p<0.01 when compared with the* $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

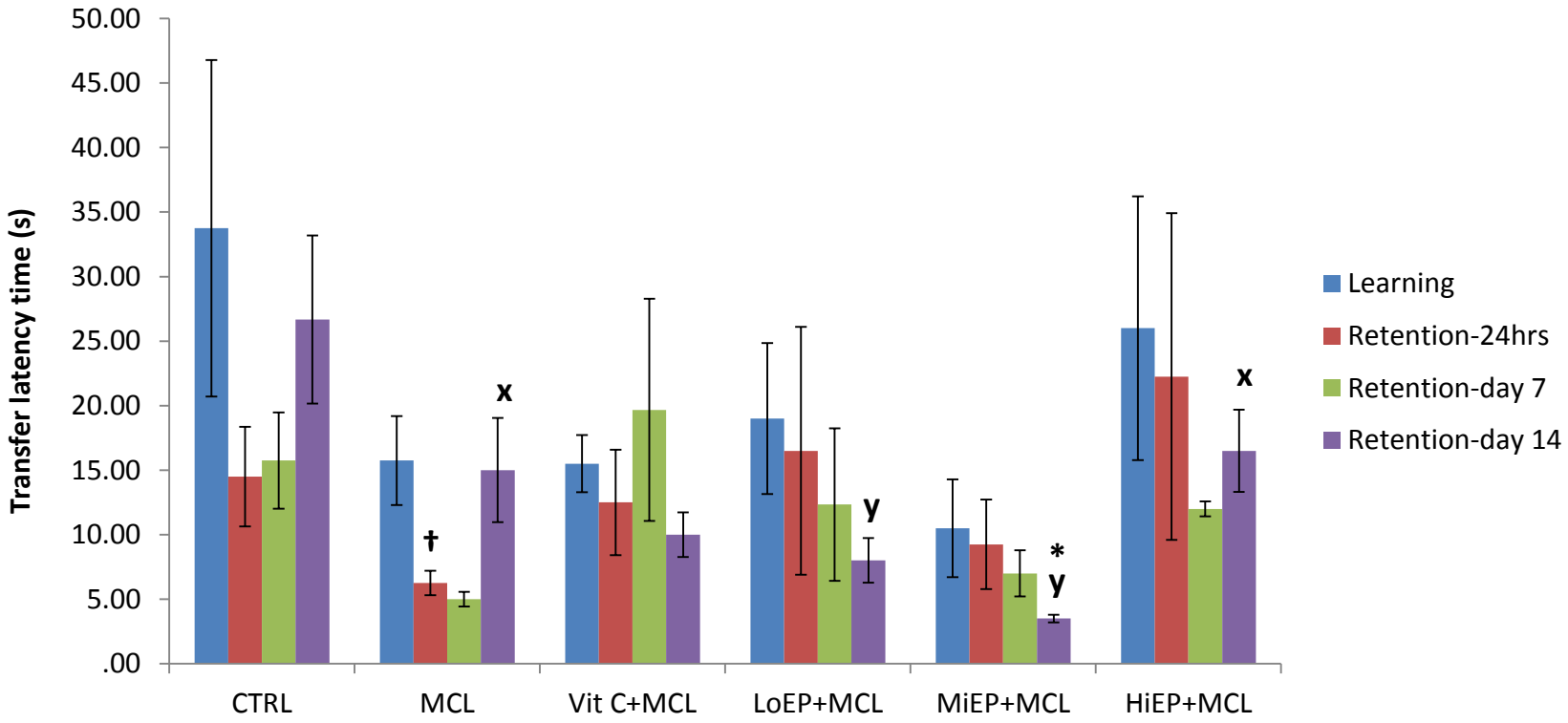


Figure 4.4b: Effect of ethanol fruit extract of *P. dactylifera* on transfer latency of Wistar rats in Elevated plus maze open arm

n = 4; mean ± SEM; Paired sample *t*- test; †=p<0.05 *when compared with the* learning; One way ANOVA *LSD post hoc test.* ***a/x** = p<0.05; **b/y** = p<0.01 when compared with the* control (day-7/ day-14); ***** = *p<0.05 when compared with the* $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

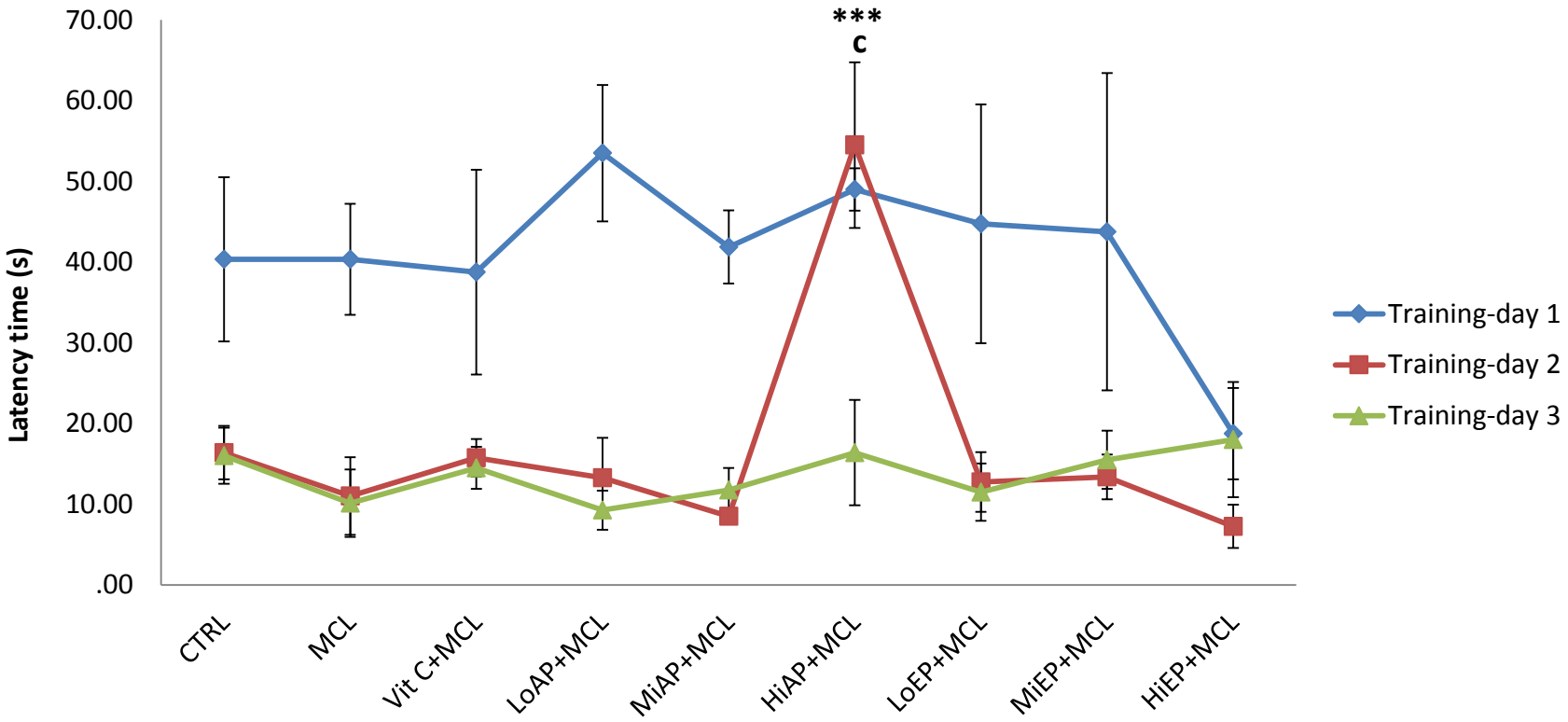


Figure 4.5a: Transfer latency of Wistar rats on Morris water maze habituation.

n = 4; mean ± SEM; One way ANOVA *LSD post hoc test.* ***c*= *p<0.001 when compared with the* control; ***= *p<0.001 when compared with the* $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

Reduction in latency in all the Wistar rats (of both study categories) compared to the pre-treatment indicated improvement on learning and memory. However, at day-14 of treatment, mercury-treated group showed learning and memory impairment by increased latency, while vitamin C-treatment showed decreased latency when compared to the control. Administration of AFPD, especially at day-14 of treatment, ameliorated memory impairment following mercuric intoxication by decreased latency when compared to the mercury-treated group (**Figure 4.5b**).

At day-14 of treatment, administration of EFPD revealed a dose dependent decrease in latency time when compared to the mercury-treated group (**Figure 4.5c**).

4.5.4 FGS Studies

Forelimb grip strength test was used as a measure of the muscular strength in the forelimbs (forepaws) of the Wistar rats. Forelimb grip strength time (latency time-to-release-grip) reflects the FGS. Remarkable ($p<0.05$) improvement on FGS was observed with vitamin C-treated group when compared to the control and mercury-treated groups. Across the period of study (day-7 through day-14 of treatment), administration of AFPD had no effect on FGS when compared to the mercury-treated group (**Figure 4.6a**).

Administration of EFPD at dose 1,000 mg/kg significantly ($p<0.05$) improved FGS when compared to the control and mercury-treated groups across the period of study (**Figure 4.6b**).

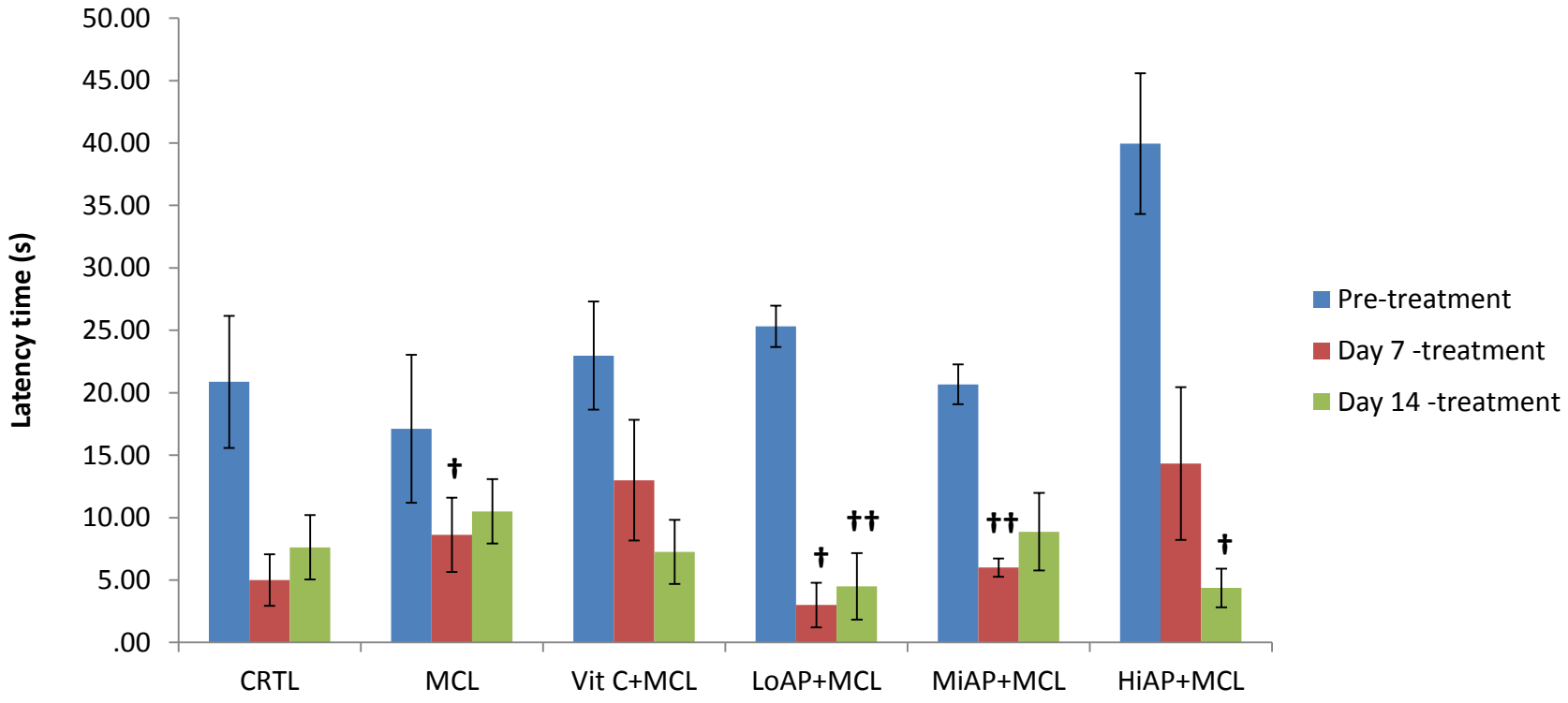


Figure 4.5b: Effect of aqueous fruit extract of *P. dactylifera* on transfer latency of Wistar rats in Morris water maze

n = 4; mean ± SEM; Paired sample *t*- test; † = *p<0.05*; †† = *p<0.01*; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test:* ***p>0.05;*** *when compared with the* control (day-7/ day-14)/ $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

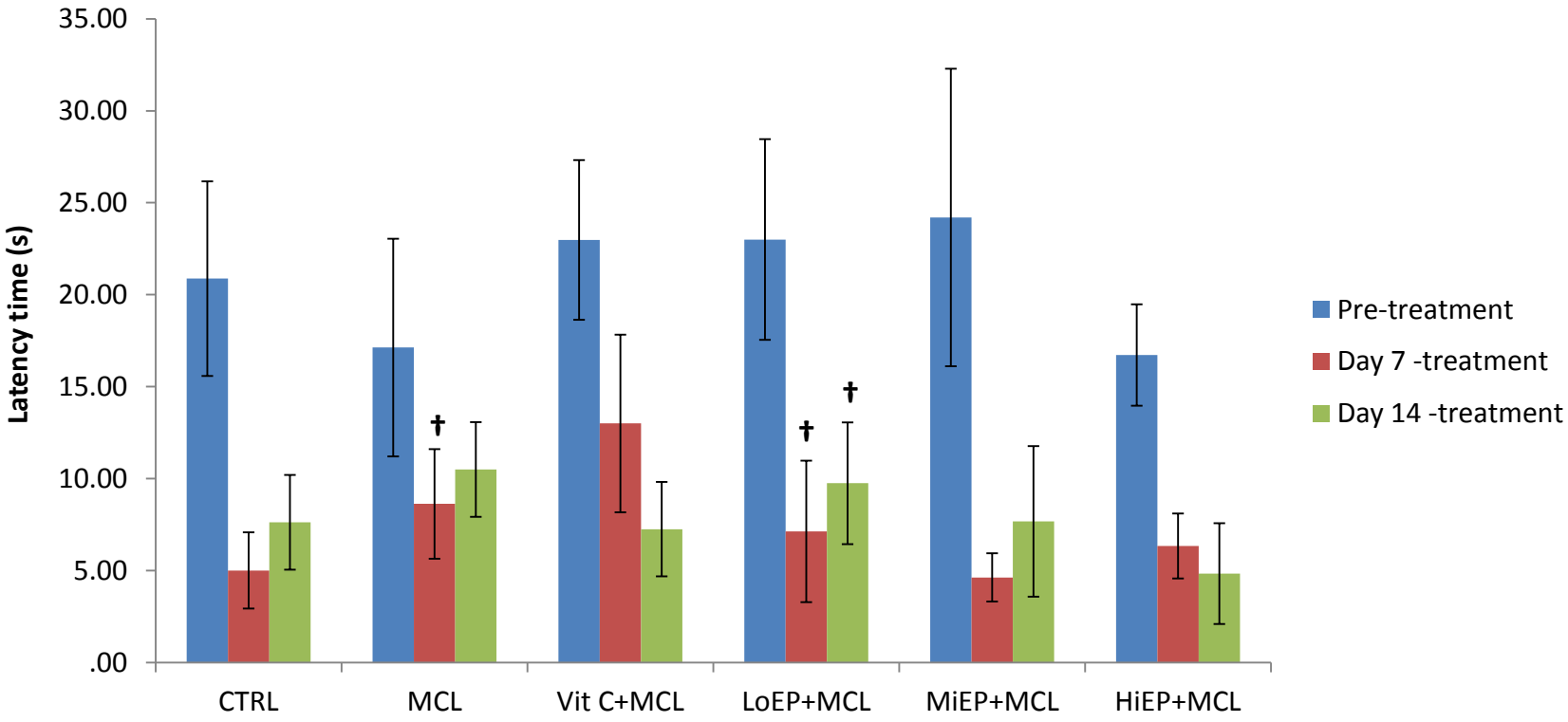


Figure 4.5c: Effect of ethanol fruit extract of *P. dactylifera* on transfer latency of Wistar rats in Morris water maze

n = 4; mean ± SEM; Paired sample *t*- test; †= $p<0.05$; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test:* ***p>0.05;*** *when compared with the* control/ $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

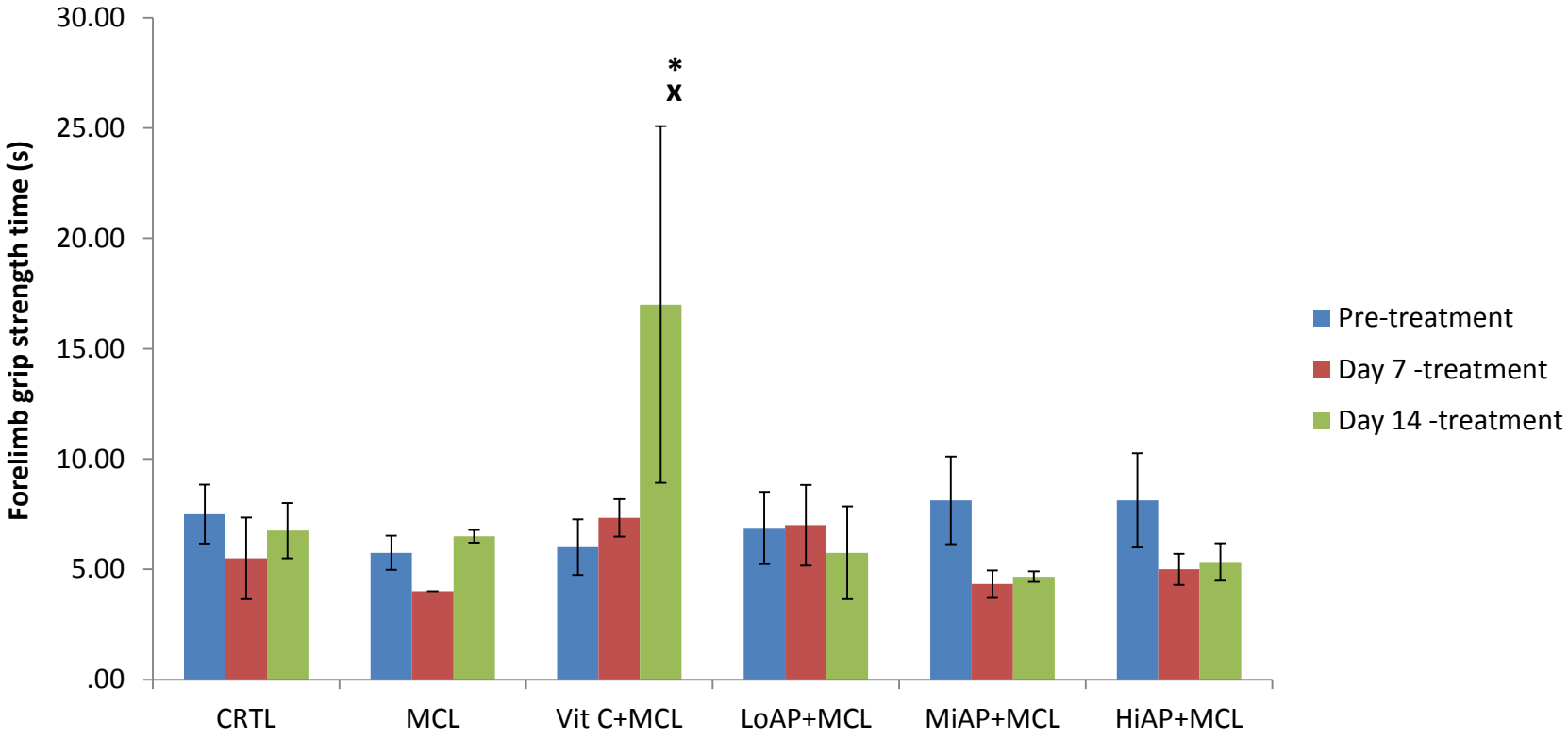


Figure 4.6a: Effect of aqueous fruit extract of *P. dactylifera* on Forelimb grip strength of Wistar rats

n = 4; mean ± SEM; Paired sample *t*- test; *p>0.05*; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test:* ***x/*= p<0.05 when compared with the*** control (day-14)/ $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

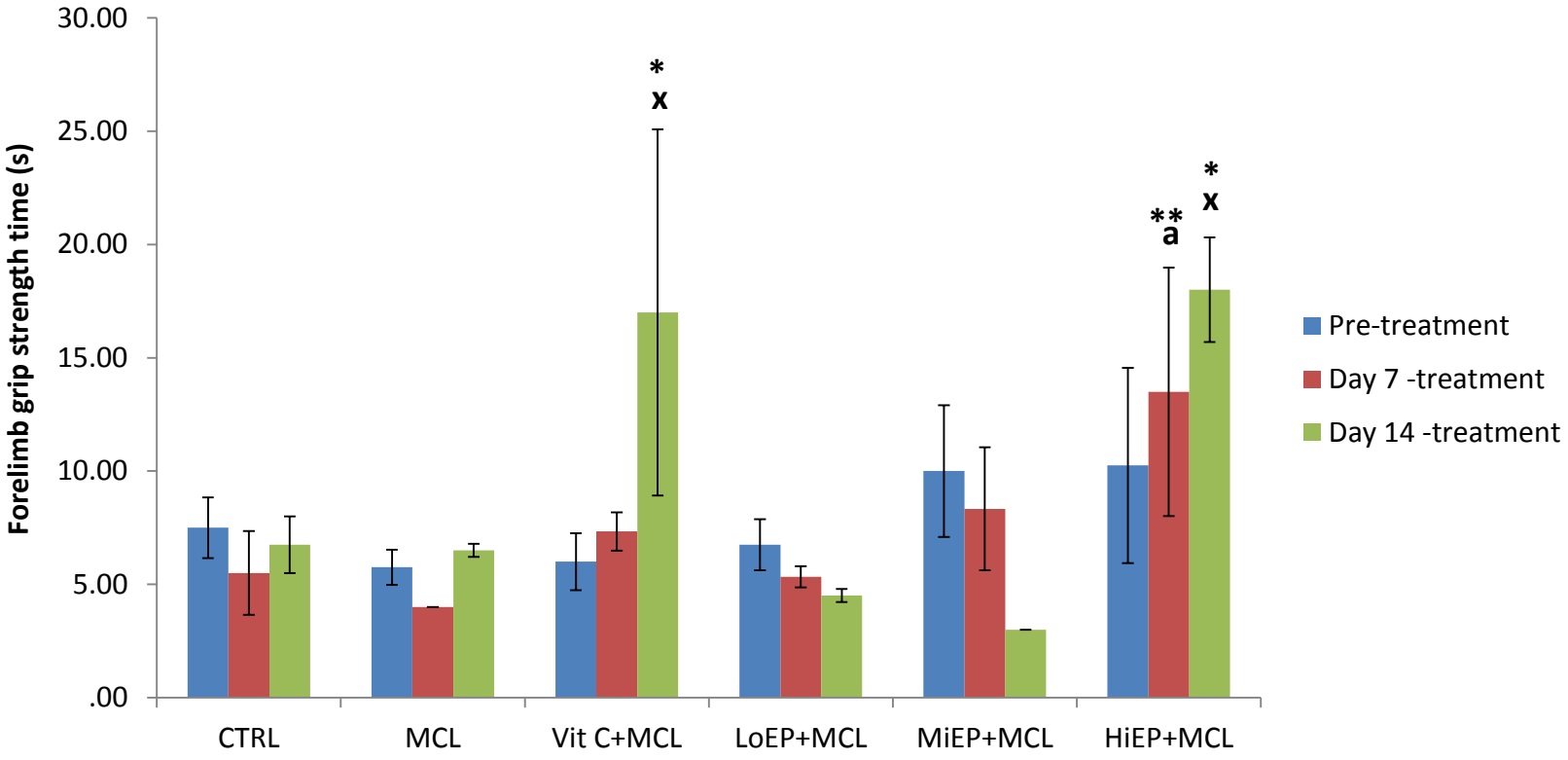


Figure 4.6b: Effect of ethanol fruit extract of *P. dactylifera* on Forelimb grip strength of Wistar rats

n = 4; mean ± SEM; Paired sample t- test; *p>0.05*; when compared with the Pre-treatment. One way ANOVA LSD post hoc test: **a/x** = *p<0.05*; when compared with the control (day-7/ day-14); ***** = *p<0.05*; ******= *p<0.01*; when compared with the $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

4.5.5 BWT Studies

Beam walking test was used to assess motor coordination and balance in the Wistar rats. Beam walking ability was measured using beam (foot-slips) score (a 6-point scoring system scale) and latency (time) until the Wistar rat's nose entered the goal box.

Across the period of study (pre-treatment through day-14 of treatment), administration of extract (AFPD or EFPD) had no effect on beam score when compared to the control and mercury-treated groups. In the study categories (A and B), comparison between pre-treatment and treatments revealed deficit in motor coordination and balance, especially at day-14 of treatment, remarkable ($p<0.05$) in the AFPD (1,000 mg/kg)-treated group (**Figure 4.7a and 4.7b**).

Relative to beam walk latency, administration of AFPD had no effect on beam walking performance when compared to the control across the period of study (day-7 through day-14 of treatment). However, comparison with mercury-treated group showed improvement on motor coordination and balance, especially ($p<0.05$) in AFPD (1,000 mg/kg)-treated group at day-7 of treatment (**Figure 4.7c**).

Administration of EFPD, especially ($p<0.01$) 1,000 mg/kg EFPD-treated group, showed improvement on motor coordination and balance in comparison with mercury-treated group at day-7. However, deficit in beam walking performance ($p<0.01$) was observed with 250 mg/kg EFPD-treated group when compared to the control and mercury-treated groups at day-14 (**Figure 4.7d**).

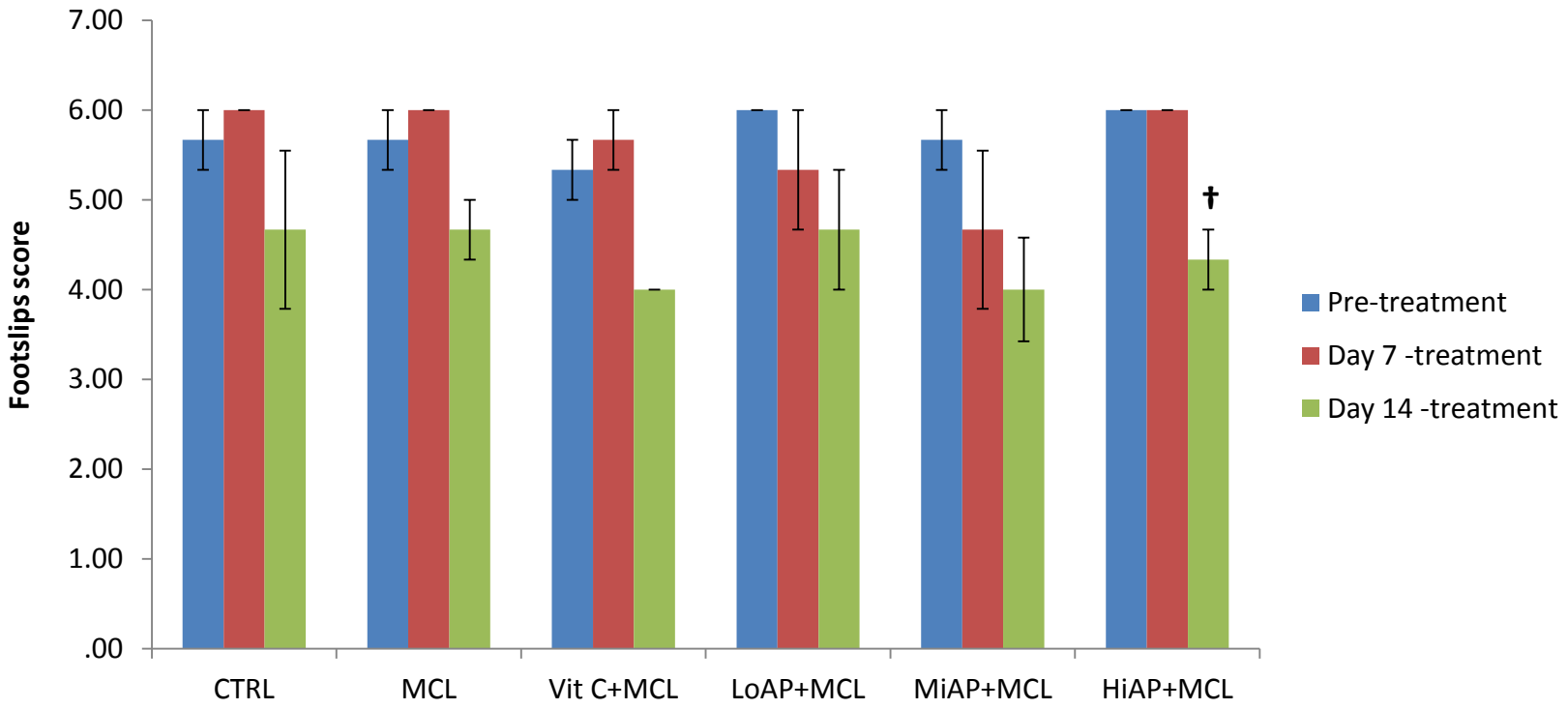


Figure 4.7a: Effect of aqueous fruit extract of *P. dactylifera* on Beam walking test of Wistar rats

n = 4; mean ± SEM; Paired sample *t*- test; †= p<0.05; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test:* ***p>0.05*** *when compared with the* control/ $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

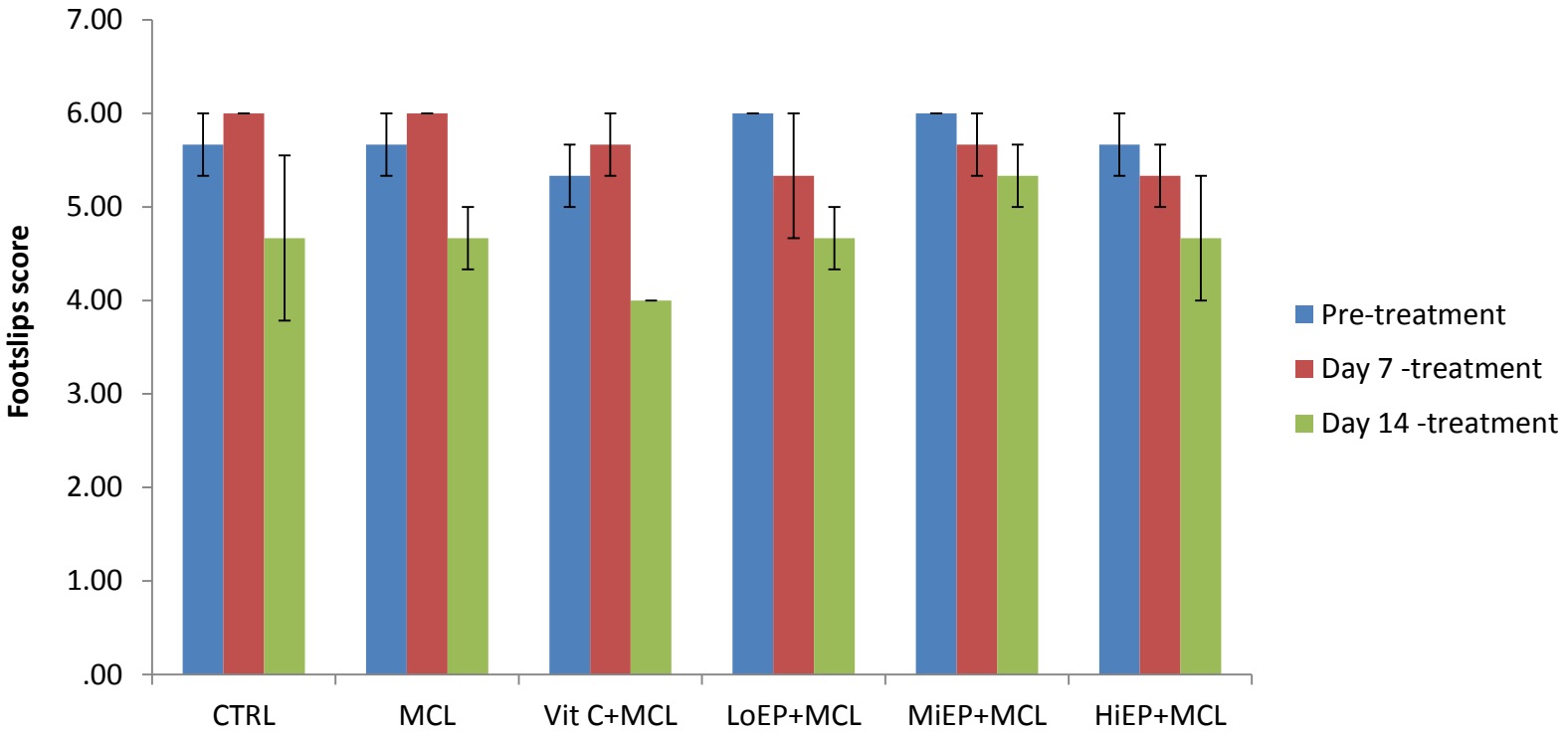


Figure 4.7b: Effect of ethanol fruit extract of *P. dactylifera* on Beam walking test of Wistar rats

n = 4; mean ± SEM; Paired sample *t*- test; *p>0.05*; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test:* ***p>0.05*** *when compared with the* control/ $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

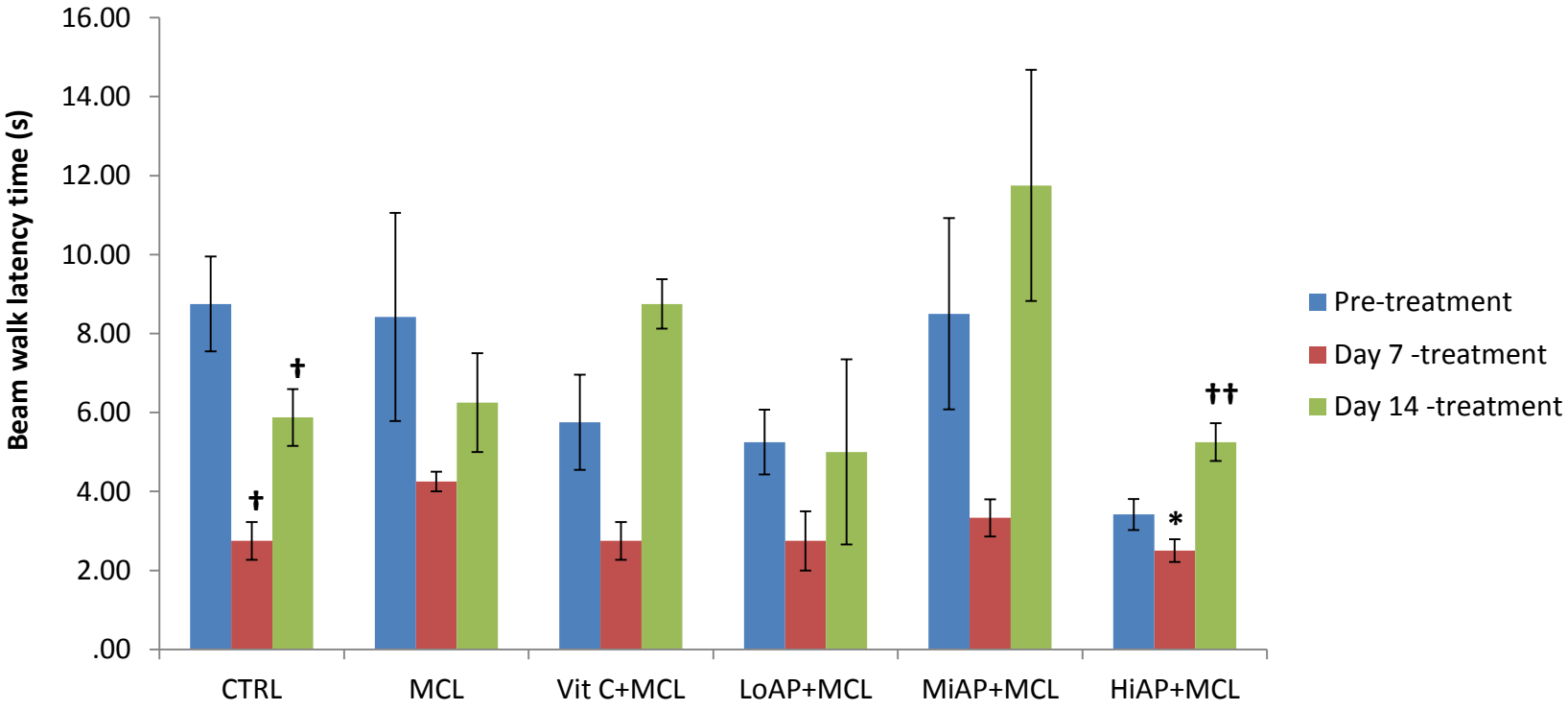


Figure 4.7c: Effect of aqueous fruit extract of *P. dactylifera* on Beam walking test of Wistar rats

n = 4; mean ± SEM; Paired sample *t*- test; † = p<0.05; †† = p<0.01; *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test:* ***p>0.05;*** *when compared with the* control; ****=*** p<0.05 *when compared with the* $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

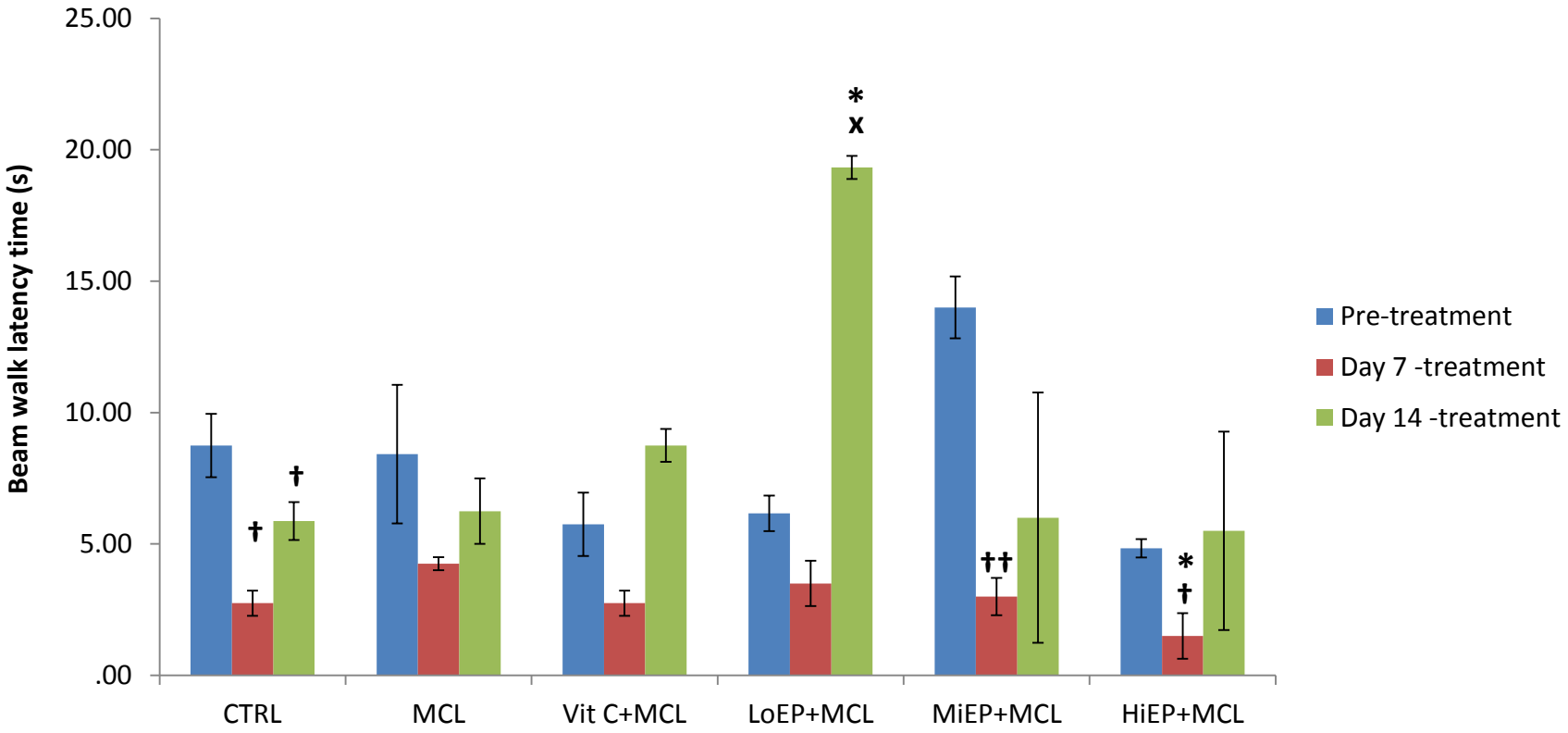


Figure 4.7d: Effect of ethanol fruit extract of *P. dactylifera* on Beam walking test of Wistar rats

n = 4; mean ± SEM; Paired sample *t*- test; †= p<0.05; ††= p<0.001 *when compared with the* Pre-treatment. One way ANOVA *LSD post hoc test:* ***x*/*= *p<0.01; when compared with the* control/ $HgCl_2$ group. **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

4.6 Neurochemical/ Biochemical Studies

Neurochemical analysis for metal estimation assessed the concentration of neuro-trace elements (copper, Cu; iron, Fe; manganese, Mn and zinc, Zn) in tissue (brain) homogenate. Biochemical analysis for lipid peroxide levels and antioxidant enzyme activity (malondialdehyde, MDA; superoxide dismutase, SOD; catalase, CAT and glutathione peroxidase, GPx) were assessed in blood serum and brain-tissue homogenate, and acetylcholinesterase activity for endogenous enzyme in brain-tissue homogenate of Wistar rats.

4.6.1 Neurochemistry of Neuro-trace Elements

Remarkably ($p<0.05$) elevated level of manganese (Mn) concentration in the brain tissue of Wistar rats was observed in the mercury-treated group compared to the control. Vitamin C-treatment significantly ($p<0.05$) depressed the mercury-induced elevated Mn concentration level when compared to the mercury-treated group. Similarly, administration of AFPD, except 1,000 mg/kg AFPD-treatment, significantly ($p<0.05$) and dose dependently depressed the elevated levels of Mn concentration when compared to the mercury-treated group.

Iron (Fe) concentration levels, compared to the control, was elevated in the mercury-treated group and depressed with administration of AFPD, except 1,000 mg/kg AFPD-treatment. However, differences in Fe concentration levels were not remarkable (**Figure 4.8a**).

Administration of EFPD significantly ($p<0.05$) depressed the elevated levels of Mn concentration when compared to the mercury-treated group. Elevated Fe concentration level was depressed with administration of EFPD, especially with 500 mg/kg EFPD-

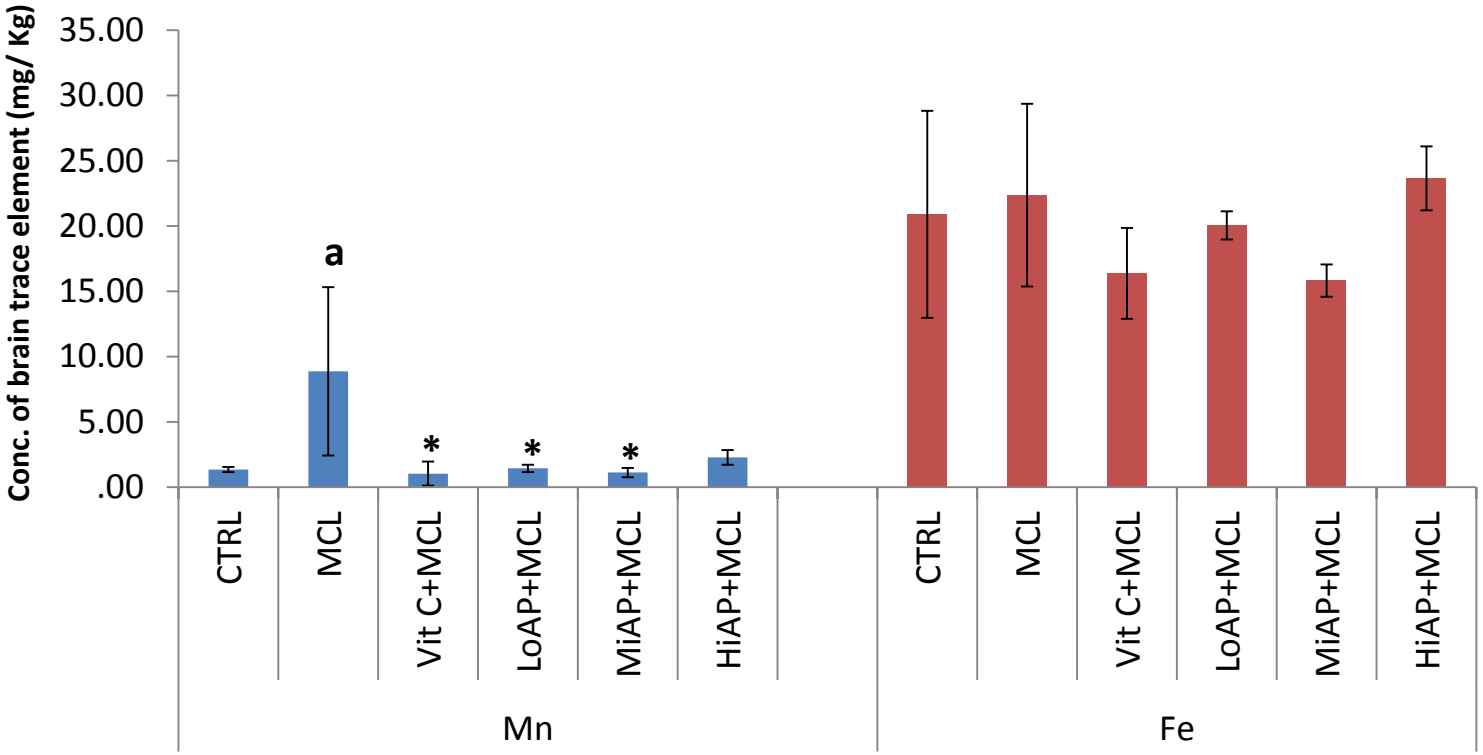


Figure 4.8a: Effect of aqueous fruit extract of *P. dactylifera* on concentration of brain trace elements (Mn and Fe) of Wistar rats

n = 4; mean ± SEM; One way ANOVA LSD post hoc test: **a/***=p<0.05; when compared with the control/ $HgCl_2$ group. **Mn**= Manganese; **Fe**= Iron; **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

treatment. However, differences in Fe concentration levels were not remarkable (**Figure 4.8b**).

Elevated ($p>0.05$) level of zinc (Zn) concentration in the brain tissue of Wistar rats was observed in the mercury-treated group compared to the control. Vitamin C- and AFPD administration, especially ($p<0.05$) 500 mg/kg AFPD-treatment, depressed the elevated levels of Zn concentration when compared to the mercury-treated group.

Copper (Cu) concentration levels, compared to the control, was elevated in the mercury-treated group, although not significantly ($p>0.05$). Vitamin C- and AFPD treatment depressed the elevated levels of Cu concentration when compared to the mercury-treated group. Difference was remarkable ($p<0.05$) with vitamin C-treatment (**Figure 4.8c**).

Administration of EFPD significantly ($p<0.05$) depressed the elevated levels of Zn concentration when compared to the mercury-treated group. Elevated Cu concentration level was depressed with administration of EFPD, especially ($p<0.05$) with 500 mg/kg EFPD-treatment when compared to the mercury-treated group (**Figure 4.8d**).

4.6.2 Biochemistry of Lipid Peroxide Levels and Antioxidant Enzyme Activity

The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assay was used to estimate the lipid peroxidation levels, while antioxidant enzymatic activity was estimated by assaying for superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in blood and brain tissue.

Tissue MDA levels increased significantly ($p<0.001$) in all the treated groups (categories **A** and **B** study) when compared to the control. Serum MDA levels increased in all the

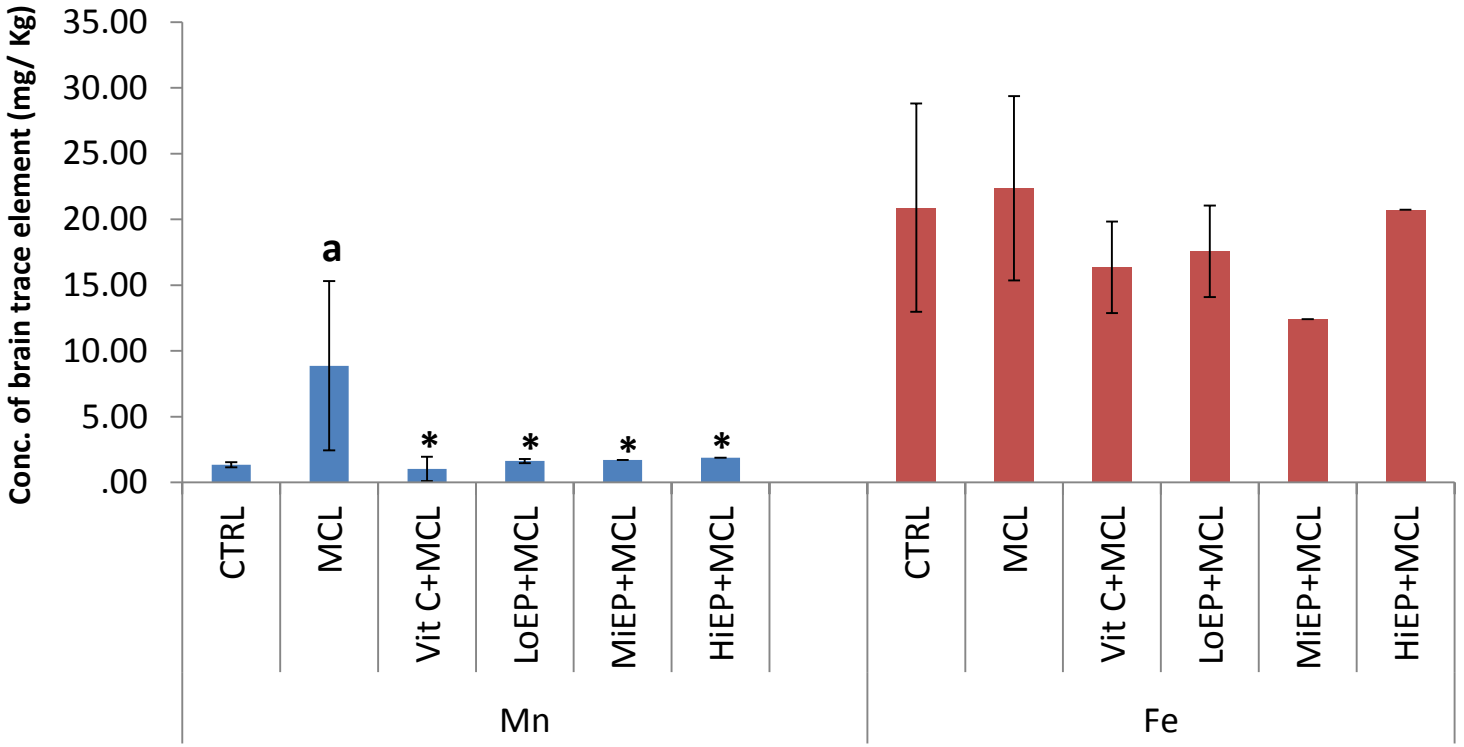


Figure 4.8b: Effect of ethanol fruit extract of *P. dactylifera* on concentration of brain trace elements (Mn and Fe) of Wistar rats

n = 4; mean ± SEM; One way ANOVA LSD post hoc test: **a/***=$p<0.05$; when compared with the control/ $HgCl_2$ group. **Mn**= Manganese; **Fe**= Iron; **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

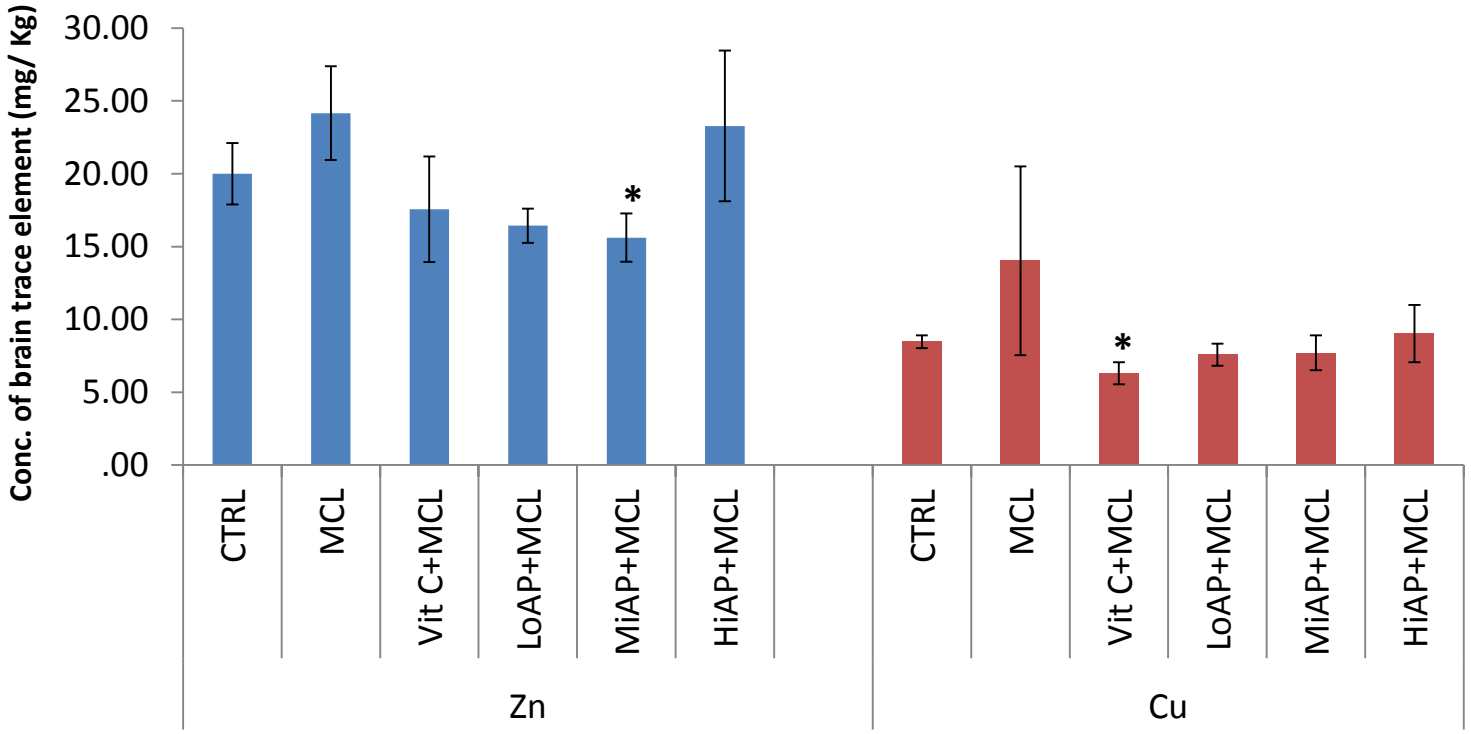


Figure 4.8c: Effect of aqueous fruit extract of *P. dactylifera* on concentration of brain trace elements (Zn and Cu) of Wistar rats

n = 4; mean ± SEM; One way ANOVA LSD post hoc test: $p>0.05$ when compared with the control; *****=$p<0.05$; when compared with the $HgCl_2$ group. **Zn**= Zinc; **Cu**= Copper; **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

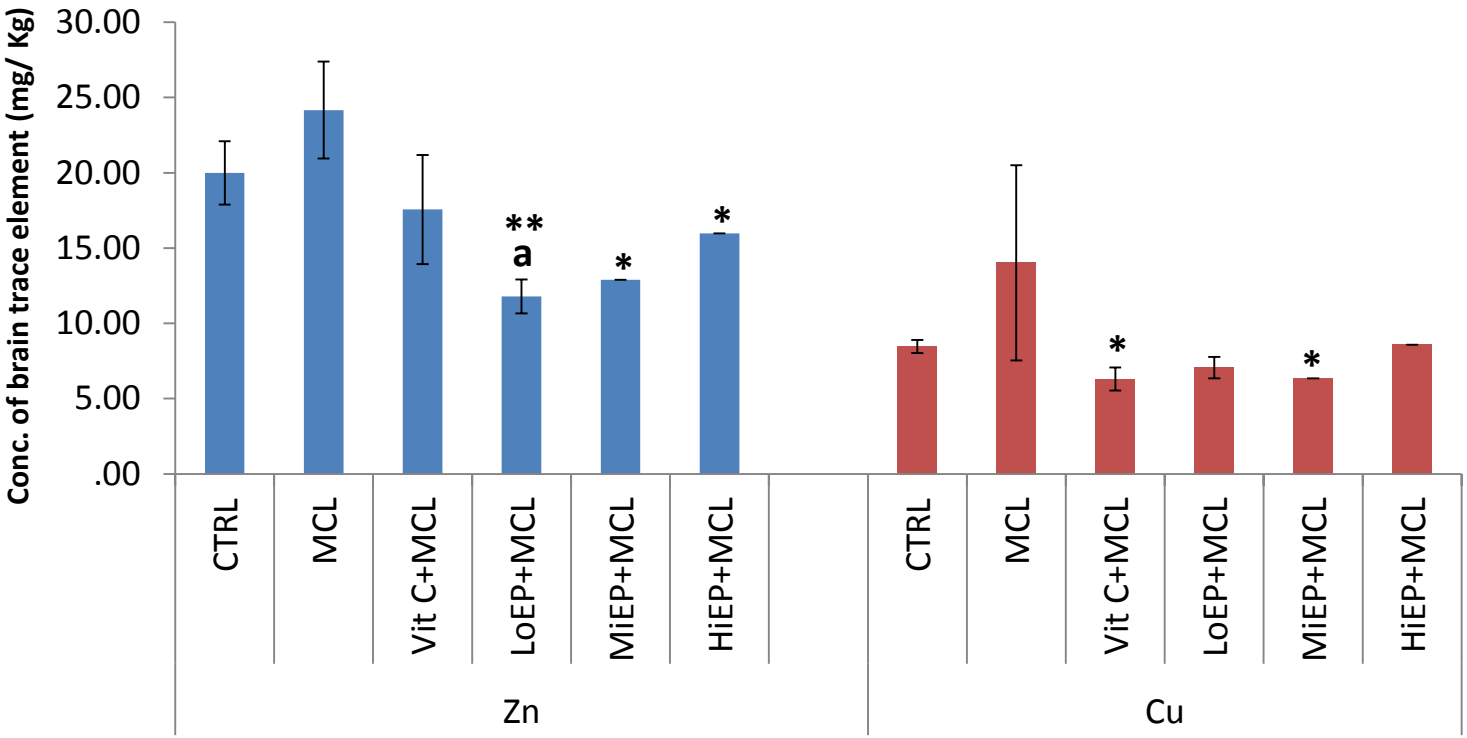


Figure 4.8d: Effect of ethanol fruit extract of *P. dactylifera* on concentration of brain trace elements (Zn and Cu) of Wistar rats

n = 4; mean ± SEM; One way ANOVA LSD post hoc test: **a**=p<0.05 when compared with the control; *=p<0.05, **=p<0.01 when compared with the $HgCl_2$ group. **Zn=** Zinc; **Cu=** Copper; **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

treated groups, especially with AFPD treatment (category A study) compared to the control. Serum MDA levels of EFPD (1,000 mg/kg)-treated group showed no difference when compared to mercury-treated group (**Figures 4.9a and 4.9b**).

Tissue SOD activity levels decreased remarkably ($p<0.05$) in all the treated groups (categories A and B study) when compared to the control. Tissue SOD activity levels increased ($p>0.05$) in EFPD (500 and 1,000 mg/kg)-treated groups when compared to mercury-treated group. Serum SOD activity levels decreased remarkably ($p<0.05$) in all the treated groups, except vitamin C-treated, compared to the control (**Figures 4.10a and 4.10b**).

Tissue CAT activity levels in all the treated groups (categories A and B study) showed no remarkable ($p>0.05$) difference, except vitamin C-treated, 1,000 mg/kg AFPD and 250 mg/kg EFPD -treated groups when compared to the control. Decreased levels in serum CAT activity were observed in all the treated groups, especially in mercury-, 1,000 mg/kg AFPD and 250 mg/kg EFPD -treated groups, when compared to the control (**Figures 4.11a and 4.11b**).

Tissue GPx activity levels decreased in all the treated groups (categories **A** and **B** study), especially ($p<0.05$) in mercury-, 1,000 mg/kg AFPD and 250 mg/kg EFPD -treated groups when compared to the control. Tissue GPx activity levels were observed to be higher in all the treated groups, except 1,000 mg/kg AFPD and 250 mg/kg EFPD-treated groups relative to mercury-treated group. Decreased serum GPx activity levels ($p<0.05$) were observed in all the treated groups, except in Vitamin C-treated group, when compared to the control (**Figures 4.12a and 4.12b**).

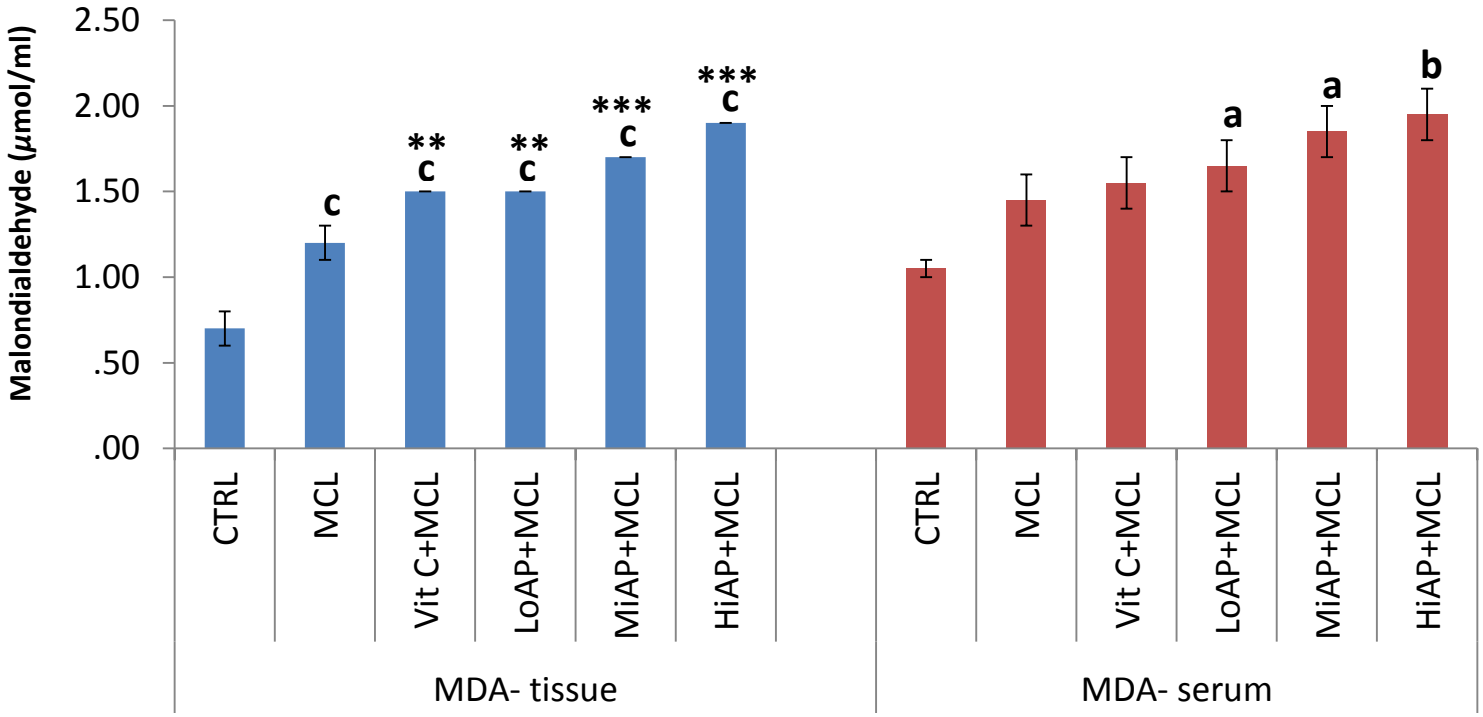


Figure 4.9a: **Effect of aqueous fruit extract of *P. dactylifera* on tissue (brain) and serum MDA concentration of Wistar rats**

n = 4; mean ± SEM; One way ANOVA *LSD post hoc test:* ***a*= *p<0.05*; *b*= *p<0.01; c*= *p<0.001 when compared with the* control; ***= *p<0.01;* ***= *p<0.001; when compared with the* $HgCl_2$ group. **MDA=** Malondialdehyde; **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

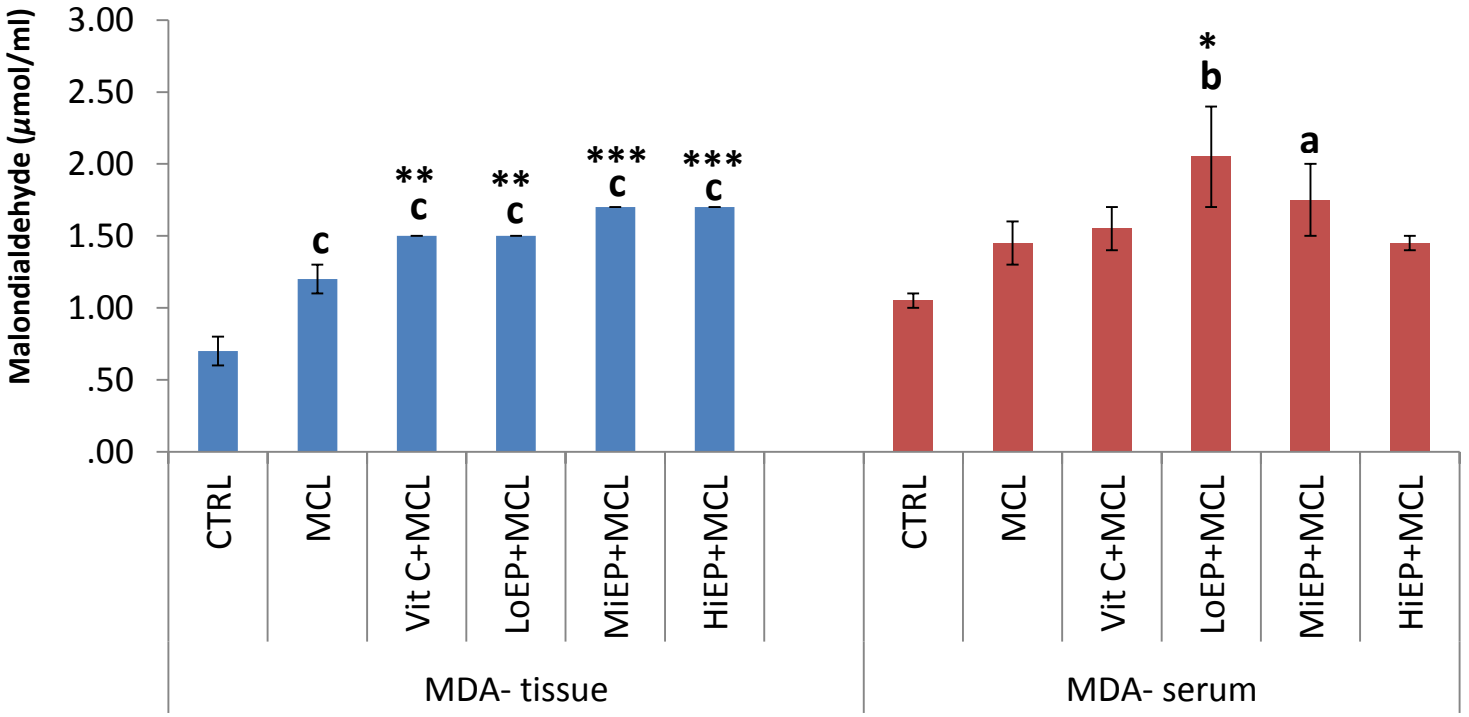


Figure 4.9b: **Effect of ethanol fruit extract of *P. dactylifera* on tissue (brain) and serum MDA concentration of Wistar rats**

n = 4; mean ± SEM; One way ANOVA *LSD post hoc test:* ***a*****=** *p<0.05;* ***b*****=** *p<0.01****; c*****=** *p<0.001 when compared with the* control; ***=** *p<0.05;****=** *p<0.01;* ****=** *p<0.001; when compared with the* $HgCl_2$ group. **MDA=** Malondialdehyde; **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

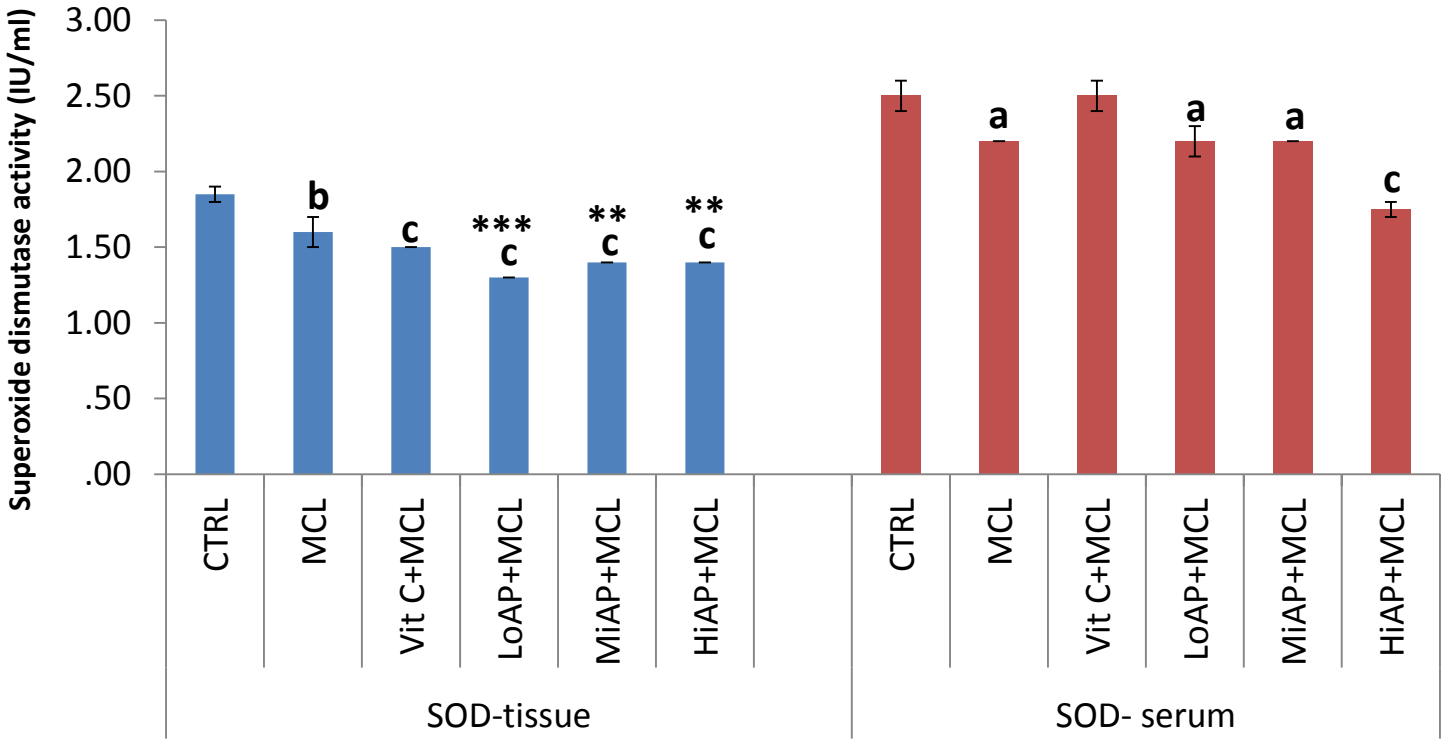


Figure 4.10a: **Effect of aqueous fruit extract of *P. dactylifera* on tissue (brain) and serum SOD activity of Wistar rats**

n = 4; mean ± SEM; One way ANOVA *LSD post hoc test:* ***a*= *p<0.05;* *b*= *p<0.01;* *c*= *p<0.001 when compared with the* control; ***= *p<0.01;* ****= *p<0.001; when compared with the* $HgCl_2$ group. **SOD=** Superoxide dismutase; **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

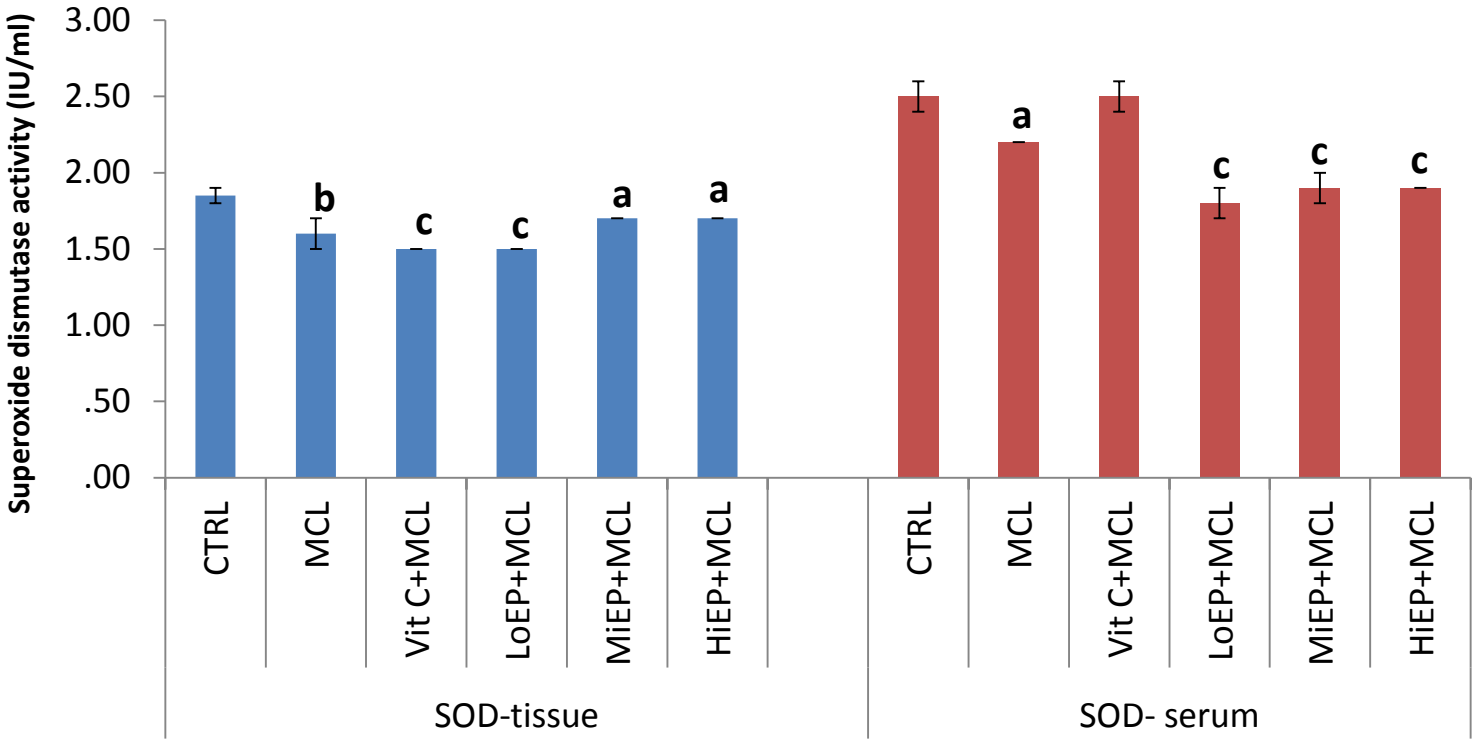


Figure 4.10b: **Effect of ethanol fruit extract of *P. dactylifera* on tissue (brain) and serum SOD activity of Wistar rats**

n = 4; mean ± SEM; One way ANOVA *LSD post hoc test:* ***a****= p<0.05;* ***b****= p<0.01;* ***c****= p<0.001 when compared with the* control. **SOD=** Superoxide dismutase; **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

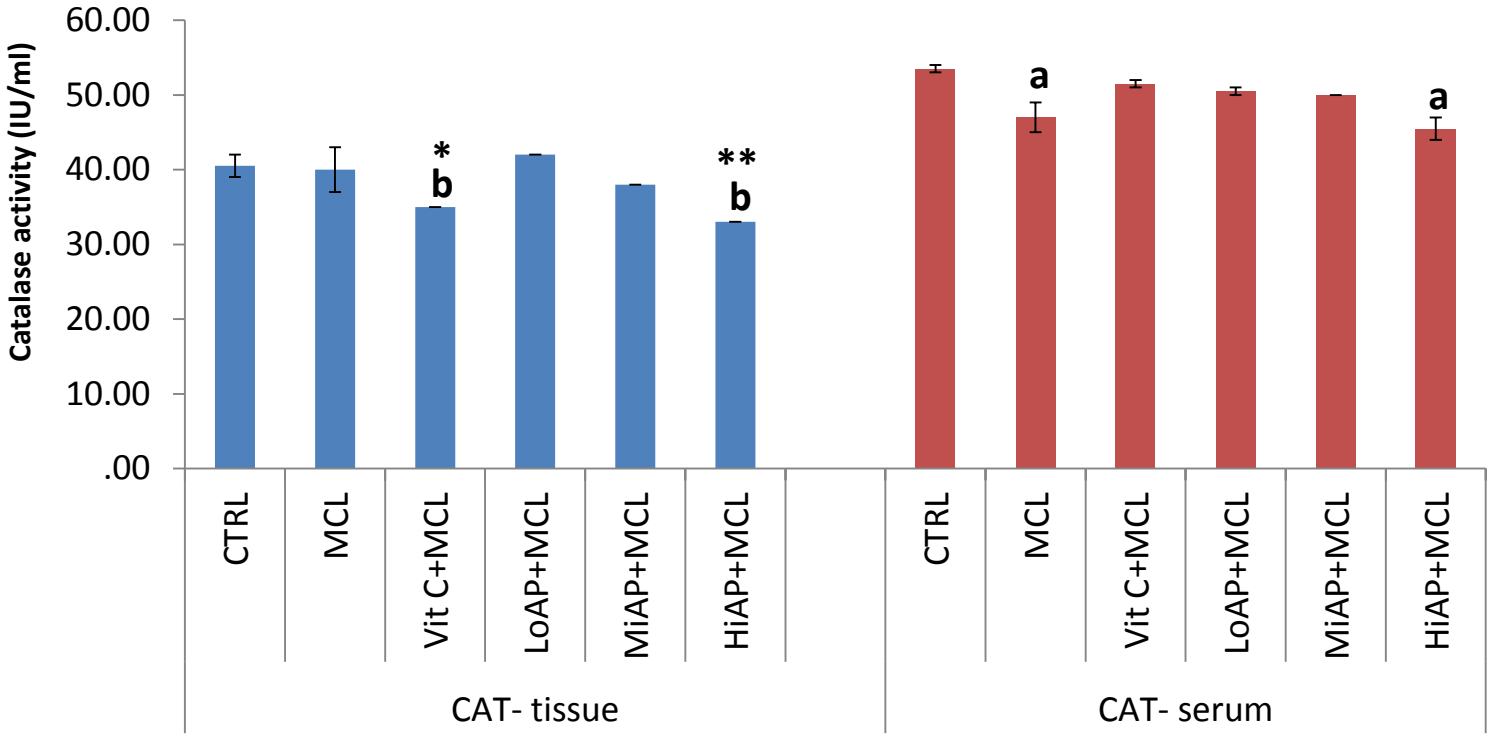


Figure 4.11a: Effect of aqueous fruit extract of *P. dactylifera* on tissue (brain) and serum CAT activity of Wistar rats

n = 4; mean ± SEM; One way ANOVA LSD post hoc test: **a**= *p<0.05*; **b**= *p<0.01* when compared with the control; ***= *p<0.05*; ****= *p<0.01*; when compared with the $HgCl_2$ group. **CAT=** Catalase; **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

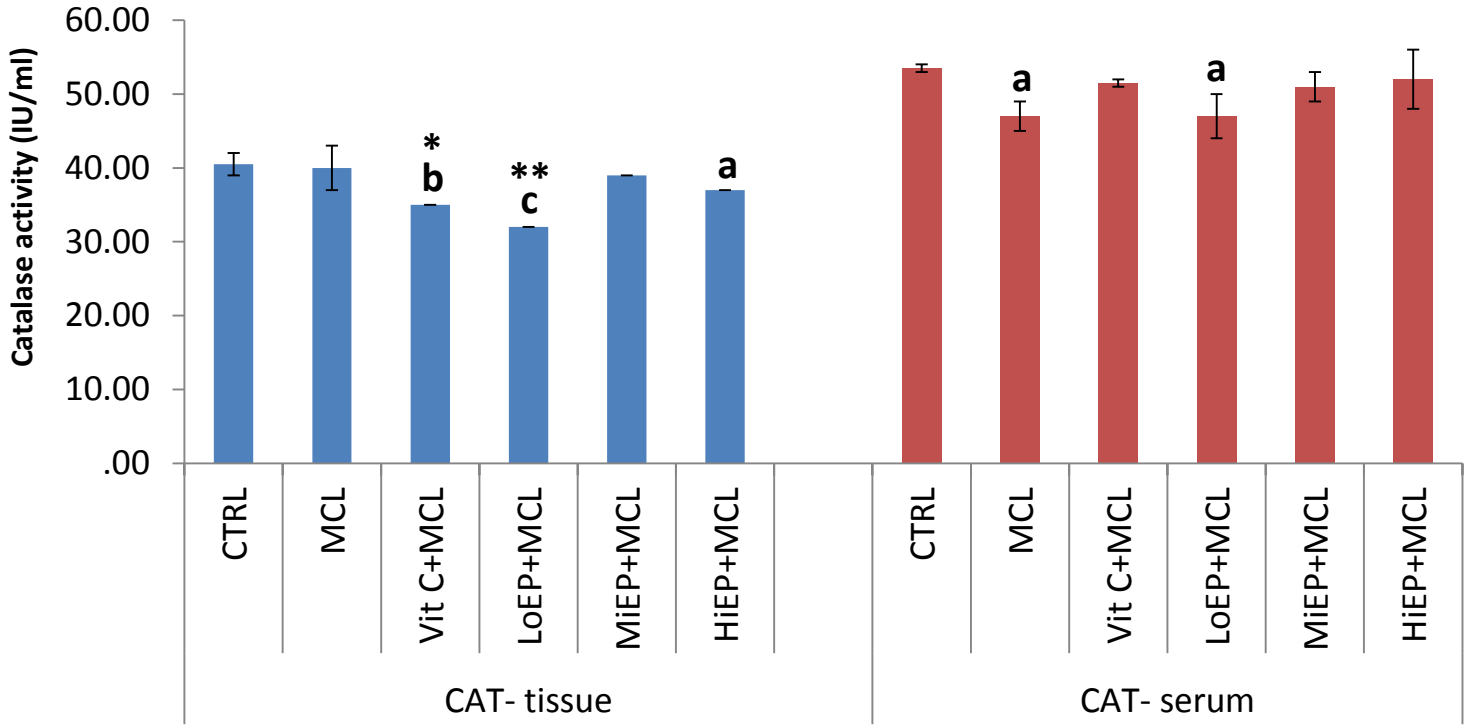


Figure 4.11b: Effect of ethanol fruit extract of *P. dactylifera* on tissue (brain) and serum CAT activity of Wistar rats

n = 4; mean ± SEM; One way ANOVA LSD post hoc test: **a**= *p<0.05*; **b**= *p<0.01;* ***c***= *p<0.001* when compared with the control; ***=p<0.05;* ****= *p<0.01* when compared with the $HgCl_2$ group. **CAT=** Catalase; **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

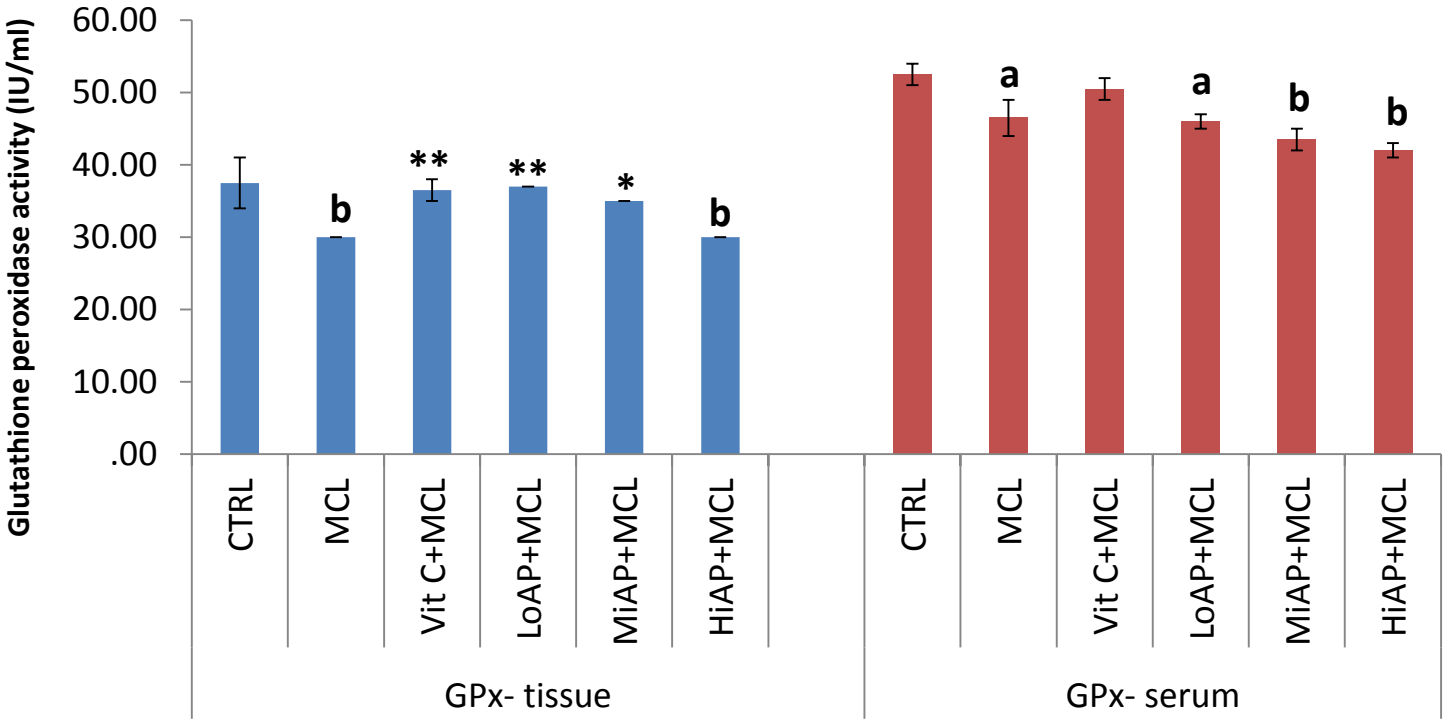


Figure 4.12a: **Effect of aqueous fruit extract of *P. dactylifera* on tissue (brain) and serum GPx activity of Wistar rats**

n = 4; mean ± SEM; One way ANOVA LSD post hoc test ***a***=*p<0.05;* ***b***=*p<0.*01 when compared with the control; ***=*p<0.05;* ****=*p<0.01*; when compared with the $HgCl_2$ group. **GPx=** Glutathione peroxidase; **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

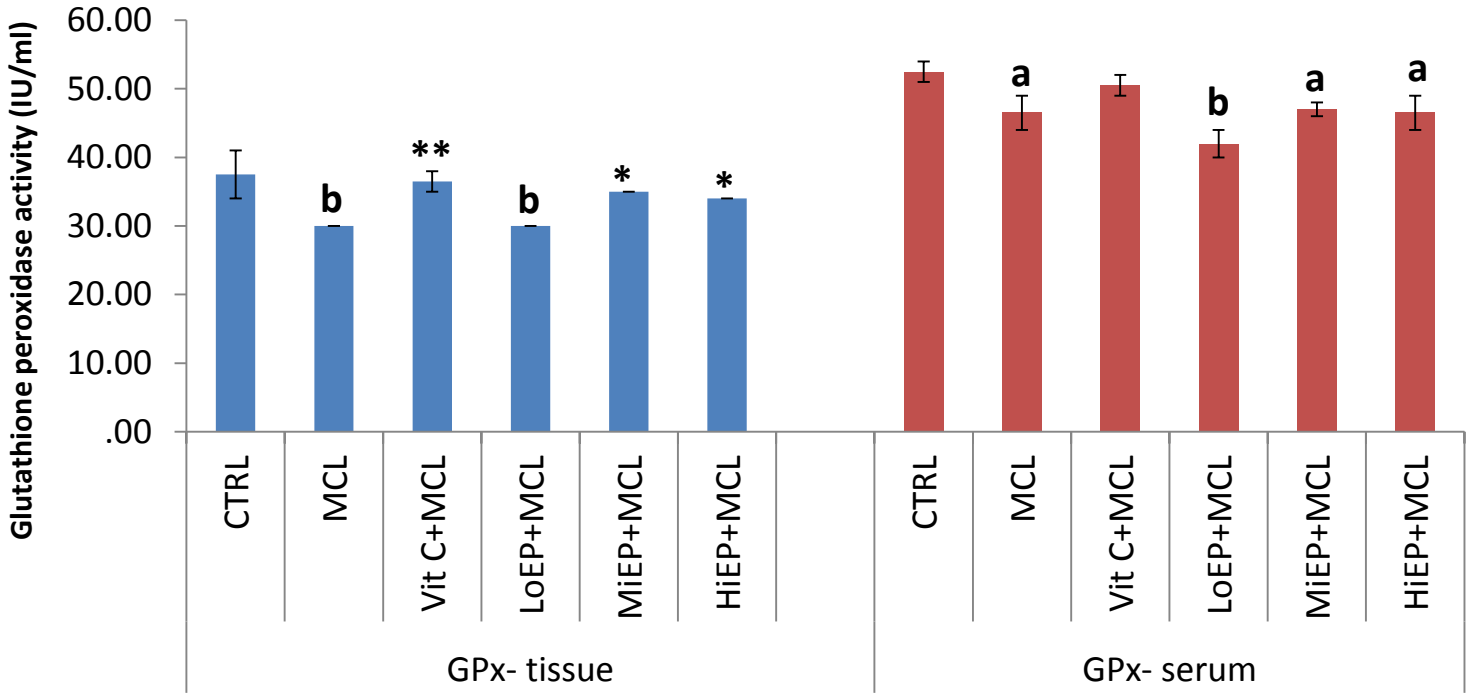


Figure 4.12b: **Effect of ethanol fruit extract of *P. dactylifera* on tissue (brain) and serum GPx activity of Wistar rats**

n = 4; mean ± SEM; One way ANOVA LSD post hoc test ***a***=*p<0.05;* ***b***=*p<0.*01 when compared with the control; ***=*p<0.05;* ****=*p<0.01*; when compared with the $HgCl_2$ group. **GPx=** Glutathione peroxidase; **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

4.6.3 Biochemistry Endogenous Enzyme Activity

Endogenous enzyme was estimated by assaying acetylcholinesterase (AchE) activity in brain tissue of Wistar rats. AchE activity levels remarkably ($p<0.05$) increased in all the treated groups (categories **A** and **B** study), except EFPD (500 mg/kg and 1,000 mg/kg)-treated groups, when compared to the control. Strikingly ($p<0.01$) decreased AchE activity levels were observed in EFPD-treated groups when compared to mercury-treated group (**Figures 4.13a and 4.13b**).

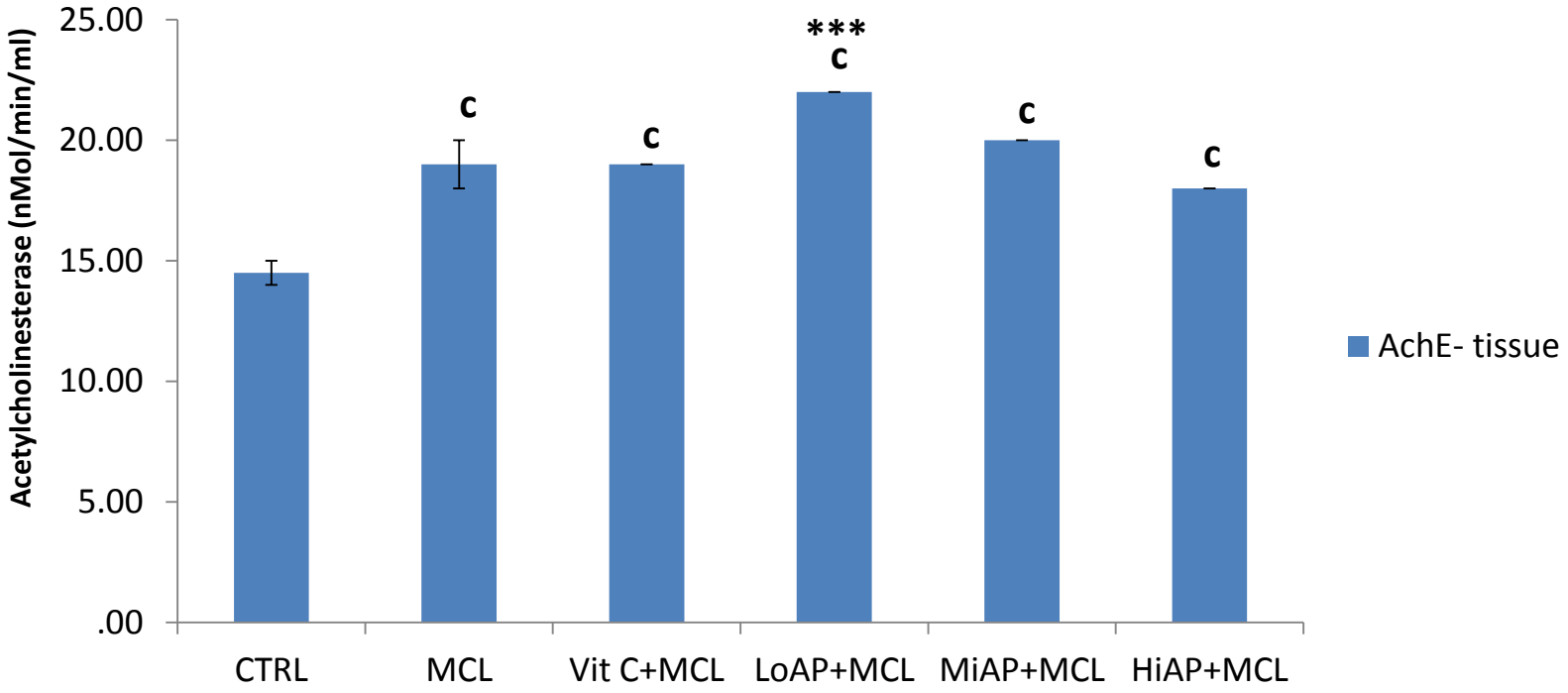


Figure 4.13a: **Effect of aqueous fruit extract of *P. dactylifera* on tissue (brain) AchE activity of Wistar rats**

n = 4; mean ± SEM; One way ANOVA *LSD post hoc test:* ***c*/*****=*p*<*0.001 when compared with the* control/ $HgCl_2$ group. **AchE=** Acetylcholinesterase; **CTRL**= Control (distilled H_2O 0.5 ml); **LoAP, MiAP and HiAP=** *Aqueous fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively); **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg**).**

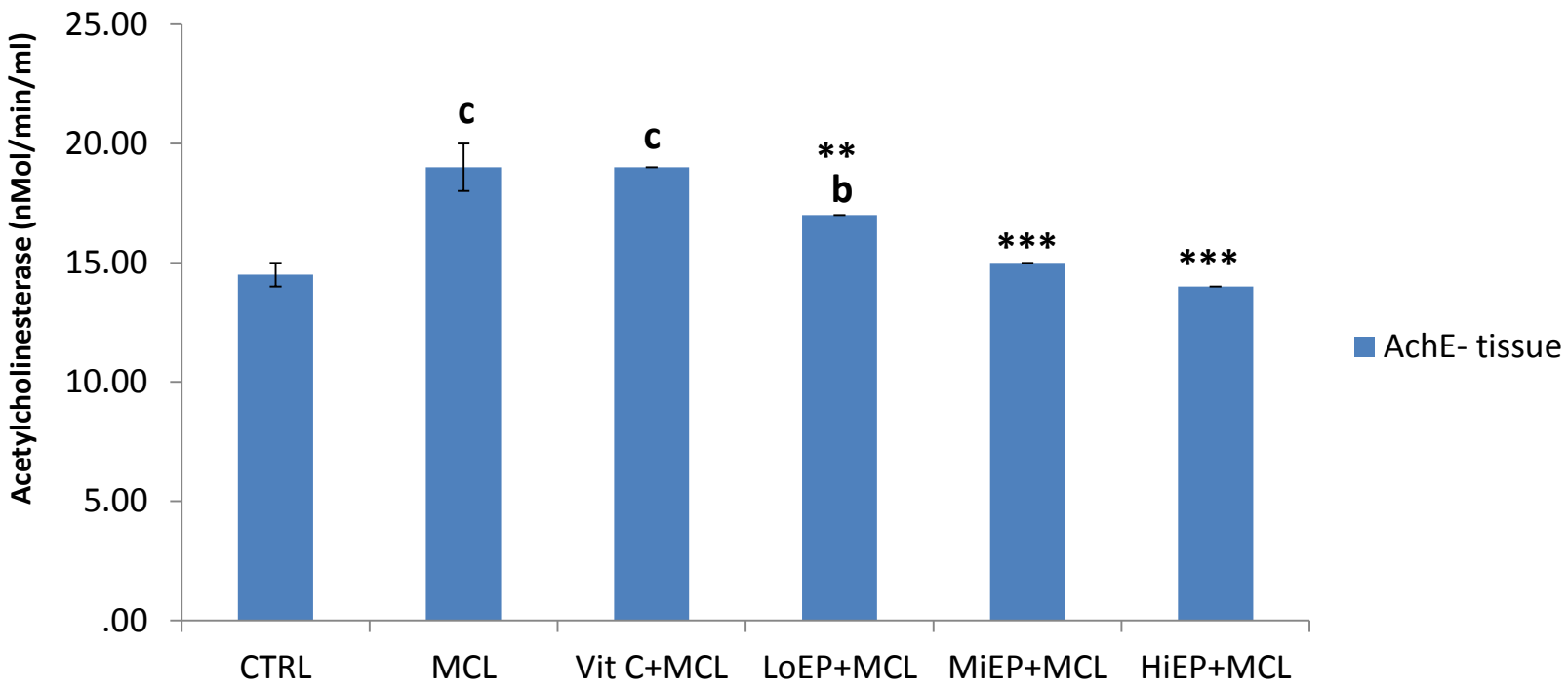


Figure 4.13b: **Effect of ethanol fruit extract of *P. dactylifera* on tissue (brain) AchE activity of Wistar rats**

n = 4; mean ± SEM; One way ANOVA *LSD post hoc test:* ***c/*******=*p<0.001 when compared with the* control/ $HgCl_2$ group. **AchE=** Acetylcholinesterase; **CTRL**= Control (distilled H_2O 0.5 ml); **LoEP, MiEP and HiEP=** *Ethanol fruit extract of P. dactylifera* (250, 500 and 1,000 mg/kg respectively)*;* **MCL**= $HgCl_2$ (5 mg/kg); **Vit C**= Vitamin C **(**100mg/kg).

CHAPTER FIVE

5.0 Discussion

In this study, preliminary phytochemical screening was carried out, and the protective effect of aqueous and ethanol fruit extracts of *Phoenix dactylifera* on mercury-induced cerebral, cerebellar and hippocampal neurotoxicity in Wistar rats assessed using morphologic and histometric, histologic and histochemical, neurobehavioural, neurochemical and biochemical investigations.

5.1 Phytochemical studies

Phytochemical screening of the plant (*Phoenix dactylifera*) fruit extracts (aqueous and ethanol), in this study, revealed the presence of flavonoids, saponins, tannins and alkaloids which have been reported to exert neuroprotective actions in animal and cell culture models of neurological disorders (Selvam, 2008; Lobo *et al.,* 2010; Chang *et al.,* 2012; Kumar and Khanum, 2012; Hwang *et al.,* 2015).

5.2 Physical Observation

Decreased physical activity exhibited by mercury-treated Wistar rats reflects treatment-related toxicity. This is in concordance with reports on drug-related toxicity; altered physical activity manifesting as sluggishness and loss of appetite indicates drug-related toxicity (Toma *et al.,* 2009; Salawu *et al.,* 2009). Body weight changes serve as a sensitive indication of the general health status of animal (Salawu *et al.,* 2009), and used as an indicator of adverse effect of drugs and chemicals (Mukinda and Syce, 2007). Changes in the body weight of Wistar rats treated with mercury were similar to those of the extract

treated. This was at variance with the reports of Jadhav *et al.* (2007) and Wadaan (2009) who reported remarkable weight changes in rats exposed to mercury.

5.3 Morphological Studies

Morphometry involves studying variations in shapes and the detection of possible shape changes between groups. Evaluation of shape changes in the brain is a key step in the development of new rodent models, the monitoring of different pathologies and the measuring of environmental influences (Scheenstra *et al.,* 2010). In this study, morphometry of brain dimensions and the relative organ weights were computed and compared with the control. Relative organ weight increase in treated groups relative to the control, though not remarkable statistically, could be indicative of treatment-related toxicity. Increased relative brain weight has been reported with heavy metal intoxication (Amal and Mona, 2009). Ghusoon *et al.* (2012) reported macroscopic brain lesions of rats treated orally with mercuric chloride appeared swollen and edematous, with severe congestion of meningeal blood capillaries. Relative organ weight comparable to the control in EFPD (1,000 mg/kg)-treated group could be indicative of neuroprotective activity. Phytochemicals from medicinal plants play a vital role in maintaining the brain's chemical balance by influencing receptors' functions (Kumar and Khanum, 2012).

In this study, brain dimensions of treated Wistar rats revealed differences relative to the control. Striking differences in cerebral and cerebellar length and width, especially in extract (AFPD and EFPD)-treated groups, could be suggestive to be related to treatment. However, prudence is required in the interpretation of morphometry results. Detecting striking difference actually implies a significant difference in intensity between the two

groups, which could be due to a morphological difference between the structures of the two groups or could be caused by other factors (Scheenstra *et al.,* 2010).

Histometry provides a sound basis for comparison of histological observation. It offers objectivity, increases precision compared with direct visual appraisal and makes statistical analysis easier. It improves assessment of certain histological change, which though may be recognizable by the eye, are accurately graded and their progression better appreciated by histometric quantization (Ahmad *et al.,* 1989; Asuquo *et al.,* 2007; Huda and Zaid, 2007).

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In this study, histometry of the cell area and perimeter of neurones (pyramidal cells of cerebral cortex- *layer V* and CA1 and CA3 hippocampal regions, and Purkinje cells of the cerebellar cortex) of the Wistar rats were conducted. Relative to the control, histometric analysis of cerebral cortex pyramidal cells of the treated groups (AFPD and EFPD) revealed no remarkable differences in the histometric characteristics. The cerebellar Purkinje cells which are among the largest cells in the central nervous system, are of primary interest, and have been the subject of several investigations concerning number and size, as these cells constitutes the only output elements of the cerebellar cortex (Andersen *et al.,* 1992; Korbo & Andersen, 1995; Jelsing *et al.,* 2006). Remarkable difference in histometric characteristics (particularly, cell area) of the Purkinje cells of cerebellar cortex is indicative of treatment-related toxicity. Histometric parameters are directly applied and correlated to tissue function (Pearse and Mark, 1974; Huda and Zaid, 2007). Relative to mercury-treated group, striking difference in cerebellar cortex Purkinje cell area with EFPD-treatment, especially 250 and 1,000 mg/kg doses indicates

neuroprotective activity; as cell areas are comparable to the control. Decrease in the perikaryal size – neuronal cytoplasmic shrinkage, has been attributed to stress-induced structural changes at the light microscopic level (Insausti *et al.,* 1997; Mohammad *et al.,* 2012).

Histometric analysis of the hippocampus revealed remarkable difference, especially in CA1 pyramidal cells area, when extract (AFPD and EFPD)-treated groups were compared to the mercury-treated group. Pyramidal neurones of CA1 region of the hippocampus are particularly vulnerable to hypoxic-ischemic events (Seidman, 2011). Heavy metals, such as mercury, have been reported to undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radical and nitric oxide in biological systems (Jomova and Valko, 2011; Farina *et al.,* 2013). Degenerative changes, such as shrinkage of neurones have been reported as direct toxic effect or role of mercuric chloride as anoxic poison (Ramzi and Stanely, 1994; Ghusoon *et al.,* 2012). Striking difference revealed in AFPD 500 mg/kg and EFPD 500 mg/kg treatments relative to the mercury-treated group was suggestive of neuroprotective activity. The vast majority of studies on health benefits of phytochemicals have focused on the fact that, many of the active chemicals possess antioxidant activity. Neuroprotective effects of various phytochemicals are associated with reduced levels of oxidative stress (Kumar and Khanum, 2012).

5.7 Histological and Histochemical Studies

In this study, light microscopic examination of routinely (Haematoxylin and Eosin, H&E) and histochemically (Cresly Fast Violet and Silver–*Golgi*) stained histological sections of

Wistar rats' brain regions: cerebral cortex–layer V, cerebellar cortex and hippocampus were conducted.

Neurodegeneration is a process involved in both neuropathological conditions and brain ageing (Kumar and Khanum, 2012). Histoarchitectural distortion of neural tissue manifesting as neuronal degenerative changes are indicative of neurotoxicity in the central nervous system (Nahla *et al.,* 2011; Kalantaripour *et al.,* 2012). Degenerative changes observed as, cortical neuronal shrinkage and perineuronal vacuolations, loss of pyramidal neurone processes, necrosis and chromatolysis in cerebral section of mercury-treated Wistar rats when compared with the cerebral section of control indicates treatment (mercury) related neurotoxicity. This is in concordance to the findings of previous worker on heavy metals, such as mercury, lead, cadmium, arsenic and other organic compounds, who reported that these metals have the capacity to induce nervous tissues damage (Amal and Mona, 2009; Fakunle *et al.,* 2013; Farina *et al.,* 2013). Ibegbu et *al.* (2013) reported that, administration of mercuric chloride at different doses produced clumping of cerebral cortical cells and necrosis of cells in adult Wistar rats. Mercury has been reported to interact with a wide range of cellular targets and affect multiple cellular functions. These toxic effects include, but are not limited to, the inhibition of neuronal ion channels (Pekel *et al.,* 1993; Sirois and Atchison, 1996; Peng *et al.,* 2002), disruption of presynaptic transmitter release and postsynaptic receptor function (Castoldi *et al.,* 2001; Yuan and Atchison, 2005), damage to neuronal cytoskeleton components and DNA structures (Castoldi *et al.,* 2000; Juarez *et al.,* 2005), and alteration of Na^+/K^+ ATPase and mitochondrial function (Insug *et al.,* 1997; Limke and Atchison, 2002; Xu *et al.,* 2012).

Neuronal injury may result in reversible or irreversible cell damage or cell death (Seilhean, *et al.,* 2004). Cells death has been reported to result from neuronal degeneration (Waters *et al.,* 1994). Cell death may result from necrosis, a pathologic type of cell death that occurs from extrinsic insults to the cells or after abnormal stresses, such as chemical injury or toxin, thermal, traumatic and mechanical factors (Kumar *et al.,* 2009). Mild histoarchitectural distortion, such as chromatolysis, necrosis and satellitosis/ gliosis and indistinct neurones due to reduced staining intensity compared to the control observed from cerebral sections of Wistar rats treated with vitamin C and extracts (AFPD and EFPD) followed by the administration of mercury is indicative of mercury induced neurotoxicity. Chromatolysis (central chromatolysis) is a histopathologic change seen in the cell body of a neurone, where the chromatin and cell nucleus are pushed to the cell periphery, in response to axonal injury (Holland, 1996); it is a morphologic manifestation of a reparative response to injury (Seilhean *et al.,* 2004) and, response is associated with increased protein synthesis to accommodate for axonal sprouting (Holland, 1996). Necrosis is an indication of treatment-related toxicity (Kumar *et al.,* 2009). Cresyl fast violet (CFV) is a stain for Nissl bodies (essentially the rough endoplasmic reticulum, RER). Degenerating neurones stain very poorly (rather than darker) with CFV as a result of disassociation of ribosomes from the RER which occurs in the early stages of cell degeneration (Garman, 2011).

Ascorbic acid (Vitamin C) is a known antioxidant compound (Aydin *et al.,* 2005). In this study, histological features of the vitamin C- treated group were comparable with the control. Thus indicates neuroprotection. This finding is in consistence with the reports of Ibegbu *et al.* (2013); ascorbic acid ameliorated mercury-induced cerebral cortex damage.

Antioxidants play significant roles in the reversion of the toxicity of mercury by forming inert complexes and inhibiting their toxicity on the dopaminergic neurones (Burger and Gochfeld, 2011).

Treatment of Wistar rats with AFPD and EFPD showed preserved histoarchitecture of the cerebral cortex parenchyma and cytoarchitectural preservation of neuronal cell Nissl substance. This implies that, the extract has a protective effect against mercury-induced neurotoxicity. Results from this study are in consistence with reports on the neuroprotective effect of *P. dactylifera* extract (Asadi-Shekaari *et al.,* 2008; Majid *et al.,* 2008). Kalantaripour *et al.* (2012) reported that, extract of *P. dactylifera* protected cortical neurones against ischemia reperfusion induced insults in Wistar rats. In this study, maximum protective effects of AFPD were observed with doses 250 mg/kg and 500 mg/kg, and lower protective effects with dose 1000 mg/kg. This is consistent with the findings reported by Majid *et al.* (2008).

The Purkinje cells and the granule cells are the most important targets in cerebellum for toxic substances (Fonnum and Lock, 2000). Neurodegenerative changes, such as, perineuronal vacuolations, neuronal cytoplasmic shrinkage and gliosis, Pukinje cell and internal granule cell necrosis, and indistinct neurones due to reduced staining intensity observed in mercury-treated Wistar rats implies treatment related toxicity. Findings are in consonance to studies related to heavy metals induced cell injury on cerebellar structures (Palmer *et al.,* 1984; Benlahcen *et al.,* 2009). Heavy metals disrupt the mechanisms release of neurotransmitters regulated by calcium, but also induced damage to mechanisms of DNA repairs (Hartwig *et al.,* 1994). Oral administration of mercuric chloride has been

reported to induce cerebellar degenerative changes (Ghusoon *et al.,* 2012). Inorganic mercury, a more reactive form of mercury, can cross the blood–brain barrier and accumulate in the brain at higher concentrations (Aschner and Aschner, 1990; Charleston *et al.,* 1996). It interacts with many macromolecules and exhibits a long latency of neurotoxicity (Allen *et al.,* 2001; Castoldi *et al.,* 2001). Mercuric chloride disturbs neuronal network structure and induces cell apoptosis in cortical neurones. These actions involve a degradation of β-tubulin, an important component of the neuronal cytoskeleton, and these effects are evoked by NMDA receptor function (Xu *et al.,* 2012). Rough endoplasmic reticulum and free ribosomes appear as basophilic granular areas (Nissl bodies) with Cresy Fast Violet staining in light microscopy. Reduction in Nissl boches has been reported in relation to neuronal degeneration (Martin *et al.,* 1998). The observed neuronal degeneration: loss of Nissl bodies with consequent reduced staining intensity of the Nissl substances in the cerebellar section of the mercury-treated Wistar rats in this study is in concordance with reports by the earlier findings of Classen *et al.* (1999) and Ajibade *et al.* (2009; 2012), reporting loss of Nissl substance in cerebellar neurones and shrinkage of the nucleus following chemically induced toxicity.

Mild histoarchitectural distortion, such as perineuronal vacuolations, satellitosis, outer molecular cell and internal granule cell necrosis compared to the control observed from cerebellar sections of Wistar rats treated with vitamin C and extracts (AFPD and EFPD) followed by the administration of mercury suggests mercury induced neurotoxicity. The brain is made up of neurones and neuroglia, the neurones being responsible for sending and receiving nerve impulses or signals. The microglia and astrocytes are essential for ensuring proper functioning of neurones and, are quick to intervene when neurones

become injured or stressed. As they are sentinels of neurone well-being, pathological impairment of microglia or astrocytes could have devastating consequences for brain function. It is assumed that neuroglial activation is largely determined by neuronal signals (Halliwell, 1992). Satellitosis, a condition marked by an accumulation of neuroglia cells around damaged or necrosed neurones of the central nervous system, often as a prelude to neuronophagia (Manickam *et al.,* 2009), as observed in this study is indicative of treatment related toxicity. Acute injury causes neurones to generate signals that inform neuroglia about the neuronal status. Depending on how severe a degree of neuronal injury, neuroglia will either nurse the injured neurones into regeneration or kill them if they are not viable. These types of neuroglial responses are considered to represent normal physiological and neuroprotective responses (Kumar and Khanum, 2012).

Administration of AFPD and EFPD showed preserved histoarchitecture of the cerebellar cortex parenchyma and cytoarchitectural preservation of neuronal cell Nissl substance in Wistar rats. This implies that, the extracts have a protective effect against mercury-induced neurotoxicity. Results from this study are in consistence with reports on the neuroprotective effect of *P. dactylifera* extract (Agbon *et al.,* 2014). Pujari *et al.* (2011) reported that, pretreatment with extract of *P. dactylifera* attenuated cortical neurones against bilateral common carotid artery occlusion induced oxidative stress neuronal damage in Wistar rats. Wan Ismail and Mohd Radzi (2013) reported that, neuronal damage in the form of shrinkage, atrophy and necrosis of neurones is greatly reduced and there is an increase in the levels of endogenous antioxidants in the brain of rats treated with *P. dactylifera* (date palm) fruit extract.

AFPD administration conferred preservation of the histoarchitecture dose-dependently compared to the control. However, EFPD administration revealed lower ameliorative effects with dose 1,000 mg/kg. This is consistent with the findings reported by Majid *et al.* (2008); fruit extract of *P. dactylifera* at dose of 1000 mg/kg showed negative effect on the rate of neuronal death in CA1 hippocampus following focal cerebral ischemia, which may be due to high concentration of antioxidants that may be harmful (Kuhn, 2003).

Observed histoarchitectural distortion of the hippocampus (regions CA1 and CA3), such as, irregular arrangement of CA1 hippocampal neurones, pkynosis and basophilic necrosis is indicative of treatment (mercury) induced neurodegenerative changes. Finding is in accordance to previous report on heavy metal induced neurotoxicity (El-Sokkary and Awadalla, 2011). Mercuric chloride is reported to be five times as potent as methyl mercury (MeHg) in blocking L-type Ca^{2+} channels in hippocampal neurones (Sziics *et al.*, 1997). It is also a more potent inhibitor of glutamate reuptake and glutamine synthetase activity than MeHg (Allen *et al.*, 2001). Hippocampal CA1 neurones, characteristically, are packed together; arranged in one or two very dense rows (Slomianka, 2011). Histoarchitecture of CA1 region is important, because of the particular vulnerability of the neurones in the pyramidal layer to toxic events (Seidman, 2011). Irregular arrangement of CA1 neurones in this study implies treatment related degenerative changes. Towfighi *et al.* (2004) reported a preferential degeneration of deep CA1 pyramidal cells was observed during early postnatal development in rabbits as a consequence of pilocarpine-induced seizures. A higher incidence of depolarizing responses to anoxia has been found in rat deep CA1 pyramidal cells when compared with superficial cells (Morris *et al.* 1995). Differential effects of ischemia on CA1 pyramidal cells (Fan *et al.* 2005) have also been

reported (Ruan *et al.* 2007). Basophilia of cytoplasm and pkynosis of neurones, which histologically characterizes neuronal atrophy (Mena *et al.,* 2004) as observed in this study is indicative of treatment related toxicity. Neuronal atrophy, a descriptive term given to a wide variety of irreversible neuronal injuries resulting to slow cell death, occurs in many degenerative disorders (Seilhean *et al.,* 2004).

Mild neurodegenerative changes in CA1 and CA3 regions of the of hippocampus, such as, irregular arrangement of CA1 hippocampal neurones, chromatolysis, eosinophilic necrosis, karyorrhexis, pyknotic necrosis, satellitosis and perineuronal vacuolations compared to the control in Wistar rats treated with vitamin C and extracts (AFPD and EFPD) followed by the administration of mercury suggests mercury induced neurotoxicity. Acute neuronal necrosis occurs in a variety of acute injuries, including anoxia and ischemia (Seilhean *et al.,* 2004). Neurodegenerative changes have been reported as direct toxic effect of mercuric chloride as anoxic poison (Ramzi and Stanely, 1994; Ghusoon *et al.,* 2012). Acute neuronal necrosis involves the cytoplasmic organelles and the cell membrane, which ruptures, leading to cell death. Features include neuronal cytoplasmic shrinkage, disappearance of Nissl bodies and intense eosinophilia ("red neurone") (Mena *et al.,* 2004; Seilhean *et al.,* 2004).

In this study, administration of ascorbic acid (vitamin C) ameliorated mercury-induced hippocampal damage. Result is in concordance with previous studies on neuroprotective activity of ascorbic acid following heavy metal intoxication (El-Sokkary and Awadalla, 2011). Raghu Jetti *et al.* (2014) observed neurodegenerative changes in CA1 and CA3 regions of hippocampus of fluoride exposed animals were less severe in ascorbic acid

treated animals. Treatment with extracts ameliorated mercury-induced hippocampal damage by conferring histoarchitectural preservation of the hippocampus, dose dependently with AFPD, while EFPD conferred histoarchitectural preservation, especially with dose 500 mg/kg EFPD compared to the control. Neuroprotection refers to the relative preservation of neuronal structure and/ or function (Casson *et al.,* 2012). Relative preservation of neuronal integrity implies a reduction in the rate of neuronal damage in the presence of a neurodegenerative insult, such as mercury. Findings are in consistence with reports related to neuroprotective activity of plant/ plant products which had been shown to exert neuroprotective effects (Watanabe *et al.,* 2001; Levites *et al.,* 2002; Rausch *et al.,* 2006; Kim *et al.,* 2008). Most of these studies attribute the neuroprotective effects to the antioxidant properties (Norfaizatul *et al.,* 2010; Hwang *et al.,* 2012). The phytochemical characteristics, such as, flavonoids and tannin constituents of plant extracts have been reported to exhibit great antioxidant activities which provides protection against oxidative stress (Chang *et al.,* 2012). Antioxidant effects have been implicated for neuroprotective activity of *P. dactylifera* (Panahi *et al.,* 2008; Zangiabadi *et al.,* 2011).

5.5 Neurobehavioural Studies

In this study, a battery of neurobehavioural test was conducted to assess a variety of endpoints, such as cognition, sensory-motor activity and, motor coordination and balance in the Wistar rats. Elevated plus maze (EPM) was used to assess anxiety-related behaviour; transfer latency (TL) on EPM open arm and Morris water maze (MWM) to evaluate learning and memory; forelimb grip strength (FGS) test to assess muscular strength in the forelimbs and beam walking test (BWT) to monitor motor coordination and balance of Wistar rats.

The EPM is a widely used behavioural assay for rodents and it has been validated to assess the anti-anxiety effects and to define brain regions and mechanisms underlying anxiety-related behaviour (Walf and Frye, 2007; He *et al.*, 2011). It is a validated and reliable test for detecting both anxiolytic- and anxiogenic-like effects of agents (Kochenborger *et al.*, 2014; Skelly and Weiner, 2014). In this study, specific variables, such as time spent in the open/ closed arm, entry frequencies to the open/ closed arm, grooming, rearing and head dipping were used to characterize the level of anxiety in Wistar rats. Elevated anxiety levels observed as increased entry frequency into the closed arm; increased grooming, rearing and head dipping frequencies (from day-7 to day-14 treatment) in the mercury-treated group implies anxiogenic effect of mercury. This finding is in line with previous studies related to heavy metals, such as lead and cadmium, exposure on anxiety-like behaviour in the rat (Laret *et al.*, 2003). Similarly, exposure to mercuric chloride ($HgCl_2$) induced anxiety-related responses (Teixeira *et al.*, 2014).

An increase of the time and proportion of the entries into the open arms without a changed locomotor activity is regarded as a powerful marker for an anxiolytic substance effect (Pellow *et al.*, 1985). In this study, administration of vitamin C and plant extracts (AFPD and EFPD) remarkably decreased anxiety levels by reduced entry frequency into the closed arm across the period of study when compared to the control. Anxiogenic response, such as, grooming, rearing, and head dipping grooming have been demonstrated as credible indices in measuring rodent response to stress (Puurunen, 2001; He *et al.*, 2011). Striking decrease in anxiety-like response, such as, grooming, rearing, and head dipping frequencies were observed with vitamin C and plant extracts treatments. Increase in the

duration and frequency of the entries into the open arms in EPM test had been confirmed as a potent sign of an anxiolytic substance effect (Skelly and Weiner, 2014).

Extensive research has been conducted to reveal multiple neural substrates and mechanisms that contribute to the etiology of depression and anxiety, among which the imbalance between oxidation and the antioxidant defense system has gained attention (Ding *et al.,* 2014). Some studies have demonstrated the role of oxidative stress in anxiety of rodents (Vollert *et al.,* 2011; de Almeida *et al.,* 2014). Increased anxiety has been correlated positively with the increase of reactive oxygen species levels (Komaki *et al.,* 2015). Oxidative stress in the hippocampus of adult rats has been reported to be anxiogenic (Souza *et al.,* 2007). Teixeira *et al.* (2014) correlated accumulation of $HgCl_2$ in both the hippocampus and cortex of the brain of $HgCl_2$-exposed rats to induce anxiety-related responses, short- and long-term memory impairments and motor deficits. In this study, increased anxiety-like behaviour was ameliorated by treatment with an antioxidant; vitamin C. Result is in concordance with previous studies that reported, increase in anxiety-like behaviour to be reversed by treatment with the antioxidant and suggested direct involvement of oxidative stress in mediating anxiety-like behaviour in rats (Salim *et al.,* 2010; Komaki *et al.,* 2015). Administration of plant extracts ameliorated treatment (mercury)-induced anxiety like behaviour, although with exceptions in some doses of either extracts. Thus, suggests that, many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds (phytochemicals) acting at a single target site or at multiple target sites (Adwan *et al.,* 2006). Several researchers have reported that flavonoid groups exhibited a wide range of biological activities, such as antioxidant, anti-inflammatory, and antiangiogenic effects (Anyasor *et*

al., 2010; Popovic-Milenkovic *et al.,* 2014). It is possible that phytochemical components with antioxidant activities, such as flavonoid, play essential roles in the anxiolytic properties of the plant extracts (AFPD and EFPD) as observed in this study. This is in consistence with report related to anxiolytic effect of plant extracts (Komaki *et al.,* 2015).

Memory is an organism's mental ability to store, retain and recall information (Chakravarthi *et al.,* 2012). In this study, elevated plus maze served as an exteroceptive behavioural model to evaluate learning and memory in Wistar rats (Kulkarni *et al.,* 2011; Veni Bharti *et al.,* 2013). Learning behaviour of Wistar rats is reflected by transfer latency (TL) of first day (pre-treatment) and retention of information or memory reflected by TL of 24 hours, day-7 and day-14 of treatment. Administration of vitamin C and plant extracts (AFPD and EFPD), especially at day-14 of treatment, remarkably improved learning and memory by reduction in TL when compared to the control.

The Morris water maze is one of the most widely used tasks in behavioural neuroscience for studying the psychological processes and neural mechanisms of spatial learning and memory (Brandeis *et al.,* 1989; He *et al.,* 2011). Learning and memory of Wistar rats is reflected by escape latency compared to the performance at the pre-treatment session. Increased latency as observed with mercury-treatment, at day-14 of treatment, indicates learning and memory impairment. Previous works have elucidated that distinct forms of memory are mediated by different CNS regions, such as the primary cortex (*that is,* prefrontal cortex) and limbic structures (*that is,* hippocampus). Memory forms can be classified as *declarative or explicit* (the ability to recall past events deliberately) and are hippocampus-dependent; and *non-declarative or procedural* (also called implicit), defined by unconsciously performed skills (motor or cognitive) that are mainly dependent on the

striatum and cerebellum (Packard and McGaugh, 1992; McDonald and White, 1993). The hippocampus plays an important role in learning and memory (Chakravarthi *et al.,* 2012). Teixeira *et al.* (2014) reported that, exposure of rats to $HgCl_2$ induced short- and long-term memory impairments, and correlated cognitive dysfunction to accumulation of $HgCl_2$ in the hippocampus. Results are in accordance with Yasutake *et al.* (2010) which infer that acute doses of inorganic mercury induced cognitive damage in mice. Recent study has shown that memory functions of hippocampus was damaged when mercury caused damage to the hippocampus (Wu *et al.,* 2016).

Vitamin C-treated rats showed decreased latency when compared to the control, and administration of extracts (AFPD and EFPD) especially at day-14 of treatment, ameliorated memory impairment following mercuric intoxication by decreased latency. Administration of plant extracts has been reported to successfully reverse chemical-induced memory deficits in rodents (Chandrasekaran *et al.,* 2001; Kulkarni *et al.,* 2011), and correlated memory enhancing properties is by virtue of their bioactive phytochemical constituents (Kumar and Khanum, 2012). Phytoconstituents include: flavanoids, steroids (sitosterol and stigmasterol), terpenoids and organic acids (ascorbic, benzoic, shikimic and vanillic acid). Memory enhancing action shown by medicinal plants is by the increased supply of oxygen, and free radical elimination from the body, thereby improving memory. Recently, there has been intense interest in the potential of flavonoids to modulate neuronal function and prevent age-related neurodegeneration. Flavonoids exert a multiplicity of neuroprotective actions within the brain, including a potential to protect neurones against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning, and cognitive function. These effects

appear to be underpinned by their interaction with critical protein and lipid kinase signaling cascades in the brain leading to an inhibition of apoptosis triggered by neurotoxic species and to a promotion of neuronal survival and synaptic plasticity. Through these mechanisms, the consumption of flavonoid-rich foods throughout life holds the potential to limit neurodegeneration, decrease neuroinflammation, and prevent or reverse age-dependent loses in cognitive performance (Vauzour *et al.,* 2013). Dietary intervention studies in several mammalian species, including humans, using flavonoid rich plant or food extracts have indicated an ability to improve memory and learning, by protecting vulnerable neurones, enhancing existing neuronal function or by stimulating neuronal regeneration. Result suggests that extracts (AFPD and EFPD) may serve as a potential neuroprotective agent against chemical-induced cognitive dysfunction.

The motor cortex has long been viewed to play an important role in fine motor control and fractionation of movement (Evarts *et al.,* 1983), sensorimotor integration and higher order cognitive-motor movements (Sanes and Donoghue, 2000). In this study, muscular strength in the forelimbs (forepaws) of the Wistar rats was assessed using forelimb grip strength (FGS) test. Time-to-release-grip (forelimb grip strength time) reflects the FGS. Forelimb grip strength decline has been reported with heavy metal intoxication in rats (Saritha *et al.*, 2014). Daya *et al.* (2005) reported forelimb grip strength decline in animals exposed to mercury. Administration of vitamin C and extract, especially EFPD at dose 1,000 mg/kg, significantly improved FGS compared to the control and mercury-treated groups across the period of study. Several antioxidants such as, vitamins C and E, carotenoids, and flavonoids have been used to protect cells from oxidative stress and muscle cell damage (Halliwell and Gutteridge, 1999; Zoppi *et al.,* 2006).

The cerebellum is involved in the control of movements, particularly those linked to the voluntary nervous system and movements where timing is an important aspect. It co-ordinates the different muscle groups so that the muscle exerts movements fluently and precisely. The cerebellum receives continual feedback information about intended movement and actual movements. The cerebellum should be regarded as a stabilizing control system which receives forewarning of each motor impulse. Atrophy of the cerebellum is usually accompanied by different forms of ataxia and an unstable gait (Fonnum and Lock, 2000). In this study, beam walking test was used to assess motor coordination and balance in the Wistar rats. Beam walking ability was measured using foot-slips score and latency (time) until the Wistar rat's nose entered the goal box of the beam walking apparatus.

Across the period of study administration of extract (AFPD or EFPD) had no effect on foot slips score when compared to the control and mercury-treated groups. Relative to beam walk latency, however, administration of AFPD and EFPD, especially at dose 1,000 mg/kg, revealed improved beam walking performance in comparison with mercury-treated, thus, indicating improvement on motor coordination and balance. Excitotoxicity, characterized by a cascade of reactions resulting in a rise in intracellular calcium, and free radical formation play an important role in cerebellar toxicity involving granular cells or Purkinje cells since both cells have glutamatergic receptors. Excitotoxicity can be initiated by glutamate agonists and by inhibitors of neuronal metabolism (Fonnum, 1998; Fonnum and Lock, 2000). Albrecht and Matyja (1996) reported mercury neurotoxicity may be mediated by excitotoxic activity of glutamate. In recent studies, mercury-induced neuronal degeneration is thought to invoke glutamate-mediated excitotoxicity (Aschner *et al.,* 2000;

Allen *et al.,* 2001; Xu *et al.,* 2012). Limiting excitotoxicity and oxidative stress is a very important aspect of neuroprotection (Zádori *et al.,* 2012). The polyphenolics including flavonoids, found in many plant extracts, have been shown to be strong reactive oxygen species (ROS) scavengers, antioxidants and protectors of neurones from lethal damage (Rice-Evans, 2001; Pujari *et al.,* 2011). In this study, improvement on motor coordination and balance with EFPD-treatment could be related to antioxidant activity and possibly acted as glutamate antagonists, which aim to limit excitotoxicity.

5.6 Neurochemical/ Biochemical Studies

Trace elements are of widespread neurological importance and have critical actions in neurodevelopment, neurotransmitter synthesis, energy metabolism, antioxidant defence and DNA synthesis. Thus, trace elements are important for cognitive function (Jones *et al.,* 2008; Dauncey, 2009).

Antioxidants have been suggested to be useful in conditions associated with oxidative stress (Halliwell and Gutteridge, 1999; Norfaizatul *et al.,* 2010). Oxidative stress which occurs due to the imbalance between production and detoxification of reactive oxygen species (ROS) has also been implicated in many neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Gilgun-Sherki *et al.,* 2001; Trushina and Mcmurray, 2007). The brain is more susceptible to oxidative stress due to its high oxygen consumption, relatively low antioxidant capacity and high contents of lipid and ferum. Increased oxidative stress will further lead to lipid peroxidation, DNA damage, protein damage and induction of apoptosis (Halliwell and Gutteridge, 1999; Crichton *et al.,* 2007).

In this study, neurochemical analysis assessed the concentration of neuro-trace elements (copper, Cu; iron, Fe; manganese, Mn and zinc, Zn) in brain-tissue homogenate, while biochemical analysis for lipid peroxide levels and antioxidant enzyme activity (malondialdehyde, MDA; superoxide dismutase, SOD; catalase, CAT and glutathione peroxidase, GPx) were assessed in blood serum and brain tissue homogenate, and acetylcholinesterase (AchE) activity for endogenous enzyme in brain tissue homogenate of Wistar rats.

Many trace elements are known to cause abnormal mental function when either a deficiency or excess exists. When a nutritional mineral accumulates excessively in the body, it can become toxic (Watts, 1990). Recent findings suggest that optimal concentrations of Cu, Zn and Fe exist for optimal cognitive function in adults (Lam *et al.*, 2008).

In this study, elevated levels of neuro-trace elements (Mn, Fe, Zn and Cu) were observed in the mercury-treated group compared to the control. Excess iron (Fe) accumulation affects the CNS. Increased Fe has been observed in the lesion site of a number of neurodegenerative disorders and models of neurodegeneration (Olanow *et al.*, 1990). Increased Fe deposition in regions of the brain has been implicated in Parkinson's disease (PD) (Watts, 1990). Fe levels are also increased at sites of degeneration in Alzheimer disease (AD); detected in the hippocampus, nucleus basalis, frontal cortex and motor cortex of AD patients (Conner *et al.*, 1992; Richardson, 1993). There is growing evidence to suggest that heavy metal ions can act synergistically with Fe ions to increase free radical damage (Olanow *et al.*, 1990). Manganese toxicity in humans is associated with Parkinsonian-like symptoms, ataxia and altered balance may develop (Watts, 1990).

Copper is one of many metal ions that are required for essential body functions but are toxic in excess (Cox and Moore, 2002; Desai and Kaler, 2008). Copper is present throughout the brain and is most prominent in the basal ganglia, hippocampus, cerebellum, numerous synaptic membranes, and in the cell bodies of cortical pyramidal and cerebellar granular neurones (Madsen and Gitlin, 2007). Excess copper has been implicated directly or indirectly in the pathogenesis of numerous neurological diseases, such as depression, AD, PD and Wilson disease (Scheinberg, 1976; Desai and Kaler, 2008). The hippocampus is particularly rich in zinc (Dreosti, 1984). Acute oral zinc toxicity has been reported to produce drowsiness and somnolence (Watts, 1990).

Exposure to heavy metals has been shown to induce significant changes in the cerebral and cerebellar content of Fe, Zn and Cu in rats (Abdel-Maksoud *et al.,* 2004; Tripathi *et al.*, 2011). Elevated levels of heavy metals in brain have been associated with serious neurological diseases (Cannata *et al.,* 1998), which has been attributed to accumulation of such metals in the brain of affected individuals (Lovell *et al.,* 1993). Oral exposure to heavy metals result in accumulation of such metals in hippocampus of brain and thus affect some essential elements (Zn, Fe, Cu and Ca) contents in the hippocampus at different degrees (Yang *et al.,* 2002). Previous studies have correlated neurological disorders to accumulation of $HgCl_2$ in brain in rats (Berlin *et al.,* 2007; Bernhoft, 2012; Teixeira *et al.*, 2014). Thus, findings of this study corroborate reports on the association of trace elements toxicity with mercury toxicity.

In this study, administration of Vitamin C and extracts (AFPD and EFPD), especially with 500 mg/kg treatment, depressed the elevated levels of the studied neuro-trace elements concentrations. Findings is in consistence with studies related to neuroprotective activity of

ascorbic acid against heavy metal neurotoxicity; vitamin C binds heavy metals such as copper, lead, cadmium, and mercury, and facilitates their excretion by the kidneys (ARL, 1989). Yousef (2004) reported ascorbic acid (vitamin C), being antioxidant, ameliorated the adverse effect of heavy metal administration in rabbits, by decreasing the free radicals, cholesterol, creatinine, and increased the activity of glutathione s-transferase. Some researchers have ascertained the fact that phenolic compounds are able to chelate metal ions (Komaki *et al.*, 2015). The antioxidant activity of *P. dactylifera* (dates) has been attributed to the wide range of phenolic compounds, such as, p-coumaric, ferulic, sinapic acids, flavonoids and procyanidins (Gu *et al.*, 2003).

Flavonoids exert a multiplicity of neuroprotective actions within the brain, including a potential to protect neurones against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning, and cognitive function. These effects appear to be underpinned by their interaction with critical protein and lipid kinase signaling cascades in the brain leading to an inhibition of apoptosis triggered by neurotoxic species and to a promotion of neuronal survival and synaptic plasticity (Vauzour *et al.,* 2013). Melidou *et al.* (2005) found that intracellular binding of iron is responsible for the protection offered by flavonoids against hydrogen peroxide-induced DNA damage. Thus, antioxidant activities of the extracts could be implicated for neuroprotection. Extract of *P. dactylifera* benefits the brain through neuroprotective property, protecting the brain from the actions of ROS by utilizing its antioxidant property (Kalantaripour *et al.,* 2012; Wan Ismail and Mohd Radzi, 2013).

The brain has a large potential oxidative capacity but a limited ability to counteract oxidative stress. Oxidative stress has been implicated in mechanisms leading to neuronal

cell injury in various pathological states of the brain, including neurodegenerative disorders. Although the brain accounts for less than 2% of the body weight, it consumes about 20% of the oxygen available through respiration. Therefore, because of its high oxygen demand, the brain is the most susceptible organ to oxidative damage (Weiss and Fintelmann, 2000).

In this study, malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) were assayed for the estimation of lipid peroxidation levels, while antioxidant enzymatic activity was estimated by assaying for superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in blood and brain tissue.

Tissue and blood MDA levels increased remarkably in all the treated groups when compared to the control. Elevated tissue MDA levels suggest, enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive radicals (Himakar *et al.*, 2010). Lipid peroxidation is a free radical-related process, which is potentially harmful because its uncontrolled, self-enhancing process causes disruption of membranes, lipids and other cell components. It is also involved in oxidative stress, which plays a major role in neural-degeneration (Rao and Purohit, 2011). Abnormally high levels of lipid peroxidation and the simultaneous decline of antioxidant defence mechanisms can lead to damage of cellular organelles and lead to oxidative stress (Mahboob *et al.*, 2005). Results of this study demonstrate that, the treatment of rats with $HgCl_2$ had a significant enhancement in TBARS levels, indicative of the generation of lipid peroxides (LPO). Enhanced lipid peroxidation levels were also reported in Hg toxicity by Sener *et al.* (2007) and Agarwal *et al.* (2010). $HgCl_2$ is known to increase the production of many ROS, such as superoxide and H_2O_2, which cause lipid

peroxidation and subsequently oxidative tissue damage (Huang *et al.,* 1996; Mahboob *et al.,* 2001; Gado and Aldahmash, 2013). Mercury is a major neurotoxicant (NRC, 2000; Rao and Purohit, 2011); generates high levels of reactive oxygen species (ROS) and oxidative stress, depletes glutathione and thiols causing increased neurotoxicity from interactions of ROS, glutamate, and dopamine (Araragi *et al.,* 2003; Esmaeili *et al.,* 2012).

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators, enzymes such as SOD, CAT and GPx systems (Semiz and Sen, 2007; Uma *et al.,* 2012).

The activity of SOD is a sensitive index in oxidative damage as it scavenges the superoxide anion to form hydrogen peroxide leading to diminish the toxic effects (Khan *et al.,* 2012). In this study, tissue and blood SOD activity levels decreased remarkably in all the treated groups when compared to the control. However, serum SOD activity levels of the vitamin C-treated was comparable to the control, while tissue SOD activity levels were relatively increased in EFPD (500 and 1,000 mg/kg)-treated groups when compared to mercury-treated group. Heavy metals such as mercury, lead and thallium have been reported to reduce the anti-oxidative enzymes such as catalase, SOD, glutathione reductase and lipid peroxidase (Moreira *et al.,* 2010). Acute exposure to mercury has been reported to decrease SOD and increase LPO (Jomova and Valko, 2011). Decrease in the activity levels of anti-oxidative enzymes such as superoxide dismutase suggests the formation of free radicals and the participation of free radical induced oxidative cell injury as in the toxic effects of mercury (Jomova and Valko, 2011). Mercuric ion, one of strongest thiol-binding agents, increases the intracellular levels of ROS and induces oxidative stress, resulting in tissue damage (Reus *et al.,* 2003; Zahir *et al.,* 2005). Administration of vitamin

C, in this study, maintained the antioxidant defense system by maintaining SOD activity levels comparable to the control. This is in consistence with the reports of (Ibegbu *et al.*, 2013). Vitamin C plays a pivotal role in neutralizing free radicals; it can work both inside and outside the cells to combat free radical damage. The free radicals will seek out an electron to regain their stability; vitamin C is an excellent source of electrons so it can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity (Bindhumol *et al.,* 2003; Soujanya *et al.,* 2012). EFPD treatment moderately ameliorated mercury-induced decrease in SOD activity due to its antioxidant and free radical scavenging effect.

Catalase is a key component of the antioxidant defense system. Inhibition of these protective mechanisms risks in enhanced sensitivity to free radical-induced cellular damage (Young and Woodside, 2001). GPx is an equally important antioxidant enzyme, which reacts with hydrogen peroxide thus preventing intracellular damage caused by the free radicals (Lee *et al.,* 2007).

In this study, tissue CAT activity levels in all the treated groups showed no remarkable difference, except vitamin C-treated, 1,000 mg/kg AFPD and 250 mg/kg EFPD -treated groups when compared to the control. Decreased serum CAT activity levels were observed in all the treated groups, when compared to the control. Tissue and serum GPx activity levels decreased in all the treated groups when compared to the control. Tissue GPx activity levels were observed to be higher in all the treated groups, except 1,000 mg/kg AFPD and 250 mg/kg EFPD-treated groups relative to mercury-treated group. Decreased serum GPx activity levels were observed in all the treated groups, except in Vitamin C-treated group, when compared to the control.

Mercuric ion is one of strongest thiol-binding agents; binding of mercuric ions to thiol groups may cause decreased glutathione (GSH) levels, leading to increases in levels of reactive oxygen species (ROS), such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals, which provoke lipid, protein, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) oxidation (Clarkson, 1997; Li *et al.,* 2006). CAT, enzymatic antioxidant, widely distributed in all animal tissues decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Fang *et al.,* 2000). The activity of GPx is coupled to glutathione reductase (GSSG-R), which maintains reduced glutathione (GSH) levels (Bompart *et al.,* 1990). Using glutathione (GSH) as a reducing reagent, the GPx enzymes catalyze the reduction of H_2O_2 and organic peroxides (R-O-O-H) to water and the corresponding stable alcohol thus inhibiting the formation of free radicals. Thus, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide, linked with neurodegenerative diseases (Khan *et al.,* 2012). Remarkable mercury-induced decrease in tissue GPx activity in this study indicates the occurance of oxidative stress in brain structures. Finding is in line with the reports of Durak *et al.* (2010) and Kim *et al.* (2015).

Serum GPx activity level in Vitamin C-treated group was comparable to the control in this study. Vitamin C has been reported to have protective effect aganist mercury toxicity (Patil and Rao, 1999; Durak *et al.* 2010). Vitamin C is a strong antioxidant (Rao, 1997) having nucleophilic properties and binds to mercury ions (Hg^{+2}) to reduce mercury-induced damage (Durak *et al.,* 2010). Remarkably higher tissue GPx activity levels in the extracts (AFPD and EFPD)-treated groups relative to mercury-treated group reflects increased GPx

activity, facilitating detoxification of the H_2O_2 produced by mercury (Lu, 2013).Thus, suggestive of AFPD and EFPD neuroprotective effects through its antioxidant activity. Result is consistent with reports on medicinal plant extract exerting neuroprotective effects against mercury-induced oxidative stress through its antioxidant activities (Farina *et al.*, 2005; Lu, 2013; Kim *et al.*, 2015). Evidence indicates that plant extracts, such as AFPD and EFPD, with phytochemicals having antioxidant properties reduce the symptoms of neurodegeneration. Some studies have indicated that phenolic substances such as flavonoids are considerably potent antioxidants (Cao *et al.*, 1997; Pandit, 2011).

In this study, 1,000 mg/kg AFPD and 250 mg/kg EFPD-treated groups showed significantly reduced tissue GPx activity levels. This may be related to toxicity of medicinal plants due to mixtures of active compounds and their interactions with other herb and drugs. Plants have complex mixtures of terpenes, alkaloids, phenols and other chemicals (Azaizeh *et al.*, 2003). Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another (Himakar *et al.*, 2010).

Acetylcholine (Ach), the first neurotransmitter to be characterized, has a very significant presence in the brain; it was determined that acetylcholine is essential for learning and memory (Winkler *et al.*, 1995); that is central cholinergic system is essential for the regulation of cognitive functions (Khan *et al.*, 2012). Acetylcholine deficit, extensive loss of cholinergic neurones, among other factors, has been held responsible for senile dementia and other degenerative cognitive disorders, including Alzheimer's disease (Kumar and Khanum, 2012). Acetylcholinesterase metabolizes acetylcholine to choline and acetyl-CoA, which exist into asymmetric forms and globular forms (Lane *et al.*, 2006).

In this study, endogenous enzyme was estimated by assaying acetylcholinesterase (AchE) activity in brain tissue of Wistar rats. Remarkable elevated AchE activity levels was observed in all the treated groups, except EFPD (500 mg/kg and 1,000 mg/kg)-treated groups, when compared to the control. Strikingly decreased AchE activity levels were observed in EFPD-treated groups when compared to mercury-treated group. Findings corroborates the reports of previous studies; Castoldi *et al.* (1996) and Rajanna *et al.* (1997) reported that mercury micromolar concentrations inhibited cholinergic, glutamatergic, GABAergic and dopaminergic systems affecting both behaviour and cognition. It is well established that cognitive and motivational processes depend on the connections between prefrontal cortex (PFC) and limbic structures, which are dependent on the neurotransmitters cited above, and the hippocampus plays a key role in the functioning of these pathways (Yehuda *et al.,* 2002). Considering the high bonding affinity between mercury and sulfur compounds (*that is*, thiol groups of proteins, peptides and amino acids), interactions of mercury compounds with proteins in the CNS may explain some of their effects on neurotransmission.

Many anticholinergic agents/ drugs cause learning and memory deficits in a variety of cognitive animal models (Zimmerman and Soreq, 2006). Agonists of cholinergic receptors and inhibitors of AChE have been extensively used in order to increase endogenous acetylcholine levels and thus overcome cognitive deficits. Medicinal plant bioactive metabolites play a key role in the slowing of many pathogenesis and neurodegenerative disorders, such as, Alzheimer and dementia (Dai *et al.,* 2006). Beneficial effects of these metabolites are proposed to associate with potentiating of antioxidant defenses, which is linked to normal aging and neurodegenerative diseases (Morris *et al.,* 2006).

Dates are widely used in traditional medicine for the treatment of various disorders, such as, memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders (Nadkarni, 1976), which might be due to the presence of mainly phenolic compounds, which may have more antioxidant activity than vitamins C, E, acarotene (Vinson *et al.*, 1998; Haslam, 2006). Phenolic compounds have anticholinesterase activities, which might be valuable for the treatment of AD (Tumiatti *et al.*, 2008). In this study, administration of EFPD decreased brain AChE activity in rats, which might be due to a decrease in gene transcription, translation and enhance cholinergic activity thereby improving cognitive function (Shahidi *et al.*, 2008). Findings are in accordance to studies related to neuroprotective role of plant extracts (Kulkarni *et al.,* 2011; Tripathi *et al.,* 2011) indicating ameliorative role against mercury-induced elevated levels of AChE activity, and its potential in the attenuations of severity of neurodegenerative disease, such as, AD.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendation

6.1 Summary

In this study, evaluation of the protective effect of aqueous and ethanol fruit extracts of *Phoenix dactylifera L* on mercury-induced neurotoxicity in Wistar rats, the results of phytochemical screening revealed the presence of flavonoids, saponins, tannins and alkaloids which have been reported to exert neuroprotective actions in animal and cell culture models of neurological disorders. Exposure to mercury ($HgCl_2$) resulted in neurotoxicity, which was evident from morphologic, histometric, histologic and histochemical alterations in the brain of the rats. Mercury induced anxiety-related responses, short- and long-term memory impairments and motor deficits in neurobehavioural assessment, and altered the levels of neurochemical and biochemical constituents which could be related, at least in part, by the cortical and hippocampal mercury content that may interfere with local homeostasis. The polyphenolics including flavonoids, found in many plant extracts, have been shown to be strong reactive oxygen species (ROS) scavengers, antioxidants and protectors of neurones from lethal damage (Rice-Evans, 2001; Pujari *et al.,* 2011) and are able to chelate metal ions (Komaki *et al.*, 2015). Flavonoids exert a multiplicity of neuroprotective actions within the brain, including a potential to protect neurones against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning, and cognitive function. Thus, antioxidant activities of the extracts could be implicated for neuroprotection. Fruit extracts (aqueous and ethanol) of *P. dactylifera* benefits the brain

through neuroprotective property, protecting the brain from the actions of ROS by utilizing its antioxidant property (Wan Ismail and Mohd Radzi, 2013).

Findings of the present study reveal the potentials of the aqueous and ethanol fruit extracts of *Phoenix dactylifera* as neuroprotective agent in the following studies:

- Morphologic features of the brain and histometric characteristic of brain regions (cerebral and cerebellar cortices and hippocampus) were preserved relative to the control.
- Mercury-induced histologic and histochemical alterations in cerebral and cerebellar cortices and hippocampal regions (CA1 and CA3) were ameliorated relative to the control.
- Mercury-induced anxiety-related responses, short- and long-term memory impairments, and motor coordination and balance deficits in neurobehavioural assessment were ameliorated relative to the control. Thus extracts possess potentials as axiolytics and, cognitive-motor and memory enhancing agents.
- Alterations in the concentration levels of neuro-trace elements (Fe, Mn, Cu and Zn), MDA and endogenous antioxidants (SOD, CAT, GPx), and endogenous enzyme (AchE) activity levels were moderately ameliorated relative to the control, which protect neurones against reactive oxygen species (ROS).

Neuroprotective treatments often target oxidative stress and excitotoxicity—both of which are highly associated with CNS disorders (Zádori *et al.*, 2012). All these evidences explain the importance of plants in neurodegenerative disorders management in modern day healthcare.

The results of this study revealed that, the aqueous and ethanol fruit extracts of *Phoenix dactylifera* contains pharmacologically active substance(s) with neuroprotective properties. These properties explain the rational for the effective use of the plant as neuroprotective agent in traditional practice of medicine.

6.2 Conclusion

The result of the present study suggests that, the aqueous and ethanol fruit extracts of *Phoenix dactylifera* may prove efficacious in ameliorating mercury-induced alterations in the brain (cerebral and cerebellar cortices and hippocampus) of Wistar rats.

The neuroprotective property of extracts, relative to the standard (vitamin C), is rather similar, and is attributed to antioxidant properties of constituent phytochemicals, such as flavonoids. Thus, aqueous and ethanol fruit extracts of *P. dactylifera* are potential candidate for application in the management and treatment of ROS-induced neurodegenerative diseases, such as AD and PD.

Neuroprotective activity is dose dependent; 500 and 1,000 mg/kg doses possessing maximal activity for both extracts (aqueous and ethanol fruit extract of *P. dactylifera*).

In relation to extracts' neuroprotective activity, ethanol fruit extract of *P. dactylifera* is of more efficacy. This could be that, ethanol extract possess certain additional antioxidative and neuroprotective properties or may involve different mechanisms of constituent antioxidant activities.

6.3 Recommendation

Further studies are required to fully investigate the mechanisms responsible for the observed neuroprotective activity of aqueous and ethanol fruit extracts of *Phoenix dactylifera*, and to determine the exact compound(s) or active ingredients responsible for its neuroprotective activity.

In depth studies such as, ultra-microscopy, stereology and immunohistochemistry are required to confirm findings of the study.

Further studies are required to investigate the possible neuroprotective activity of other solvents (e.g. organic solvents, such as methanol and chloroform) extract of *P. dactylifera* fruit and other parts (such as, the pits or seeds) of the plant.

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APPENDIX I

TISSUE PROCESSING – THE ROUTINE AUTOMATIC PROCESS

1. **Fixation** in formal saline/ Bouin's fluid lasted for 48 hours.
2. **Washing** in running water if necessary
3. **Dehydration** in grades of alcohol
    - 70% alcohol for 3 hours
    - 90% alcohol for 3 hours
    - First change absolute (100%) alcohol for 1 hours
    - Second change absolute (100%) alcohol for 1 hours
    - Third change absolute (100%) alcohol for 2 hours
    - Fourth change absolute (100%) alcohol for 2 hours
4. **Clearing**
    - Put in pure xylene first change for 1.5 hours
    - Pure xylene second change for 2.5 hours
5. **Infiltration**
    - First change pure paraffin (wax bath) 3 hours
    - Second change pure paraffin (wax bath) 3 hours
6. **Embed** in paraffin
7. **Microtomy**

(Drury *et al.*, 1967)

APPENDIX II

PREPARATION OF STAINING SOLUTIONS AND STAINING

1. Haematoxylin and Eosin Stains

A. Haematoxylin (Harris' (1900) Haematoxylin)

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

Heamatoxylin was dissolvded 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 solution was made `by dissolving 5 g of eosin in 100 ml of 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 100ml.

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 - 20 minutes
    - Distilled water (washing)
    - 35% alcohol, 1 minute
    - Acid- 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
    - 90% 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 differentiate in 95% alcohol
    - Dehydrate, clear and mount in mountant.

3. GOLGI STAINING (Marsland, Glees and Erikson's (1954))

- **Fixation** in 10% formal saline
- **Section**
- **Ammoniacal silver solution**

Mix 30 ml of 20% silver nitrate with 20 ml of absolute alcohol. Add strong ammonia drop wise until resulting precipitate dissolves. Add an additional 5 drops of ammonia.

- **Technique**
  - Dewaxing using xylene followed by alcohol
  - Washing in distilled water
  - Place in 2% silver nitrate for 15 - 60 minutes at 37^{o}C
  - Washing in 10% neutral formalin for 10 - 15 seconds
  - Wash off formalin with ammoniacal solution and allowed for 30 seconds
  - Drain off silver solution and wash section with 10% neutral formalin for 1 minute.
  - Wash in distilled water and fix in 5% sodium thiosulphate for 5 minutes
  - Dehydrate, clear and mount in mountant.

(Drury *et al.*, 1967)

APPENDIX III

METHOD OF DETERMINING THE VARIOUS DOSES OF *Phoenix dactylifera* FRUIT EXTRACTS, MERCURIC CHLORIDE AND VITAMIN C SOLUTIONS

1. Dosage Calculation of Fruit Extracts of *Phoenix dactylifera* (aqueous and ethanol) Solutions

To determine the equivalent dosage of volume in **ml** to **mg/kg** body weight **(**bw), the following steps were observed:

A) Dosage in mg/kg bw (250, 500 and 1000 mg/kg bw of extract)

*Taking **1000 mg/kg bw** for instance, the dosage is calculated as follows:*

1000 mg (of extract solute) ----------1 kg (body weight of rat)

1000 mg (of extract solute) ----------1000 g (body weight of rat)

X mg (of extract solute) ----------Y g (measured body weight of rat)

$$\textbf{X mg/kg (of extract solute)} = \frac{\textbf{Y g (of rat) x 1000 mg (of extract)}}{\textbf{1000 g (1 kg bw)}}$$

B) Dosage in ml:

Calculating the dosage in ml equivalent to mg/kg bw, it is important to consider the steps taken in constituting the working solution.

- **Working Solution of Extract:**

100 g (of extract solute) ---------- dissolved in 800 ml (distilled water)

100,000 mg (of extract solute) ---------- dissolved in 800 ml (distilled water)

X mg (of extract *calculated from (A) above*) ---------- dissolved in X ml

$$\textbf{X ml (of extract solution)} = \frac{\textbf{X mg (of extract) x 800 ml}}{\textbf{100,000 mg}}$$

2. Dosage Calculation of Vitamin C (Standard Drug) Solution

To determine the equivalent dosage of volume in **ml** to **mg/kg bw**, the following steps were observed:

A) Dosage in mg/kg bw (100 mg/kg bw of vitamin C)

The dosage is calculated as follows:

100 mg (of standard solute) ---------- 1 kg (body weight of rat)

100 mg (of standard solute) ---------- 1000 g (body weight of rat)

X mg (of standard solute) ----------Y g (measured body weight of rat)

$$\textbf{X mg/kg (of standard solute)} = \frac{\textbf{Y g (of rat) x 100 mg (of standard)}}{\textbf{1000 g (1 kg bw)}}$$

B) Dosage in ml:

Calculating the dosage in ml equivalent to mg/kg bw, it is important to consider the steps taken in constituting the working solution.

- **Working Solution of Standard:**

10 g (of standard solute) ---------- dissolved in 300 ml (distilled water)

10,000 mg (of standard solute) ---------- dissolved in 300 ml (distilled water)

X mg (of standard *calculated from (A) above*) ----------dissolved in X ml

$$\textbf{X ml (of standard solution)} = \frac{\textbf{X mg (of stantard) x 300 ml}}{\textbf{10, 000 mg}}$$

3. Dosage Calculation of Mercuric Chloride (Toxin) Solution

To determine the equivalent dosage of volume in **ml** to **mg/kg bw**, the following steps were observed:

A) Dosage in mg/kg bw (5 mg/kg bw of mercuric chloride)

The dosage is calculated as follows:

5 mg (of toxin solute) ---------- 1 kg (body weight of rat)

5 mg (of toxin solute) ---------- 1000 g (body weight of rat)

X mg (of toxin solute) ----------Y g (measured body weight of rat)

$$\textbf{X mg/kg (of toxin solute)} = \frac{\textbf{Y g (of rat) x 5 mg (of toxin)}}{\textbf{1000g (1 kg bw)}}$$

B) Dosage in ml:

Calculating the dosage in ml equivalent to mg/kg bw, it is important to consider the steps taken in constituting the working solution.

- **Working Solution of Standard:**

50 g (of toxin solute) ---------- dissolved in 1000 ml (distilled water)

50,000 mg (of toxin solute) ---------- dissolved in 1000 ml (distilled water)

X mg (of standard *calculated from (A) above*) ----------dissolved in X ml

$$\textbf{X ml (of toxin solution)} = \frac{\textbf{X mg (of toxin) x 1000 ml}}{\textbf{50, 000 mg}}$$

- **The calculated volume (in ml) of the extract/ standard/ toxin was then administered depending on group and body weight of the experimental animals (Wistar rats).**

APPENDIX IV

ANALYTICAL METHOD FOR DETERMINING METALS IN BIOLOGICAL TISSUES

1. **Tissue preparation/ digestion** (EMMI, 1997)

- Take 1 ml (0.25 g) of sample (homogenized tissue; 1 g in 4 ml of phosphate buffer) into boiling tube and add 2 ml of concentrated HNO_3
- Heat (100 ^oC) for 2 hours and allow to cool
- Add 0.3 ml of 30 % H_2O_2
- Heat (100 ^oC) for 1- 2 hours and allow to cool
- Filter using (Whatmann filter paper)

2. **Dilution/ deionization**

- Make volume (of digested sample) into 20 ml using deionized water

3. **Analytical method:** Atomic Absorption Spectrophotometry (AAS)

4. **Computation of element concentration**

*Assuming the result obtained from AAS analysis was **X** conc.ppm for sample 1*. To determine the concentration of element in parts per million (**ppm**) or **mg** (of element)**/ kg** (of tissue), the following steps were observed:

Sample 1 = $\frac{\textbf{X x 20 ml} \text{ (dissolution factor)}}{\textbf{0.25 g (}\text{sample weight in 1 ml of homogenized tissue}\textbf{)}}$ = **A**

Blank sample = $\frac{\textbf{Y x 20 ml} \text{ (dissolution factor)}}{\textbf{2.3 ml (}2 \text{ ml of } HNO_3 \text{ and } 0.3 \text{ ml } H_2O_2\text{)}}$ = **B**

Total concentration of element in tissue (*ppm* or *mg/kg*) = **A – B**

APPENDIX V

HISTOMETRIC METHODS USING THE AMSCOPE MICROSCOPE CAMERA

1. Photomicrography

- Using a light microscope with a x40 objective (x 400 magnification) and computer running image processing soft ware (AmScope MT *version* 3.0.0.5, USA) for microscopy, digital microscopic images are captured.

2. Histometry and calibration (Using the AmScope Microscope Cameras; Psi Wavefunction, 2009)

- Firstly, a micrometer slide (microscope slide with a scale on it), come as 1 mm graduated in 0.01 mm units (that is divided 10x into 100 μm units) is captured at the same magnification and quality (pixels) the micrograph was photographed.
- Secondly, select the line tool, and carefully measure from start of one major line (of the captured microscope slide) to the start of the next, or end to end.

  Recorded length in pixels; in this study was, 649 pixels/ 100 μm. Thus, under given conditions, the 40x scale is 649 pixels/ 100μm, or 6.49 pixel/ μm
- Next, use the polygon tool to measure the area and perimeter of the desired cells. Values are computed and presented automatically by the software.

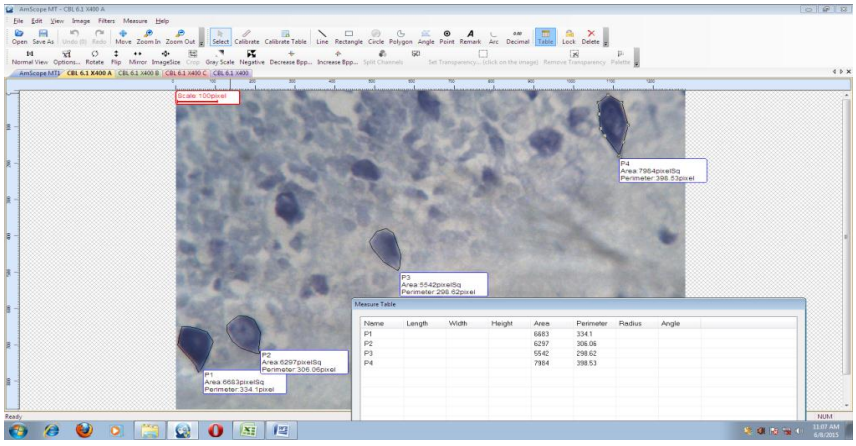


Plate 3.1: Histometry using a Cresly fast violet stained micrographs (digital microscopic images) with a computer running imaging soft ware (AmScope MT *version* 3.0.0.5, USA

APPENDIX VI

DATA TABLES AND FIGURES

Table 2.1: Description of some varieties of Date palm

S/No.	Variety	Brief description
1.	**'Barhi'**	Nearly cylindrical, light amber to dark brown when ripe; soft, with thick flesh and rich flavor; of superb quality.
2.	**'Dayri'** (the "Monastery Date")	Long, slender, nearly black, soft. *Palm requires special care.*
3.	'Deglet Noor'	It is semi-dry, not very sweet; keeps well; much used for cooking. *The palm is high yielding but not very tolerant of rain and atmospheric humidity.*
4.	**'Halawy'** ('Halawi')	Soft, extremely sweet, small to medium; may shrivel during ripening unless the palm is well-watered. *It is especially tolerant of humidity.*
5.	**'Hayany'** ('Hayani')	The fruit is dark-red to nearly black; soft. *The palm is one of the most cold-tolerant; is sold fresh; is not easy to cure.*
6.	**'Khadrawy'** ('Khadrawi')	it is a soft date of the highest quality. It is early-ripening; does not keep too well. *This cultivar is the smallest edible date palm grown in the United States and it is fairly tolerant of rain and humidity; dark in color.*
7.	**'Khastawi'** ('Khustawi'; 'Kustawy')	Small in size; prized for dessert; keeps well. The palm is large and vigorous and produces its offshoots high on the trunk in California. *The fruit is resistant to humidity.*
8.	**'Maktoom'**	Large, red-brown; thick-skinned, soft, mealy, medium sweet; *resistant to humidity.*
9.	**'Medjool'**	Large, soft, and luscious.
10.	**'Saidy'** ('Saidi')	Soft, very sweet; palm is a heavy bearer; *needs a very hot climate.*

11.	**'Sayer'** ('Sayir')	Dark orange-brown, of medium size, soft, syrupy, and sometimes some of the syrup is drained out and sold separately; *not of high quality but the palm is one of the most tolerant of salt and other adverse factors*
12.	**'Thoory'**('Thuri')	Fruit is dry; when cured is brown-red with bluish bloom with much wrinkled skin and the flesh is sometimes hard and brittle but the flavor is good, sweet and nutty. Keeps well; often carried on journeys. The palm is stout with short, stiff leaves; bears heavily, and clusters are very large; somewhat tolerant of humidity.
13.	**'Zahdi'**('Zahidi')	Of medium size, cylindrical, light golden-brown; semi-dry but harvested and sold in 3 stages: soft, medium-hard, and hard: very sugary; keeps well for months; much used for culinary purposes. The palm is stout, fast growing, heavy bearing; drought resistant; has little tolerance of high humidity.

(Morton *et. al.*, 1987).

Table 2.2: Dried Dates (edible parts) Nutritional Value per 100 g (3.5 oz)

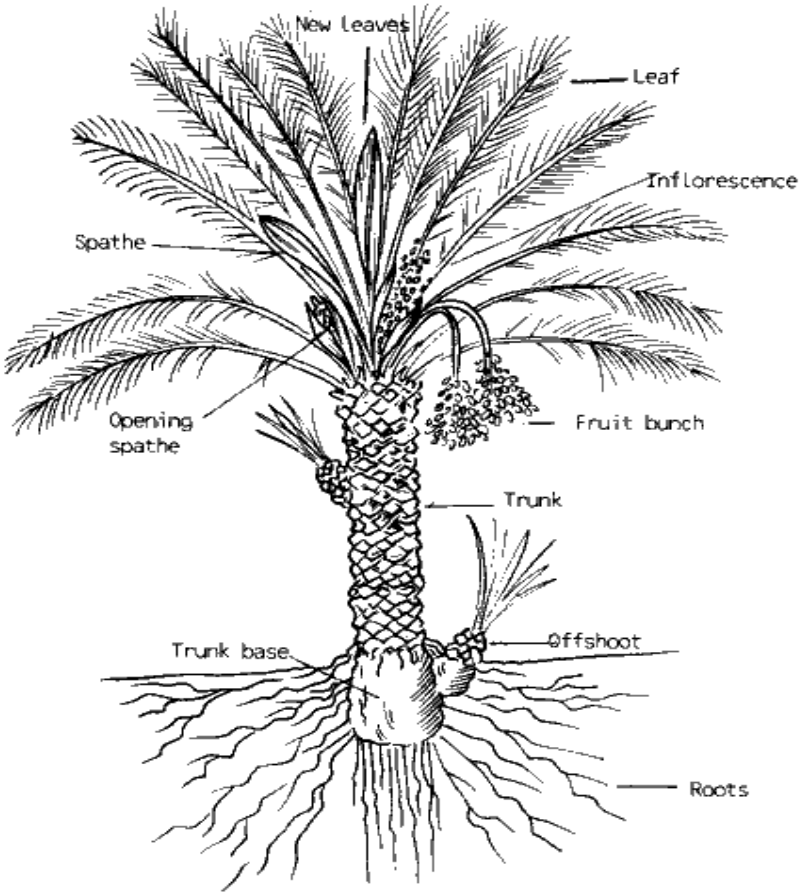
Energy	1,180 kJ (280 kcal)
Carbohydrates	75 g
Sugars	63 g
Dietary fibre	8 g
Fat	0.4 g
Protein	2.5 g
Water	21 g
Vitamin C	0.4 mg (1%)
Manganese	0.262 mg
Percentages are relative to US recommendations for adults. *Source*: USDA Nutrient database	

Table 2.3: Comparing the Nutritional Value of *Fresh* and *Dried* Date Palm Fruit.

Food Value per 100 g of Edible Portion*

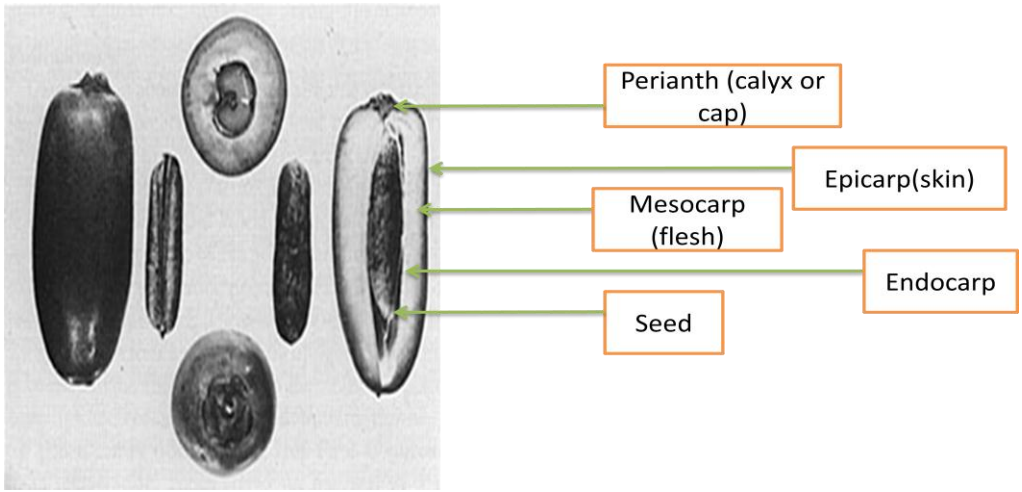
	Fresh, uncooked	***Dried***
Calories	142	274 293
Moisture	31.9 78.5 g	7.0 - 26.1
Protein	0.9 2.6 g	1.7 - 3.9 g
Fat	0.6 1.5 g	0.1 - 1.2 g
Carbohydrates	36.6 g	72.9 - 77.6 g
Fiber	2.6 4.5 g	2.0 - 8.5 g
Ash	0.5 2.8 g	0.5 - 2.7 g
Calcium	34 mg	59 - 103 mg
Phosphorus	350 mg	63 - 105 mg
Iron	6.0 mg	3.0 - 13.7 mg
Potassium	?	648 mg
Vitamin A (ß carotene)	110-175 mcg	15.60 mg
Thiamine	?	0.03 - 0.09 mg
Riboflavin	?	0.10 - 0.16 mg
Niacin	4.4-6.9 mg	1.4 - 2.2 mg
Tryptophan	?	10 - 17 mg
Ascorbic Acid	30 mg	0

*Based on standard analyses. (Sawaya *et al.*, 1983).



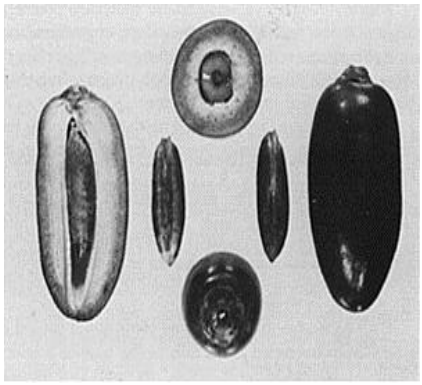
(Barreveld, 1993).

Figure 2.1 Date palm tree and parts

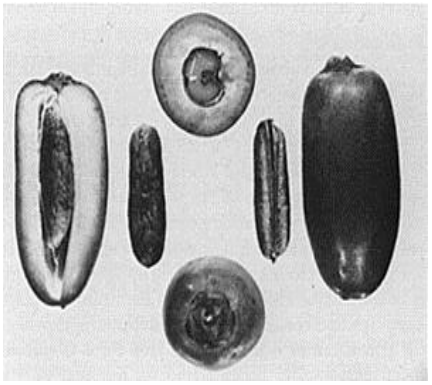


(Barreveld, 1993; Morton *et. al*., 1987).

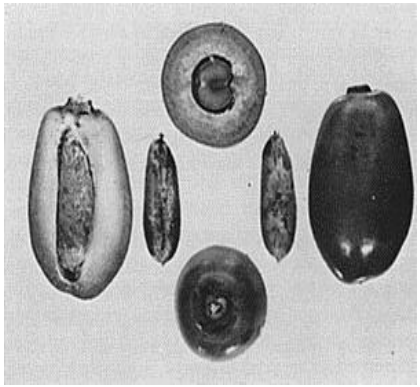
Figure 2.2 Date palm fruit and parts



'Deglet Noor'

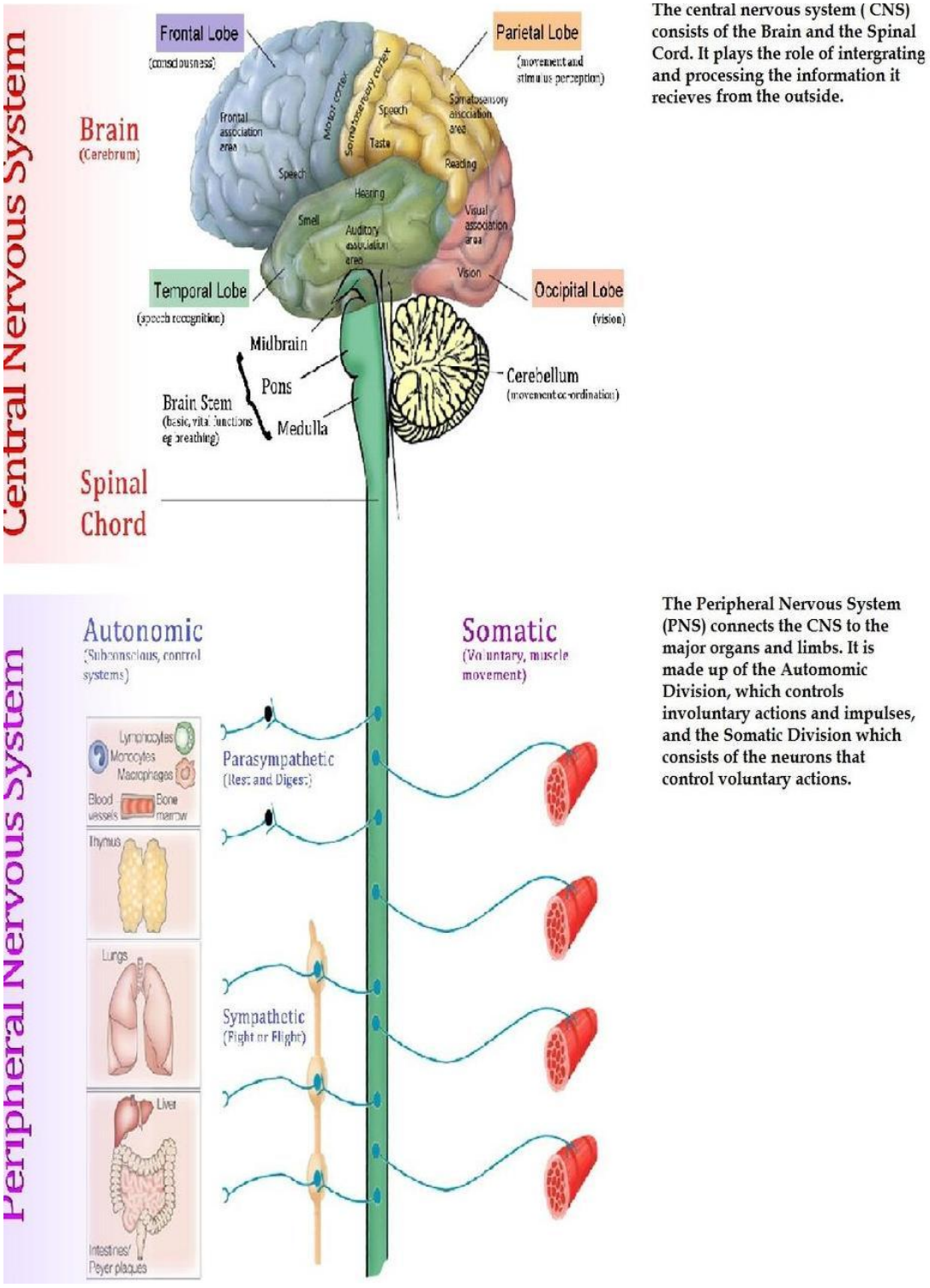


'Halawi'



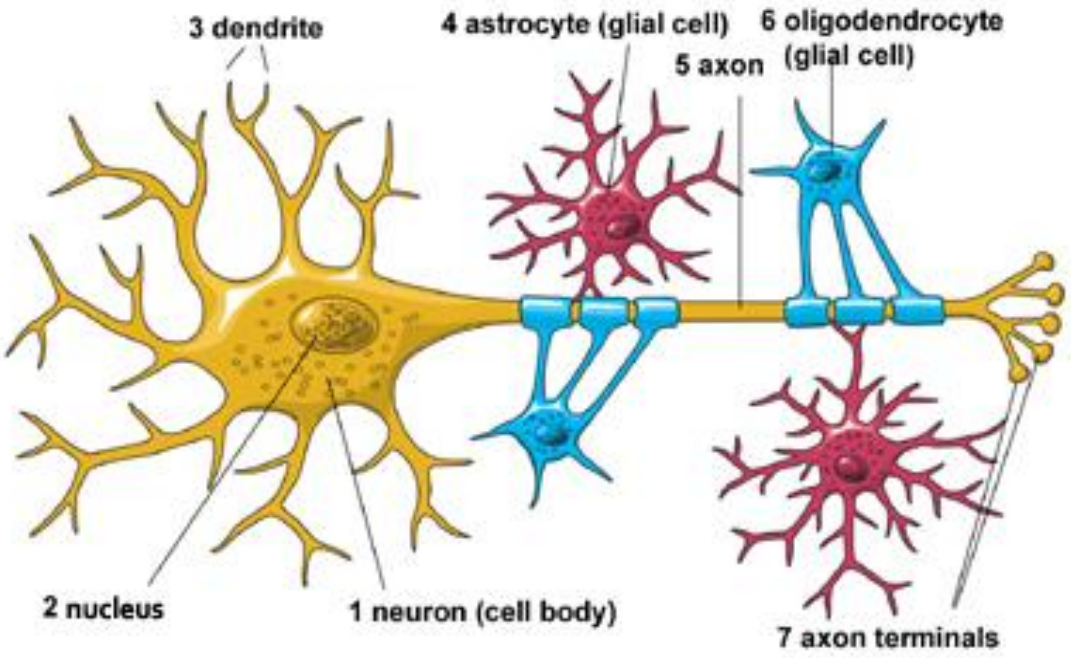
'Zahdi' (Morton *et. al.*, 1987).

Figure 2.3: Some varieties of date palm



(Source: *www.livelancaster.org*)

Figure 2.4: The Central and peripheral nervous systems



(Source: *www.ninds.nih.gov*)

Figure 2.5: Neurone and glial cells

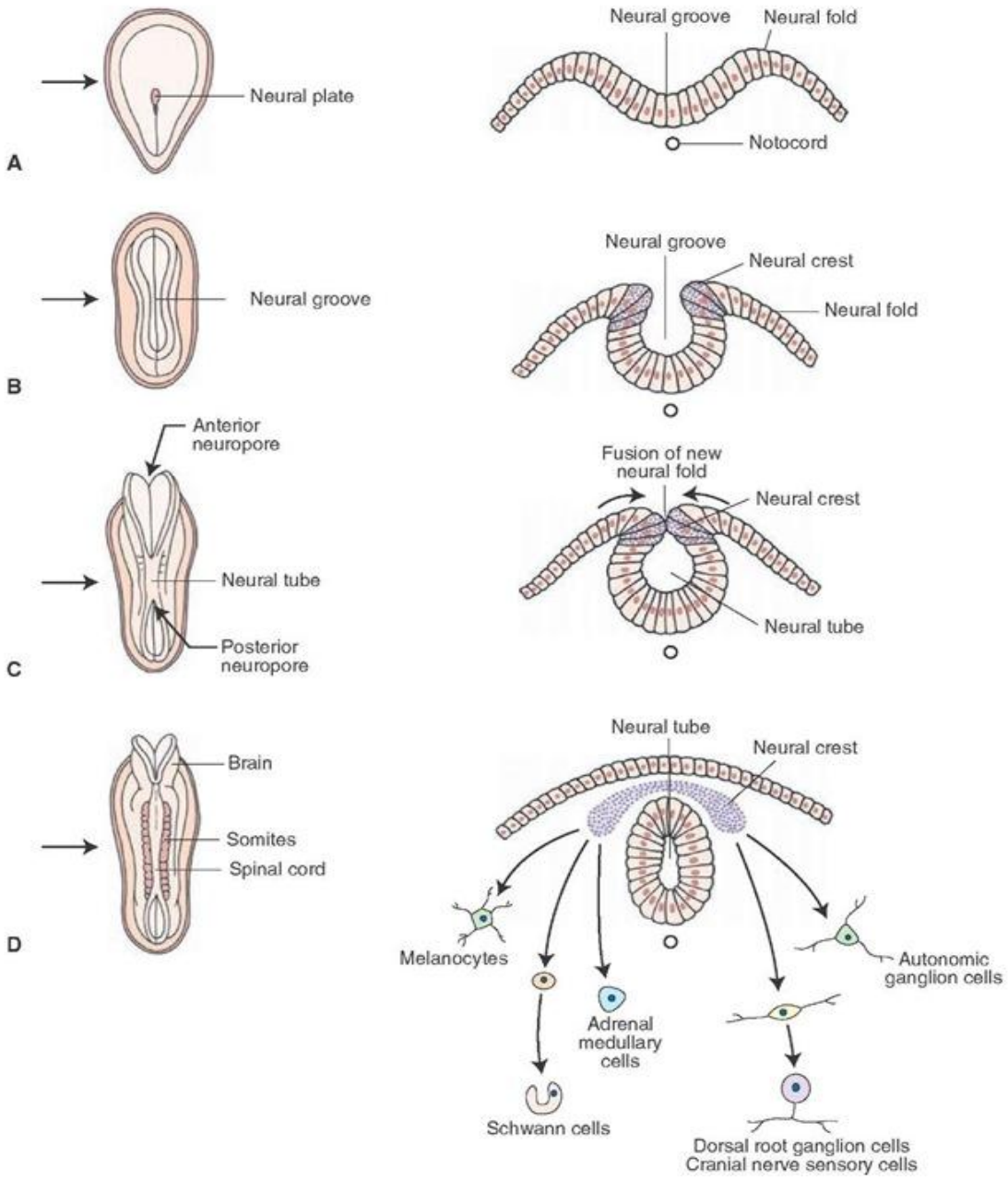
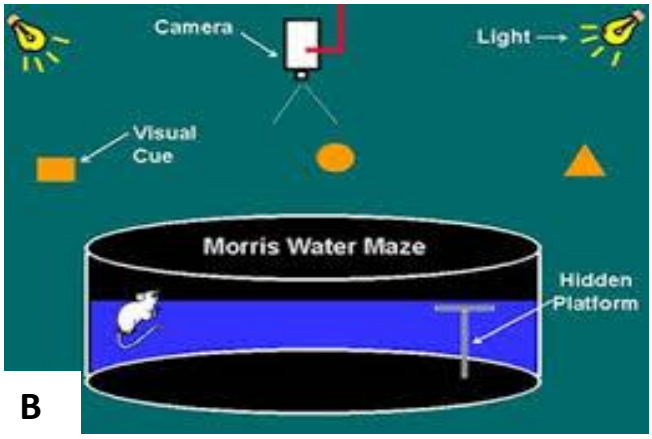


Figure 2.6: Neural plate and development of the nervous system

Panels A-D depict early development (at the third and fourth weeks of gestation) in which the neural plate (A), neural groove (B), and neural tube (C) are formed from the dorsal surface of the embryo. The left side of each panel depicts the developing embryo in a dorsal view, and the right side shows cross sections through the nervous system cut at the levels indicated by the arrows. Note also the cells formed from differentiated cells of the neural crest (D).

(Source: *what-when-how;* *In Depth Tutorials and Information*)





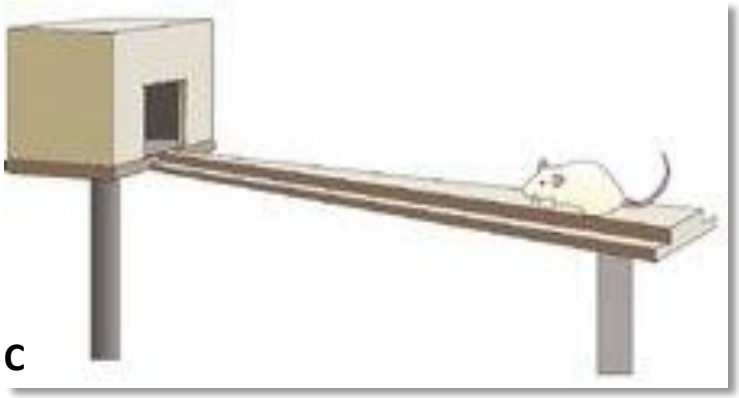


Figure 2.7: Apparatuses for neurobehavioural studies.

A= Morris water maze; B= Elevated plus maze; C= Beam walk set-up

(*Source*: Neuroscience: Nature reviews; Drug Discovery: Nature reviews (2005))