THE COMPARATIVE EFFECTS OF AQUEOUS AND ETHANOL FRUIT EXTRACTS OF *PHOENIX DACTYLIFERA* ON THE HIPPOCAMPUS AND CEREBELLAR CORTEX OF ARTESUNATE-AMODIAQUINE TREATED ADULT WISTAR RATS

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FACULTY OF BASIC MEDICAL SCIENCES
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ZARIA, NIGERIA

JULY, 2018

DECLARATION

I, Michael Ndomi BUDAYE, hereby declare that this research work reported in this dissertation entitled "**The comparative effects of aqueous and ethanol fruit extracts of *Phoenix dactylifera* on the hippocampus and cerebellar cortex of artesunate-amodiaquine treated adult Wistar rats"** was carried out by me in the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Science, Ahmadu Bello University Zaria, under the supervision of Prof. S. S. Adebisi and Dr. A. A. Buraimoh. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work was previously presented for another degree or diploma at this or any other Institution.

_____ _____

Michael Ndomi BUDAYE **Date**

CERTIFICATION

This Dissertation titled "THE COMPARATIVE EFFECTS OF AQUEOUS AND ETHANOL FRUIT EXTRACTS OF *Phoenix dactylifera* ON THE HIPPOCAMPUS AND CEREBELLAR CORTEX OF ARTESUNATE-AMODIAQUINE TREATED ADULT WISTAR RATS" by Michael Ndomi BUDAYE meets the regulations governing the award of the degree of Master of Science (M.Sc.) of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

THIS DISSERTATION IS DEDICATED

TO MY LOVING PARENTS AND BELOVED SIBLINGS

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ABSTRACT

Amidst the current rise in the usage of artemisinin-based combination therapies as the first line treatment of falciparum resistant malaria in the Tropics, artesunate-amodiaquine combination therapy have been found to have degenerative and toxicity effects. This study was aimed at evaluating the effects of aqueous and ethanol fruit extracts of *Phoenix dactylifera* on the hippocampus and cerebellar cortex of artesunate-amodiaquine (AS-AQ) treated Wistar rats, as well as its effects on neurobehaviour, neuro-trace elements and oxidative stress markers. Thirty six (36) adult Wistar rats (150-180g) were used; they were randomly divided into nine groups. The rats in Group 1 served as the control; Group 2 was administered 2.86/ 8.75mg/kg AS-AQ. Groups 3, 4 and 5 were administered 2.86/8.75mg/kg AS-AQ + 500mg/kg aqueous extract of *P. dactylifera* (AEPD), 2.86/8.75mg/kg AS-AQ + 1000mg/kg AEPD and 2.86/8.75mg/kg AS-AQ + 1500mg/kg AEPD respectively. Groups 6, 7 and 8 were administered 2.86/8.75mg/kg AS-AQ + 500mg/kg ethanol extract of *P. dactylifera* (EEPD), 2.86/8.75mg/kg AS-AQ + 1000mg/kg EEPD and 2.86/8.75mg/kg AS-AQ + 1500mg/kg EEPD respectively. Group 9 served as the standard drug group and was administered 2.86/8.75mg/kg AS-AQ + 100mg/kg ascorbic acid. Once weekly, for 3 weeks, behavioural tests using the Morris water maze for spatial learning and memory, and Beam walk test setup for coordination and balancing were carried out. The drugs administration lasted for 28 days after which the animals were sacrificed, their brain excised and processed for histological (using Hematoxylin and Eosin, Cresyl fast violet, and Bielschowsky stains), biochemical (Superoxide dismutase, Catalase, Glutathione reductase, and Malondialdehyde), and neuro-trace elements (Zinc, Iron, Copper, and Manganese) studies. The histoarchitecture of the AEPD and EEPD treated groups were preserved particularly the 1000mg/kg and 1500 mg/kg doses, when compared to the AS-AQ and ascorbic acid treatments. The AS-AQ had no significant effect on the spatial learning and memory, and motor coordination and balance evidenced by the time latencies observed in the neurobehavioural study. Neurotoxicity, evidenced by necrosis, chromatolysis, vacuolations, was observed in the cerebellar cortex and hippocampus of the AS-AQ treated group. In the biochemical study, oxidative stress markers (SOD, CAT, and GSH) revealed no difference, while MDA concentration was significantly elevated ($p<0.05$) in the AS-AQ treatment when compared to the control. The neuro-trace elements result of trace metals (Zn, Fe, Cu and Mn) showed only Mn to have decreased significantly in the 1500mg/kg AEPD treated group. It was concluded that the

amelioration of the severity of neurodegeneration, stability of the neurobehavioural index, alteration of oxidative stress markers and neuro-trace elements by the extracts indicated their ameliorative effects by antioxidant activities ascribed to the phytochemicals present in the extracts.

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LIST OF ABBREVIATIONS

AA	Amino acid
AAS	Atomic absorption spectrophotometer
ACH	Acetylcholine
ACTs	Artemisinin-based Combination Therapy
AEPD	Aqueous extract of *P. dactylifera*
AL	Artemether + Lumefantrine
ALP	Alanine phosphatase
ALT	Alanine transaminase
AS + AQ	Artesunate + Amodiaquine
AS + MQ	Artesunate + Mefloquine
AS + SP	Artesunate + Sulfadoxine-pyrimethamine
AST	Aspartate transaminase
Bw	Body weight
CAT	Catalase
CCl_4	Carbon tetrachloride
CFV	Cresyl fast violet
CNS	Central nervous system
Cu	Copper
DG	Dentate gyrus
DHA + PPQ	Dihydroartemisinin + piperaquine
DNA	Deoxyribonucleic acid
EEPD	Ethanolic extract of *Phoenix dactylifera*

EMMI	Environmental Monitoring Method Index
EPD	Extract of *Phoenix dactylifera*
G6PD	Glucose-6-Phosphate Dehydrogenase
GAMA	Gamma aminobutyric acid
GPx	Glutathione peroxidase
GSH	Glutathione reductase
Kg	kilogram
LD_{50}	Lethal dose (Medium)
Mg	milligram
Ml	milliliters
Mn	Manganese
NIDA	National Institute on Drug Abuse
NPC	Neuroprogenitor cells
P. dactylifera	*Phoenix dactylifera*
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TAG	Triacyglycerol
TBARS	Thiobarbituric acid reactive substances
TP	Total protein
WHO	World Health Organization
Zn	Zinc

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Malaria is one of the deadliest diseases of the tropics, and in particular Africa (Adebisi, 2007). It is an important cause of death and illness in children and adults, especially in tropical countries. Malaria is the commonest cause of outpatient consultations and a major cause of morbidity and mortality in Nigeria It is highly endemic in Nigeria, and accounts for over 60% outpatient and 30% hospital admission in Nigeria (Federal Ministry of Health report, 2010).

According to the 2012 World Malaria Report, there were an estimated 219 million cases of malaria in 101 malaria endemic countries in 2011, 80 % of which were reported in Africa. In the same year, 3.17 billion people globally were at risk of malaria (WHO, 2012). The number of malaria deaths was estimated to be 660,000 in 2010. Of these, 86 % were children under 5 years of age and again >90% of malaria deaths occurred in the WHO Africa region (WHO, 2011). The disproportionately high mortality and morbidity associated with malaria in Africa is attributed to the differential distribution of *Plasmodium* species as well as economic development. *Plasmodium falciparum*, the most virulent species, is preponderant in tropical Africa. Two countries, Nigeria and the Democratic Republic of Congo, account for 23 % of the global *P. falciparum* burden (Hay *et al*., 2010)

The severity of the 'malaria burden' became all the more pronounced as the parasite developed resistance to almost all available anti-malarials - chloroquine, sulfadoxine-pyrimethamine and mefloquine from the 1960s to 80s. It was therefore a welcome development that the Chinese

research effort was bearing fruits in the isolation and purification of artemisinin, a potent and rapidly acting schizontocide from crude extracts of Artemisia *annua*. With this came revived optimism that the world-wide efforts at malaria control can transform to eradication. This optimism has been rewarded as the last decade (2000-2010) has seen a drastic downturn in the prevalence and mortality of malaria. Much of this has resulted from the advent and 'judicious' use of artemisinin and its derivatives in the fight against malaria (Garcia, 2015)

The broad collapse of preventive efforts, increasing parasite resistance and failure of single drug treatment of malaria in many endemic countries of Africa has led to a widespread promotion of artemisinin-based combination therapies (ACTs) as a strategy for effective management of *Plasmodium falciparum* malaria (Trape, 1998) Chloroquine was the official first-line antimalarial drug in Nigeria until February, 2005 when the Federal Ministry of Health announced the change to artemisinin (Cotexin® and Artemisinin combination therapies) (Lyda *et al*., 2007)

Artemisinin-based combination therapy for malaria is the simultaneous use of an artemisinin and another blood schizonticidal drug where both have different biochemical targets in the parasites. This has exploited the synergistic and additive potential of the individual drugs. Artemisinin kills majority of the parasites at the start of the treatment, while the slow-acting partner drug clears the remaining parasites (Oreagba, 2010).

Artemisinin-based combination therapies could be fixed combination therapy where compounds are co-formulated in the same tablets. It can also be in free combination therapy where components are co-administered separately (Chanthap *et al*., 2005)

The ACTs options now recommended for treatment of uncomplicated falciparum malaria include

1. Artemether plus Lumefantrine (AL)

2. Artesunate plus Amodiaquine (AS + AQ)
3. Artesunate plus Mefloquine (AS + MQ)
4. Artesunate plus Sulfadoxine-pyrimethamine (AS + SP)
5. Dihydroartemisinin plus Piperaquine (DHA + PPQ) (WHO, 2010).

Artesunate is a part of the artemisinin group of drugs used to treat malaria, especially chloroquine-resistant malaria in Nigeria. It is the most widely used member of artemisinin derivatives (Nwanjo and Oze, 2007). It is a semi-synthetic derivative of artemisinin that is water-soluble (Van Agtmael *et al*., 1999). It is used in combination with other antimalarial drugs such as Mefloquine (Looareesuwan *et al*., 1996; Nosten *et al*., 2000) or Amodiaquine (Adjuik *et al*., 2002). This is done so as to avoid development of resistance of the malaria parasite.

Among its contemporaries, artesunate, a semi-synthetic artemisinin anti-malarial, is presently being esteemed above others as a ready combatant against the malaria menace plaguing most parts of the tropics; one, for being less or non-resisted by the parasite and, two, less allergy-provoking to the users. However, recent clinical observations and experimental studies had implicated this drug to be embryo-fetal toxic and has since generated research interests. In spite of its excellent antimalarial activity, it was observed, through pharmacovigilance programs, that prophylactic use of artesunate caused severe side effects including hepatitis, aplastic anemia and agranulocytosis (Adebisi, 2010).

Amodiaquine belongs to a class of drugs known as the 4-amino-quinoline compounds. The synthesis of this drug was first reported at the meeting of the American Chemical Society in 1946 and is in wide use mainly as an anti-malarial drug (Neftel *et al*., 1986; Misra *et al*., 1995)

In Africa, amodiaquine is significantly more effective than chloroquine in parasite clearance and a faster clinical recovery (Schellenberg and Menendez, 2001). It is also more effective in areas of high chloroquine resistance (Olliaro *et al*., 1996).

The 4-aminophenol moiety of amodiaquine has been identified as a 'structural alert' for toxicity. Bioactivation of this moiety to a reactive quinine imine metabolite has been identified as the likely cause of amodiaquine toxicity. This reactive metabolite acts by covalently binding to cellular macromolecules causing drug-induced toxicity and cell damage directly or via immunological mechanisms (Kalgutkar *et al*., 2005).

The emergence of resistance and tolerance to the existing drugs has created a decreased efficiency of these drugs in use. Several attempts targeted at overcoming this problem have been tried by increasing the drug delivery to the target site by the use of polymers (Khalid *et al.,* 2009; Hussain *et al.,* 2011) or through nanotechnology (Naz *et al*., 2012; Ehsan *et al*., 2012), synthesis of new drugs, either by the use of proteomics (Qadir, 2011; Qadir and Malik, 2011), or synthesis from lactic acid bacteria (Masood *et al*., 2011), or marine microorganisms (Javed *et al*., 2011). Nowadays, however, the trend is being changed from synthetic drugs to the natural drugs either from plants or microbes to control diseases. The natural products are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory (Qadir, 2009), hypotensive (Qadir, 2010), hepatoprotective (Ahmad *et al*., 2012; Ali *et al*., 2013), hypoglycemic (Nisa *et al*., 2009; Qadir and Malik, 2010), anti-fertility, cytotoxic, anti-microbial (Amin *et al*., 2012), spasmolytic, bronchodilator (Janbaz *et al*., 2013a) , antioxidant (Janbaz *et al.*, 2012), anti-diarrheal (Agbon *et al*., 2013; Janbaz *et al*., 2013b) and anti-parkinsonism properties.

Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine (Krentz and Bailey, 2005). Traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions (Gupta and Briyal, 2004)

Phoenix dactylifera (Date palm) belongs to the Palmae (Aracaceae) family. The date palm is a palm extensively cultivated for its edible fruit (Rani *et al*., 2007). The fruits of the date palm are sweet berries with a sugar content of more than 50%. Because of its high nutritional value, great yields, and its long life, the date palm has been mentioned as the "tree of life" (Augstburger *et al.*, 2002)

Date pulps hold easily digestible sugars (70%) mostly glucose, sucrose and fructose, dietary fibers, and enclose less proteins and fat (Al Farsi and Lee, 2008). They also enclose vitamins like riboflavin, biotin, thiamine, ascorbic and folic acid that are essential for the body. The pulps of the fruit are rich in calcium, iron, copper, cobalt, magnesium, fluorine, manganese, phosphorus, potassium, sodium, Boron, silicon, zinc, and selenium (Al Farsi and Lee, 2008; Elias, 2008). The fleshy tissues of dates contain 0.2-0.5% oil, while the seed contains 7.7-9.7% oil (Walid, Al-Shahib and Richard, 2004)

The date palm is rich in phytochemicals like sterols, phenolics, carotenoids, procyanidins, anthocyanins, alkaloids, saponins, tannins, and flavonoids (Abdelhak *et al*., 2005; Abdul and Allaith, 2008). These natural compounds are known to function as free radical scavengers, anti-inflammatory, hepatoprotective and nephroprotective agents (Baliga *et al*., 2011)

In vitro studies have exposed that the aqueous extract of date fruit is a powerful scavenger of hydroxyl radicals and superoxides and to restrain protein oxidation and iron-induced lipid peroxidation in the rat brain homogenate in a concentration-dependent manner (Vayalil, 2002)

Studies conducted in which rats were treated with date fruit and seed extracts, it was found that neuronal damage in the form of shrinkage, atrophy and necrosis of neurons is greatly reduced compared to those not treated with Date. How the date palm extract works is that they decrease the peroxidation of lipid in brain and restore altered antioxidant enzymes caused by ROS (Kalantaripour *et al*., 2012). A study done by Kalantaripour *et al.,* (2012) showed that middle carotid artery occlusion induced 89.37% neuronal death in rats while the group treated with date seed extract (80 mg/kg) decreased the neuronal damage by 30.33%.

The human brain is the most complex organ in the body. It regulates basic life functions, enables one to interpret and respond to everything one experiences, shapes one's thoughts, emotions and behavior (NIDA, 2010)

The cerebellum is a region of the brain that plays an important role in motor control. It may also be involved in some cognitive functions, such as attention and language, and in regulating fear and pleasure responses (Wolf *et al*., 2009). Its movement-related functions are the most solidly established. The cerebellum does not initiate movement, but it contributes to coordination, precision, and accurate timing. It receives input from sensory systems of the spinal cord and from other parts of the brain, and integrates these inputs to fine-tune motor activity (Fine *et al*., 2002)

The hippocampus is a component of the brain 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. It is located under the cerebral cortex, and in primates it is located in the medial temporal lobe, underneath the cortical surface (Aboitiz *et al*., 2003; Amaral and Lavenex, 2006)

1.2 Statement of Research Problem

Artesunate-amodiaquine combination therapy has been found to have degenerative and toxicity effects in the liver and testes. The current rise in the usage of artemisinin-based combination therapies as the first line treatment of falciparum resistant malaria in the tropics and their established toxic and degenerative effects (Ekong *et al.,* 2009; Adebisi, 2009., 2010; Timbuak *et al*., 2014) necessitated this study

1.3 Justification of the Study

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans as well, as valuable components of seasoning, beverages, cosmetics, dyes and medicine. Most of these therapies involve the use of plant extracts or their active components (Craig, 1999)

P. dactylifera has been reported to ameliorate induced degenerative changes in the liver (Abdelrahman *et al.,* 2012; Al-Qarawi *et al*., 2004) and testes (Hassan *et al*., 2012) owing to its antioxidant and scavenging effects. In separate studies, the effects of both the aqueous and ethanol extracts of *P. dactylifera* have been reported (Al-Taher, 2008; Al-Qawari *et al*., 2005; Puri *et al*., 2000). This study thus seeks to investigate the comparative effect of both extracts.

1.4 Significance of the Study

The outcome of this study could help in understanding the potentials of the aqueous and ethanol extracts of *P. dactylifera* in buffering/countering the possible neurotoxicity of artesunate-amodiaquine combination therapy that is used in the treatment of malaria.

1.5 Aim and Objectives of the Study

1.5.1 Aim

This study assessed the ameliorative effect of the aqueous and ethanol extracts of *P. dactylifera L.* on the cerebellum and hippocampus of artesunate-amodiaquine treated Wistar rats

1.5.2 Objectives

To assess the ameliorative effects of the aqueous and ethanol extracts of *P. dactylifera L.* on the:

1. neurobehavioural changes in spatial learning and memory, and motor coordination and balancing, using Morris Water Maze and Beam Walking tests, respectively
2. biochemical changes in the levels of the oxidative stress markers (Superoxide dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA) and Glutathione reductase (GSH)) in the brain using antioxidant assays.
3. neuro-trace elements of Copper, Iron, Manganese, and Zinc.
4. microscopic changes in the cerebellar cortex and hippocampus of artesunate-amodiaquine treated adult Wistar rats using routine histological (Hematoxylin and Eosin (H and E)) and Histochemical (Cresyl Fast Violet and Bielschowsky) techniques.

1.6 Scope of the Study

The study was limited to the histological/microscopic, neuro-trace elements and biochemical changes that may occur in the cerebellar cortex and hippocampus, as well as the study of changes in spatial learning and memory that may occur as a result of exposure to artesunate-amodiaquine administration.

1.7 Study Hypothesis

Aqueous and ethanol extracts of *P. dactylifera* will ameliorate the neurotoxic effects induced by artesunate-amodiaquine combination treatment in adult Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Artemisinin

The history of the discovery of artemisinin is steeped in great antiquity and demonstrates, perhaps more poignantly and succinctly than most others the best and the worst trait of mankind (Bennett, 2011). It is doubtful that the intense effort of an entire segment of the Chinese Scientific community that ultimately resulted in the extraction and purification of Artemisinin would have arisen were it not for the events directly related to and arising from the battlefields of the Vietnamese war of 1965 to 1973: Aside from the casualties sustained in combats, malaria was taking its toll on both sides. The appearance of chloroquine resistant form of malaria further compounded the problem (Edikpo and Adikwu, 2013).

Without the kind of massive financial resources that the Americans poured into their antimalarial research program, the Vietnamese had no other alternative but to turn to their fellow Communist, China for help. Communist Party Chairman, Mao Zedong and Prime Minister Zhou Enlai, responded to their Communist allies request by immediately ordering a nation-wide research program. A meeting discussing the action plan was held on 23rd May, 1967, thus code-named "project 532" (Miller and Su, 2011; Faurant, 2011) after the date it was inaugurated. Though the immediate objective was to aid their North Vietnamese allies in their war endeavors, the long-term goal was the discovery of new antimalarial drugs by screening synthetic chemicals and traditional Chinese recipe (Miller and Su, 2011) for antimalarial pharmacological activities.

A National Steering Committee on Anti-malaria Research was set up in 1967 and an antimalarial drug discovery program was established in 1969. The project was a collaborative effort that

involved Pharmacologists, Pharmacognosists, Phytochemists, Clinicians and Traditional Chinese Medical Practitioners led by Zhenxing Wei and You-Tu Li were drawn from all over China (Willcox, 2009).

In the course of their research, these Chinese scientists investigated the most diverse plant extracts and combed through ancient literature. Screening of the traditional Chinese pharmacopeia soon led to the identification of more than ten plants with good anti-malaria activities of which *Artemisia annua* was one. It was only the crude extract of *Artemisia annua* that yielded significant antiplasmodial activity (Liwang *et al.*, 2009). The road to the extraction of the active constituent, artemisinin was long and tortuous as it is neither soluble in water nor ether and is temperature-labile (Willcox, 2009). Because of these characteristics, the crude extracts did not yield reproducible results which led to a more intense search of ancient Chinese literature. Through persistence, sweat and toil Tu came across Ge Hong's (284-363 AD) recommendation, 'A handful of qinghao immersed with 2L of water, wring out the juice and drink it all' which led her to reflect that, perhaps, the method of extraction they adopted had destroyed the active component(s). A low temperature ethyl ether extraction technique (White, 1992) yielded a product on 4th October, 1971 which neutral, non-toxic portion proved 100% effective against *P. berghei* and *P. cynomolgi* (Tu, 2011). Isolation and purification of the extract in 1972, yielded a colorless, crystalline substance with a molecular weight of 282 Dalton, a molecular formula of $C_{15}H_{22}O_5$ and a melting point of 156-157°C. This was artemisinin (or qinghaosu in Chinese) (Tu, 2011).

2.2 Chemical Structure and Mode of Action of Artemisinin

Chemically, artemisinin is a sesquiterpene lactone, with molecular weight of 282. It has a tetracyclic structure with triaoxane ring and lactone ring. The triaoxane ring contains endoperoxide bridges. The Endoperoxide Bridge is thought to be inextricably responsible for the majority of its antimalarial action (Haynes, 2001)

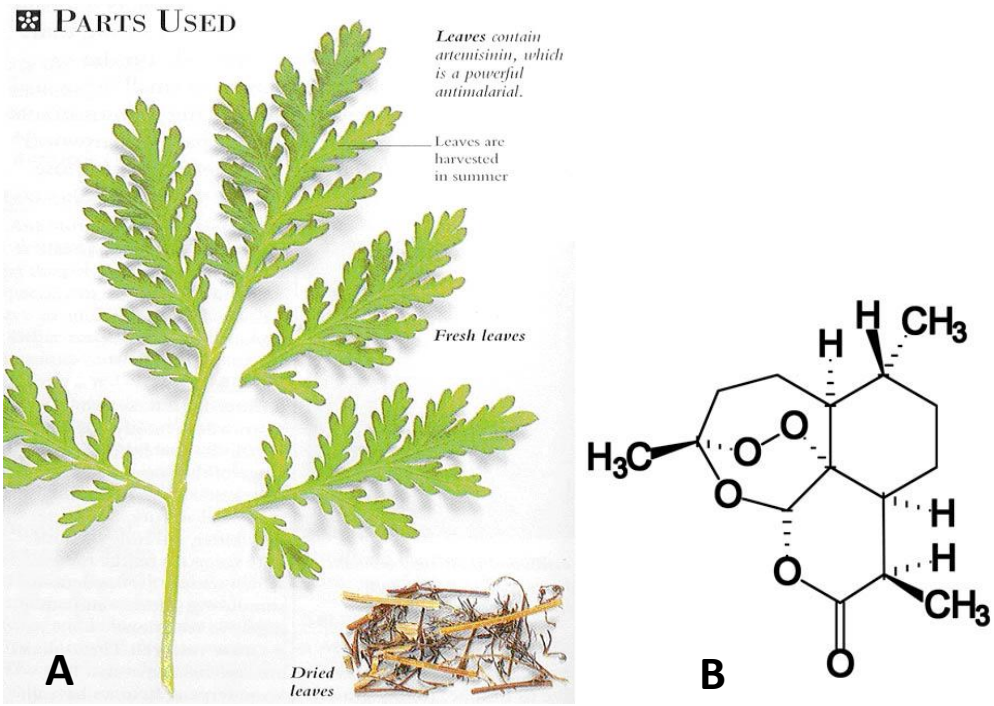


Figure 2.1: Artemisinin (A) Plant (B) Chemical structure showing endoperoxide bridge (Chevallier (1996)

Although the precise mechanism of action is still highly controversial (Posner and O'Neill, 2004), the structural determinant of the activity of artemisinin is the endoperoxide bridge which is a specific feature of this type of compounds. It has been suggested that the parasiticidal activity starts with the reaction of artemisinin with haem iron, leading to the generation of activated oxygen species, such as oxygen radicals, or of a C-centered radical of artemisinin itself, which are then further producing lethal damage to the parasite (Meshnick *et al*., 1993; Olliaro *et al*., 2001a).

This artemisinin acts via a two-step mechanism. It is first activated by intraparasitic heme-iron, which catalyzes the cleavage of the endoperoxide. A resulting free radical intermediate then forms covalent bonds with parasite proteins which may then kill the parasite by alkylating and poisoning one or more essential malarial protein(s) (Meshnick, 1994; 2002).

Secondly, the artemisinins then exert their antimalarial action by inhibiting a critical plasmodial enzyme, PfATP6 (*Plasmodium falciparum* adenosine triphosphatase). This enzyme pumps calcium from the cytoplasm of the parasite into the endoplasmic reticulum and is important in maintaining calcium homeostasis and controlling calcium signaling within the parasite; PfATP6 is the plasmodial sarcoendoplasmic reticulum calcium adenosine triphosphatase (SERCA) (Eckstein-Ludwig *et al*., 2003).

2.3 Rationale for the Use of Artemisinin-based Combination Therapy (ACTs)

The increasing resistance to the available pharmacological agents with antimalarial potentials calls for more findings on malaria control in endemic countries (Ridley, 2002). One of the chemotherapeutic drugs recommended by the World Health Organization (WHO) in the management of malaria is ACT which prevents the development of gametophyte in *Plasmodium*

vivax, Plasmodium ovale, Plasmodium malariae and the early stage *of Plasmodium falciparum* (WHO, 2011). Resistance to the classical antimalarial drugs, notably – chloroquine, sulfadoxine-pyrimethamine, mefloquine, quinine is well documented (White, 1992; Krogstad, 1996). Continued concerns of impending multi-drug resistance has caused a shift from monotherapy to combination therapy which includes drugs like the artemisinins currently recommended by WHO as a means of prolonging the effectiveness of first-line treatment regimens (WHO, 2001; Nosten and White, 2007)

Combination therapy with antimalarial drugs is the simultaneous use of two or more blood schizonticidal drugs with independent modes of action and different biochemical targets in the parasite. The ACT is a combination of an artemisinin derivative and another structurally unrelated and more slowly parasite-eliminating antimalarial (Nosten and White, 2007)

The rationale for combination therapy is to exploit the synergistic and additive potential of the individual drugs, with the aim to improve efficacy and retard development and spread of resistance to the drugs in combination (Olumese, 2007)

The ACTs are particularly known to be most effective because of their high killing rates, and they have become the mainstay chemotherapeutic agents for malaria (Haynes, 2001). Under low transmission intensity, ACTs have an additional public health benefit of reducing the overall malaria transmission and studies have demonstrated this synergistic benefit under high transmission (Amin *et al*., 2007). Artemisinin and its derivatives have a short elimination half-life and this confers the theoretical advantage that selection for drug resistant parasite is less likely. However, to avoid the higher risk of recrudescence when used in monotherapeutic regimen, artemisinins are used in combination with other antimalarials with relatively longer

elimination half-life, and this combination has been adopted as the front line drugs for the treatment of uncomplicated malaria in Nigeria (Olliaro and Tailor, 2004; Davis *et al.,* 2005).

2.4 Studies on the Effects of Artemisinin Combination Therapy.

2.4.1 Effects of ACTs on Histology

Artesunate-amodiaquine, at normal and double normal dose (for 3 and 6 days respectively), was also reported to have caused a dose and time-dependent hyperplasia of reactive astrocytes, as well as reducing staining intensities of Nissl substances in the cerebellum, reduction and shrinkage of purkinje cell layer indicative of possible cerebellar tissue damage (Ekong *et al*., 2009a; Ekong *et al*., 2009b). Arteether was reported to cause marked damage in the brainstem nuclei, ruber, superior olive, and nuclear trapezoideus which included chromatolysis, necrosis and gliosis (Genovese *et al*., 2000). It was implicated in inducing neurotoxicity which affects motor and vestibular brainstem nuclei in addition to auditory system nuclei (Brewer, 1997; Duarte *et al*., 1996).

Artesunate also was reported to cause decreased cellular population, degenerative changes, cellular hypertrophy and vacuolations which appeared in the stroma of treated rats when compared with the control section of the medial geniculate body (Eweka and Adjene, 2008).

Kasim *et al.,* (2012) also reported that the co-administration of artemether and quinine at therapeutic dose had no neurohistopathological effect on the cytoarchitecture of the cerebellum.

A disorganization of the seminiferous tubule architecture and a degeneration of the plasmalemma in the basal portion of some of the seminiferous tubules with arrest of spermatogenesis were observed in a dose and duration dependent order following artesunate treatment. The epididymal

and testicular histology showed mild to severe cell clustering, cellular hypertrophy, and interstitial cellular vacuolations with decreased sperm production (Izunya *et al*., 2010). A similar pattern of degenerative changes in the rat testes was seen following artesunate-amodiaquine administration (Timbuak *et al*., 2015). Shittu *et al* (2013) however reported that at therapeutic dose, artesunate had no significant effect on sperm quality and serum testosterone levels.

Sub-chronic exposure of artesunate, as well as artesunate-amodiaquine in rats was reported to cause hepatic damage evidenced by sinusoidal dilation, cytoplasmic vacuolations, focal necrosis, sinusoidal congestion and extensive inflammatory changes of the portal tracts (the generation of ROS led to the accumulation of lipid peroxides leading to change in permeability of the cells) (Izunya *et al*., 2010; Timbuak *et al*., 2014; Bigoniya *et al*., 2015). The same observations were made following sub-acute administration of artemether-lumefantrine on the liver (Ezike *et al*., 2013)

After artesunate administration at normal and double normal dose, Izunya *et al.,* (2011) reported that there was no effect on the histology of the heart in Wistar rats. Akinranide *et al* (2014) however reported artesunate-amodiaquine, as well as artesunate, to have deleterious effects on the histological integrity (elastic fibres and lysosomes) of the left ventricle and ascending aorta of adult Wistar rats at therapeutic, acute, and overdose treatments of the drugs. Babba (2015), however, reported artemisinin to ameliorate doxorubin mediated cardiac injury whilst enhancing its cardiotoxicity in HL-60 in a nitric oxide dependent manner. It was seen to be both anti-apoptotic and protective against myocardial ischaemic reperfusion injury as well as decreasing infarct size, and improve cellular viability significantly.

2.4.2 Effect of ACTs on Biochemical Parameters

Ewenighi *et al* (2013) also reported that artesunate-amodiaquine and amodiaquine monotherapy treatments caused no significant changes in the G6PD activity and fasting blood glucose levels. In another study, a significant decrease in ALP, ALT, and AST activities in the kidney following artesunate-amodiaquine co-administration was reported. There was, however, no significant change observed in the serum total protein level, urea level, albumin level, globulin level, ALT, ALP, and AST activities in the liver (Adegunloye *et al*., 2015).

Similarly, dihydroartemisinin-piperaquine phosphate (P-ALAXIN®) at therapeutic and double therapeutic dose was seen to significantly elevate the activities of plasma ALP, ALT, AST and GGT by 22 and 32%, 26 and 36%, 18 and 34%, 43 and 91% respectively. Plasma total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were also significantly decreased, accompanied by a corresponding significant increase in malondialdehyde level while concomitantly and significantly reducing the activities of hepatic GST, catalase and superoxide dismutase (Olayinka and Ore, 2013).

The levels of Malondialdehyde, serum total, conjugated bilirubin and the activities of alanine transaminase, aspartate aminotransferase, and alkaline phosphatase were increased after therapeutic administration of artemether-lumefantrine. Simultaneously, however, the levels of the activities of liver catalase, superoxide dismutase, and glutathione were decreased. Thus, it was implicated in having induced hepatic injury (Adegbesan *et al*., 2014).

The intracellular antioxidant system of testis-tissue incurred oxidative insult following artesunate treatment evidenced by significant decrease in activities of superoxide dismutase, glutathione,

glutathione peroxidase, and glutathione reductase and a corresponding increase in lipid peroxidase and glutathione-S-peroxidase in a dose-dependent manner (Desai *et al*., 2015)

2.4.3 Effect of ACTs on Behavioural Patterns

Experimental rats were seen to display normal balance and coordination following co-administration of artemether and quinine (Kasim *et al*., 2012). Artesunate was also reported to be harmless at its recommended dose when administered to Wistar rats as its effect was not different from the control in anthropometric parameters and behavioural activities, as well as on brain biomolecules –total protein (TP), triacyglycerol (TAG) and cholesterol unlike when taken in excess of dose and time (Ekong *et al*., 2009). Nonetheless, Onaolapo *et al.,* (2013) reported artesunate, amodiaquine, and artesunate-amodiaquine combination to have the ability to alter novelty-induced behaviour and spatial memory in healthy mice. Artesunate and amodiaquine either in combination or administered singly caused a significant increase in open field novelty-induced horizontal locomotion and rearing

Arteether was also reported to cause a significant progressive deficit in the behavioural performance of trained rats with signs of gross gait disturbances, tremor, and lethargy (Genovese *et al*., 2001). A similar profile of neurotoxicity of arteether has also been observed in dogs and primates (Brewer *et al*., 1994a; 1994b; Petras *et al*., 1997)

Ekong *et al* (2008) reported amodiaquine to increase total locomotor activity (TLA) and exploration while simultaneously reducing anxiety in Wistar rats in a dose-dependent manner. Similarly, artesunate-amodiaquine at normal dose for three and six days, and double normal dose for three days respectively, was reported to cause alterations in the cerebellum of Wistar rats

resulting in cerebellar dysfunction manifesting in some motor problems like dizziness, gait disturbances and convulsion, in a dose and duration-dependent manner (Ekong *et al*., 2009).

Following a three-day dosing regimen, Onaolapo *et al*., (2013) found artesunate-amodiaquine to alter neurobehaviour in mice by affecting their cognition and anxiety. The drugs were reported to have an anxiolytic effect in mice that significant changes became obvious after three days of dosing, suggesting a cumulative effect of artesunate-amodiaquine on anxiety. Their report equally suggested that when combined, artesunate and amodiaquine have less impairment than when used singly.

2.5 *Phoenix dactylifera* (Date Palm)

Nature is and will still serve as man's primary source for the cure of his ailments. However, the potential of plants as sources for new drugs is still largely unexplored (Oke, 2002).Medicinal plants are of great importance to the health of individual and communities. The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999)

Phoenix dactylifera belongs to the Palmae (Arecaceae) family. It is cultivated for its edible fruit (Rani *et al*., 2007). The fruits of the date palm are sweet berries with a sugar content of more than 50%. Because of its high nutritional value, great yields and its long life, the date palm has been mentioned as "the tree of life" (Augstburger *et al*., 2002)

The date fruit pulp is wealthy in phytochemicals like sterols, phenolics, carotenoids, procyanidins, anthocyanins and flavonoids. The concentration and ratio of these constituents depend on the stage of fruit picking, type of the fruit, location and soil conditions. These phytochemicals also add to the nutritional and organoleptic properties of the fruits (Abdul and Allaith, 2008)

Date fruit and its extracts in the food will increase the content of antioxidants and thus probably prevent oxidative deterioration of food (Anjum *et al.*, 2012)

The reports of Nadkarni (1976) showed that various parts of date palm are widely used in traditional medicine for the treatment of various disorders which include memory disturbance, fever, inflammation, paralysis, loss of consciousness, and nervous disorders.

The fruits of this plant are used as a detersive and astringent in intestinal troubles, 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)

2.5.1 The Plant

It is a medium sized plant, 15-25m tall, growing singly or forming a clump with several stems from a single root system. The leaves are 4-6cm long, with spines on the petiole, and pinnate, with about 150 leaflets are30cm long and 2cm wide. The full span of the crown ranges from 6-10m (Morton and Miami, 1987).

2.5.2 The Fruit

Date contain 20-70 calories each, depending on size and species. Fruits are oval, cylindrical, 3-7cm long and 2-7cm diameter, and, when ripe, range from bright red to bright yellow in colour, depending on variety.

Date palm is dioecious, having separate male and female plants. They can be easily grown from seed, but only 50% of seedling will be female and hence fruit bearing, and dates from seedling plants are often smaller and of poorer quality. Plants grown from cuttings will fruit 2-3 years earlier than seedling plants (Morton and Miami, 1987)

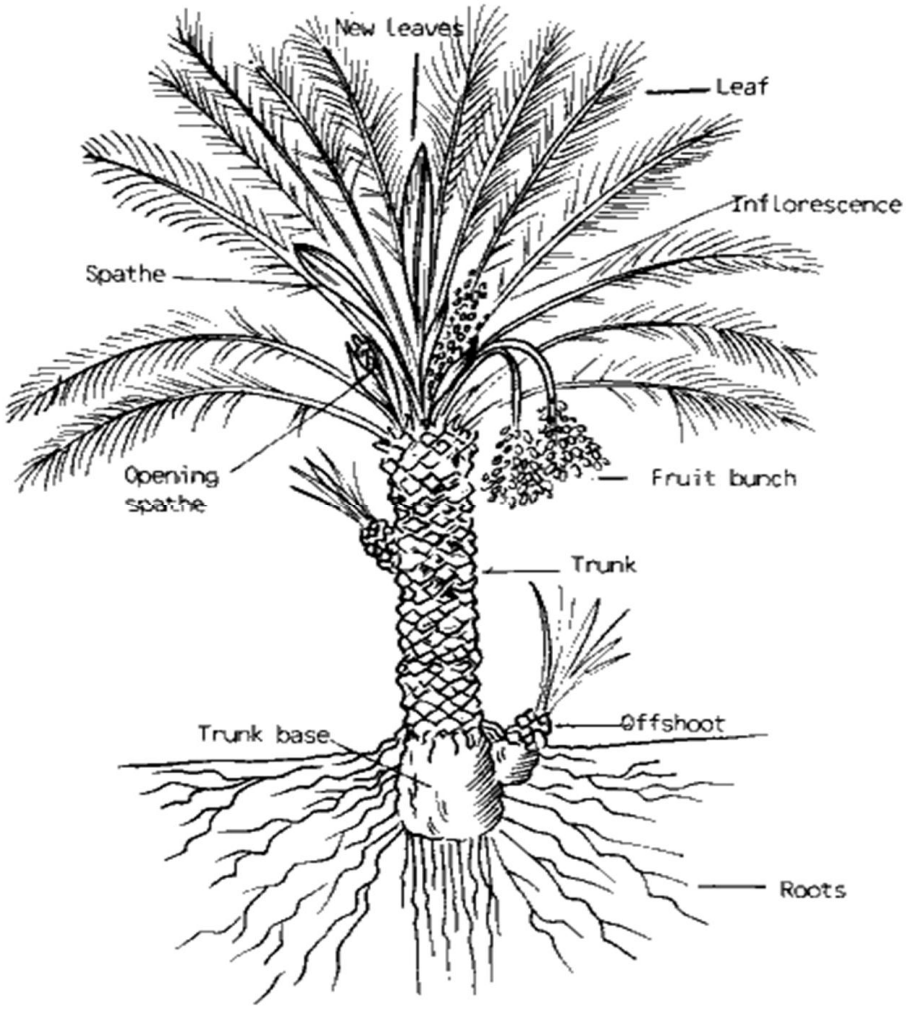


Figure 2.2: The date palm tree with fruits (Barreveld, 1993)

2.5.3 History of Dates

Date is one of the oldest known fruit crops and has been cultivated in North Africa and the Middle East for at least 5000 years. The earliest record from Iraq (Mesopotamia) shows that date culture was probably established as early as 3000 BC. Because of the long history of date culture and the wide distribution and exchange of date cultivars, the exact origin of the date is unknown, but it most likely originated from the ancient Mesopotamia area (southern Iraq) or western India. From its center of origin, date cultivation spread throughout the Arabian Peninsula, North Africa, and the Middle East. Date culture had apparently spread into Egypt by the middle of the second millennium BC. The Spanish were the first to introduce date palms outside the Arabian Peninsula, North Africa, and the Middle East/South Asia, carrying them to America (Chao and Krueger, 2007)

2.5.4 Composition of Date Fruit and Seed

Dates contain easily digestible sugars (70%) mainly glucose, fructose, sucrose, dietary fiber and contain less amount of protein and fats. They also contain vitamins like riboflavin, thiamine, biotin, folic and ascorbic acid that are essential for the body (Al-Farsi and Lee, 2008)

The pulps are rich in iron, calcium, cobalt, copper, fluorine, magnesium, manganese, potassium, phosphorus, sodium, sulfur, boron, selenium and zinc (Al-Farsi and Lee, 2008) dates contain elemental fluorine that is useful in protecting teeth against decay (Al-Farsi *et al*., 2005)

2.5.5 Phytochemistry

Phytochemicals are non-nutritive plant chemicals that have protective or disease-preventive properties. They are non-essential 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 researches demonstrate that they can also protect humans against diseases (Ziouti *et al*., 1996).

The whole plant (namely shoot tip, pollen grains, leaves, fruits, callus, embryogenesis and *invitro* leaf tissue) contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. Four free phenolic acids (protocatechnic acid, vanillic acid, syringic acid, and ferulic acid) and nine bound phenolic acids (gallic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, protocatechnic acid, p-coumaric acid, ferulic acid, and o-coumaric acid) were tentatively identified (Ziouti *et al*., 1996; Eong *et al*., 2006).

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 Benneth, 1965)

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)

2.5.6 Taxonomical Position of *Phoenix dactylifera*

Kingdom	**Plantae**
Sub-kingdom	Tracheobionta
Super-division	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida

Sub-class	Arecidae	
Order	Arecales	
Family	Arecaceae	
Genus	Phoenix	
Species	Dactylifera	(Mallhi *et al*., 2014)

2.5.7 Medicinal Uses/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 fruit is useful in dysentery (Anonymous, 1985).

The smoke produced from burning of date seeds in powder form is a useful fumigatory for piles. Paste made from ground seeds of date is said to be applied for opacity of cornea and to the head to relieve headaches and hemicrania. Furthermore, the date fruits are used as an ingredient in various aphrodisiac and tonic confections, and useful in treating asthma as well. (Anonymous, 1985)

A gum or juice obtained from the stem is used as a demulcent, diuretic and refrigerant in genitourinary infections (Nadkarni, 1976). Some doctors advise dates as consumptives as they promote expectoration, soothe the chest and prevent constipation (Anonymous, 1985). Its seed, roasted and ground into powder make a beverage-like coffee called “date coffee” (Nadkarni, 1976).

The liquid distillate obtained from spathe of *P. dactylifera* is believed to have certain mechanical uses as it relieves abdominal gases and pain especially after heavy meal and is also reported to have anti-spasmodic activity (Nadkarni, 1976)

The pollen grains of date palm have been used in Egyptian local practice to improve fertility in women (Amin *et al*., 1969) and the flower of the plant is used as a purgative (Zoghet *et al*., 2000)

The aforementioned folk mechanical claims became the basis upon which a lot of researches have been, and are still being conducted to scientifically validate them. Pharmacological studies have, in recent years been carried out on *P. dactylifera*, and it was found to have anti-ulcer activity, anti-cancer activity, anti-diabetic activity, anti-inflammatory activity, anti-oxidant activity, hepatoprotective activity, nephroprotective activity, neuroprotective activity (Vyawahare *et al*., 2009).

2.5.7.1 Nephroprotection

Studies showed that feeding rats with *P. dactylifera* fruit extract could reduce the levels of plasma creatinin and urea concentration and ameliorate gentamycin-induced damage to the proximal tubular regions of the rat kidney (Al-Qawari *et al*., 2008).

Date palm syrup showed significant hepatoprotective activity in carbon tetrachloride (CCl_4) induced hepatotoxicity among New Zealand Rabbits (Mallhi *et al*., 2014). Similar studies described the reduction of CCl_4 induced elevation in plasma enzyme and bilirubin concentration and improved morphological and histological liver damage in rats following administration of aqueous extracts of the flesh and pits of *P. dactylifera* (Al-Qawari *et al*., 2004)

2.5.7.2 Immunostimulatory Activity

Ingestion of phytochemicals to support the immune system or to combat infections has been a long standing traditional practice. Feeding of ethanol extract of dry dates to parturated mice enhanced both cell mediated and humoral immunity (Puri *et al.,* 2000).

Siwa date palm extract significantly increased the immune functions in New Zealand rabbits (IgG, IgM, and IgA) in a dose and time-dependent manner (El-Gazzar *et al*., 2009)

Dietary dates were found to reduce the adverse effects of mercuric chloride toxicity on hematological, biochemical, some immunological and pathological changes in rats (Said *et al*., 2008).

2.5.7.3 Anti-inflammatory Activity

Methanolic and aqueous extracts of date fruit produced significant increase in body weight gain and food efficiency ratio (Vyawahare *et al.,* 2009). The said extracts also suppressed foot swelling significantly by 67.8% and 61.3% respectively, while the methanolic extracts 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.

2.5.7.4 Antioxidant Activity

Free radicals cause many oxidative damages in the body and lead to carcinogenesis, mutagenesis, aging, atherosclerosis, neurodegenerative diseases and stress-induced depression. Free radicals attack on proteins, lipids and nucleic acid in biological systems and cause the said complication that leads to decline in the quality of life. (Mallhi *et al*, 2014)

The antioxidant properties of date palm cultivars from the United States of America and Saudi Arabia were analyzed by Al-Turki (2008) for their total phenolic content and antioxidant activity for two years. Results showed that total polyphenolic content of fruit ranged from 507.03mg to 225.02mg gallic acid equivalent and antioxidant activity ranged from 1400.00 to 228.06

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 invitro study (Dammak *et al*., 2008).

A linear relationship between antioxidant activity and the total phenolic content (TPC) of date fruit extract have been demonstrated on various fruits of *P. dactylifera* cultivated in Iran, Algeria and Bahrain (Allaith, 2005)

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).

2.5.7.5 Hepatoprotective Activity

Following Orchatoxin A-induced hepatotoxicity in Wistar rats, aqueous extract of the flesh of *P. dactylifera* was seen to markedly lower levels of bilirubin and ALT. Many histological alterations were enormously reduced after extract administration when compared to those treated with Orchatoxin A (Abdu, 2011).

Extract of the flesh and pits of dates were found to be prophylactic and treatment effective against CCl_4 poisoning, achieving about 80% and 70% protection respectively (Al-Qarawi, 2004; El-Gazzar *et al*., 2009).

2.5.7.6 Anti-diarrheal Activity

Aqueous extract of *P. dactylifera* can also be used for the treatment of diarrhea as proven by a study in which the same extract significantly reduced mean number of defecation when compared to control saline group. *P. dactylifera* can reduce severity of castor oil-induced diarrhea in a dose-dependent manner (Al-Taher, 2008).

At doses of 3, 6 and 12mg/kg, aqueous extract of dates produced a statistically significant reduction in both castor oil-induced intestinal transit and frequency of diarrhea in rat (Vyawahare *et al*., 2009).

The spathe aqueous extract of *P. dactylifera* at doses of 3, 6 and 12mg/kg produced a statistically significant reduction in both castor oil-induced intestinal transit and frequency of diarrhea in rats (Abdulla, 2008). The aqueous fruit extract of *P. dactylifera* at doses of 1000 and 1500mg/kg was reported to have significant anti-diarrhoeal activity due to its effect on reduction of the number of castor oil-induced diarrheal stool, delay in gastrointestinal propulsion and inhibition of fluid accumulation in the intestinal tract of rats (Agbon *et al*., 2013)

2.5.7.7 Anti-ulcer Activity

Pretreatment with date fruit ethanolic and aqueous extracts at a dose of 4mg/kg bw for 14 drugs markedly ameliorated the ulcer index, histopathological indices such as necrosis, haemorrhage, congestion and edema in stomach sections and biochemical levels of some enzymes such as gastrin in plasma, mucin and histamine in gastric ulceration in rats (Al-Qawari *et al*., 2005).

These observations lend believe to the ethnomedicinal claim that dates may be useful to humans with gastric ulcers and also as a natural laxative (Al-Qawari *et al*., 2003) and the fact that

Muslims customarily consume more of dates during the fasting month of Ramadan would be possibly to protect the gastric mucosa from the damaging effects of the gastric acid (Al-Qawari, 2005)

2.5.7.8 Neuroprotection

Pharmacologic agents that possess free radical scavenging or antioxidant properties do have the tendency to reduce brain damage that is accompanied by brain ischemia, and consequently, prevents neurological deficits as observed in *P. dactylifera* use (Gemma *et al*., 2002; Majid *et al*., 2008; Al-Taher, 2008).

Date seed extract (DSE) was reported to protect against cerebral ischemic damage induced by middle cerebral artery occlusion in rats (Kalantaripour *et al*., 2012). These properties may explain the rationale for the effective use of the plant as a neuroprotective agent in traditional medicine.

2.5.7.9 Neurobehavioural Patterns

The findings of Vyawahare *et al.,* (2009) revealed the methanol extract of *P. dactylifera* to possess significant anxiolytic, analgesic, nootropic and antipsychotic activities which could be attributed to various mechanisms such as decreased serotonergic and dopaminergic transmission and increased cholinergic transmission. The study outcome further revealed that the extract is devoid of any neurotoxicity and CNS-depressant effect.

2.6 The Hippocampus

The hippocampus is a part of the brain located in the medial temporal lobe, one in each side of the brain. It has an important role in the process of learning and memory (El-Falougy and

Benuska, 2006). It also functions as a part of the limbic system in regulation of sexual and emotional behaviours (El-Falougy and Benuska, 2006).

The hippocampus is considered an important brain structure for the treatment of spatial information (O'Keefe and Nadel, 1978). For example, lesion studies have shown that damage to the hippocampus in rats impairs the acquisition and retrieval of information required to navigate in spatial mazes (Jarrard, 1978; Morris *et al*., 1982). Studies in humans suggest that the hippocampus participates in the formation of a spatial representation of the environment (Maguire *et al*., 2000).

2.6.1 Structure of the Hippocampus

The hippocampus is a complex of six structures: dentate gyrus (DG), Ammon's horn (Lee *et al*., 2000), subiculum (SU), presubiculum (Hiens *et al*., 2001), parasubiculum (Muzio *et al*., 2005), and entorhinal cortex (EC) (El-Falougy and Benuska, 2006)

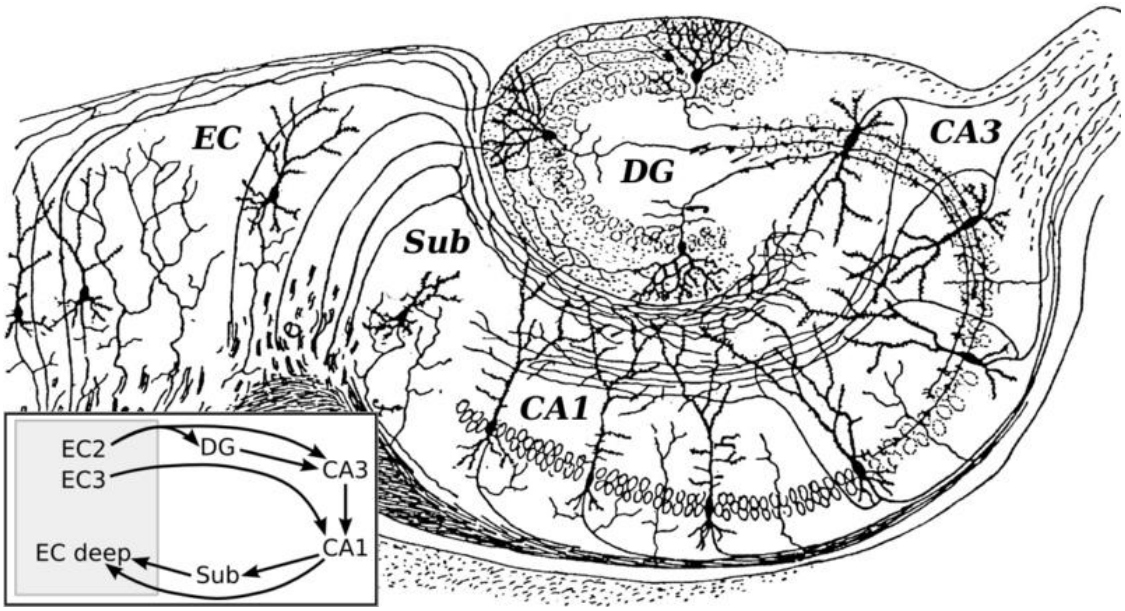


Figure 2.3: The structure of the hippocampus (Cajal, 1894)

The pyramidal cells in Ammon's horn extend from the presubiculum to the hilus of the dentate gyrus, and are divided into three regions, CA1 to CA3 (El-Falougy and Benuska, 2006; Piatti *et al*., 2006).

2.6.2 Development of the Hippocampus

Hippocampal regions begin to form from embryonic days (E15-21 in rat) and continue until post-natally (Bayer, 1980a). Hippocampal neurons migrate from the hippocampal neuroepithelium and glioepithelium. The Ammonic neuroepithelium gives rise to pyramidal cells and large neurons of the stratum oriens and radium; dentate neuroepithelium is the origin for granule cells and large neurons of stratum moleculare and hilus; and the glioepithelium generate glial cells in the fimbria. Pyramidal cells move out of the neuroepithelium and form a band of cells in the intermediate zone (Altman and Baker, 1990a; 1990b). Cells then leave this band and form the pyramidal cell layer; CA1 forms first and the CA3 appears. The granule cells of the dentate gyrus start to be generated at postnatal stage (Danglot *et al*., 2006).

The neurons in each structure arise in overlapping but sequential waves. In the rat, the hippocampal rudiment arises between E16-17, the entorhinal cortex between E15-17, the para – and presubiculum between E16-19, the subiculum between E16-18, Ammon's horn pyramidal cells between E17-19, and the hilus and molecular layer between E15-19 (Bayer, 1980a). Dentate granular cells begin to originate on E17 and most of the population forms after E18 (Bayer, 1980a)

Neurons forming the dentate gyrus granule cell layer are heterogenous because they are borne at different ages and generated even in adulthood. The granule cell layer (GCL) begins with neuroprogenitor cells (NPCs) migrating from the dentate epithelium to the secondary dentate

matrix during late embryonic development. NPCs from the secondary dentate matrix form the outer shell of the octal layer (Piatti *et al*., 2006).

The endal blade is generated and the tertiary dentate matrix is established in the prospective hilar region in early postnatal stages. NPCs migrate radically from the tertiary dentate matrix to the inner part of the GCL. Subsequently proliferative NPCs establish at the innermost layer, the subgranular zone, and will continue to generate DGCs with limited radical migration (Piatti *et al*., 2006).

2.7 The Cerebellum

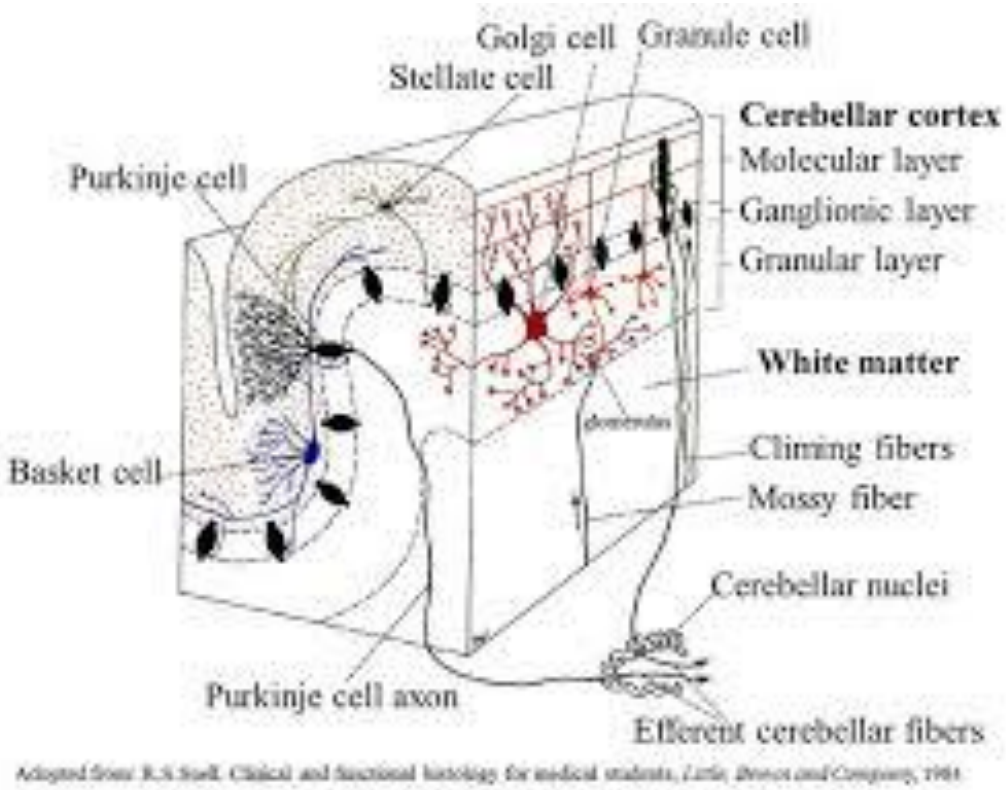
The term cerebellum is a Latin word which means "little brain." Although it is only about one-tenth the weight of the cerebrum, it contains about as many nerve cells (approximately 5 billion for each structure) (Ghez, 1991). The cerebellum consists of several subdivisions, each controlling or facilitating different behavioural functions. The cerebellum includes the left and right hemispheres and the vermis (Sullivan *et al*., 1995).

It is the largest part of the hind brain bulging back behind the pons and the medulla oblongata and overhang by the occipital lobe of the cerebrum (Martin *et al*., 2002). It contains more than 50% of all the neurons in the brain, but it only takes 10% of the brain volume. The surface of the cerebellum contains so many sulci and furrows, giving it a laminated appearance which is accentuated by several deep fissures that divide the cerebellum into several lobes (Joseph, 1982). The numerous shallow sulci on each lobe separate the individual folia from each other. The cerebellum is essential for maintenance of body balance and the synchronization of the activity among a group of muscles under voluntary control, converting muscle contraction into smooth coordinated movement (Martin *et al*., 2002)

2.7.1 Structure of the Cerebellum

The cerebellum is composed of small unpaired median portion, the vermis and two large lateral masses, the cerebellar hemispheres (Joseph, 1982).

Throughout its highly convoluted extent, the cerebellum can be divided into three cortical layers with the same basic neuronal circuitry everywhere, which involves five main cell types: Purkinje, Stellate, Golgi, Mossy and Granule cells. The most conspicuous of these are the Purkinje cells, which form an orderly monolayer interposed between the granular and molecular layers, extending their planer dendritic trees into the molecular layer above. As these cells are the sole output neurons of the cerebellar cortex, they are central to cerebellar cortical information processing. The granular layer below the Purkinje cells derives its name from the small, densely packed granule cells that send their axons into the molecular layer, where they bifurcate to become parallel fibres (Apps and Garwicz, 2005)



Adapted from: R.S Snell. Clinical and functional histology for medical students, *Little, Brown and Company*, 1984

Figure 2.4: Structure of the cerebellum (Snell, 1984)

Histologically, the cerebellar cortex is a three layered structure, the outermost molecular layer, the middle Purkinje layer, and the innermost granular layer. The granular layer is well defined due to the presence of nucleic acids in the nuclei of its numerous small cells. The Purkinje layer, composed of a single layer of large Purkinje cells perikaryons, is also easily recognizable. The molecular layer is rich in axons and dendrites as well as capillaries that penetrate deep into this layer (Singh, 2008)

2.7.2 Development of the Cerebellum

The cerebellum develops from the dorsolateral part of the allar lamina of the metencephalon (Singh and Pal, 2007). There are at first two primordial of the cerebellum, right and left primordium. This extends medially in the roof plate of the midline, as the cerebellum increases in size; fissures appear on its surface. The lateral lobes and vermis can soon be distinguished as a result of differential growth (Singh and Pal, 2007). Cell proliferation of the cerebellum takes place in different phases, with the primary proliferative cells migrating to the rhombic lips of the primordial cerebellum during the fetal period and then migrating to a secondary proliferative zone in the external granular layer of the cerebellum. This secondary wave of proliferation in the cerebellar cortex occurs postnatally in rodents and during the last trimester in humans and also continues postnatally (Rice and Barone, 2000)

2.8 Ascorbic Acid (Vitamin C)

L–Ascorbic acid, also known as L-xyloascorbic acid, 3-oxo-L-gulofuranolactone (enol form), L-3-ketothreohexuronic acid lactone, antisorbutic vitamin and vitamin C, has the chemical formula $C_6H_8O_6$ and a molecular weight of 176.12. This water- soluble vitamin is important in forming collagen, a protein that gives structure to bones, cartilage, muscles, and blood vessels. It also

helps maintain capillaries, bones and teeth and aids in the absorption of iron. As a reducing agent, it is necessary to maintain the enzyme prolyl hydroxylase in an active form, most likely by keeping its iron atom in a reduced state (Levine *et al*., 1995).

2.8.1 Food Sources of Ascorbic Acid

Ascorbic acid is widely distributed in nature, mostly rich in fresh fruits and leafy vegetables such as guava, mango, papaya, cabbage, mustard leaves, grape fruit, tomatoes, water melon, strawberries and spinach (Tee *et al*., 1997). Animal sources such as meat, fish, poultry, eggs and dairy products contain smaller amounts and are not significant sources (Levine *et al*., 1999).

2.8.2 Metabolism of Ascorbic Acid

Amino acid is reversibly oxidized to L-dehydroascorbic acid on exposure to copper, heat or mildly alkaline conditions. Both L-ascorbic acid and L-dehydroascorbic acid are physiologically active forms of vitamin C. Further oxidation of L-dehydroascorbic acid to 2,3-diketo-L-gulonic acid and oxalate is irreversible (Thurnham *et al*., 2000). The sulpation of ascorbate to ascorbate-2-sulphate is a minor pathway which at present, is not known clear biological significance for man (Bates, 1981)

2.8.3 Biological Functions of Ascorbic Acid

It is required for many metabolic functions in humans and animals. It is a co-factor for several enzymes involved in the biosynthesis, and maintenance of collagens, the basis of connective tissues, which are found in skin, ligaments, cartilages, vertebral discs, joint linings, capillary walls and the bones and teeth. Collagen protein requires vitamin C for “hydroxylation,” a process that allows the molecule to achieve the best configuration and prevent collagen from becoming weak and susceptible to damage. Further evidence indicates that vitamin C increases the level of

pro-collagen messenger RNA (Gaby and Singh, 1991). Its intake at moderate doses is important and safe for bone maintenance and therefore a factor in mitigating or delaying osteoporosis.

It is also required for wound healing and bone remodeling (Nishikimi *et al*., 1994) Furthermore, it acts as a co-factor for several other metabolic reactions e.g. synthesis of epinephrine from tyrosine and the synthesis of bile acid. It is capable of quenching potentially harmful, free radicals produced by physiological metabolic respiration processes of the body (Arigoni and Tullio, 2002), playing an important role in the antioxidant defense system, immune competence, and in strengthening resistance to infection (Hemila, 1992; 1994). It is a versatile water soluble radical scavenger widely distributed in aerobic organisms that play a central role in the protection of cellular components against oxidative damage by free radicals and oxidants that are involved in the development and exacerbation of a multitude of chronic diseases such as cancer, heart diseases, brain dysfunction, aging, rheumatism, inflammation, stroke, emphysema, and AIDs (Wimalasena *et al*., 1994)

As an antioxidant, its primary role is to neutralize free radicals. Since amino acid is water soluble, it can work both inside and outside the cells to combat free radical damages. Free radicals seek out an electron pair to regain their stability. Being an excellent source of electrons therefore, vitamin C "can donate electrons to free radicals and quench the reactivity" (Bendich, 1990).

2.9 Transition Metals

Trace elements are required for numerous metabolic and physiological processes in the human body. They play a part in the synthesis and structural stabilization of both proteins and nucleic acids. Therefore, imbalances in the optimum levels of trace elements may adversely affect

biological processes, and are associated with many diseases (Muralidhar *et al.*, 2004). Trace elements like manganese (Mn), copper (Cu), zinc (Zn) etc. have been studied in many diseases, including autoimmune diseases, neurological disorder, and psychiatric disorders (Mertz *et al.*, 1981). These elements are incorporated into the structures of proteins, enzymes, and complex carbohydrates. They take part in biochemical reactions together with enzymes (Livesay *et al.*, 2003). Various trace elements unquestionably play an important role in the physiology and pathology of the central nervous system. Some elements (e.g., Mn, Fe, Cu, and Zn) are essential for brain growth and functioning, whereas others, such as Pb and Hg may cause severe brain damage. However, the essential trace elements may also impair brain function, when they are present at elevated levels (Duflou *et al.*, 1989).

They have been suggested to play a pivotal role in the pathogenesis of numerous neurological disorders and they serve critical roles in the central nervous system (CNS) as essential cofactors, catalysts, secondary messengers and modulators of genes, enzymes and receptor activity. These elements are equally essential for normal CNS development and function, and each must be present at specific levels to avoid deficiencies or toxic excess (Lobo, 2013)

2.10 Oxidative Stress

Oxidative stress is defined as a state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them. It is a state harmful to the body which arises when oxidative reactions exceed antioxidant reactions because the balance between them has been lost (Pasupathi *et al*., 2009).

Because the body is furnished with elaborate mechanisms to remove active oxygen species and free radicals, these by-products of oxygen metabolism are not necessarily a threat to the body

under physiological conditions. However, if active oxygen species or free radicals are generated excessively or at abnormal sites, the balance between formation and removal is lost, resulting in oxidative stress. Consequently, active oxygen species and free radicals can attack molecules in biological membranes and tissues, thus inducing various diseases (Yoshikawa and Naito, 2002).

The deleterious effects of the free radicals are kept under check by a delicate balance between the rate of their production and the rate of their elimination by the body's defense systems. When there is an excessive addition of free radicals from exogenous production, the available tissue defense system becomes overwhelmed resulting in oxidative damage to the tissues (Pasupathi *et al*., 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Drugs

Camosunate

Camosunate manufactured by ADAMS Pharmaceutical (Anhui) Co., Ltd, China, was purchased from Rukayya Pharmacy, Samaru Zaria. Each packet of Camosunate is made up of artesunate and amodiaquine tablets, with each made up of twelve blistered tablets each of Amodiaquine Hydrochloride USP equivalent to Amodiaquine (AQ) base (300 mg) and Artesunate (AS) (100 mg). Solutions of the dose used were made by dissolving the tablets in distilled water and administered by oral gavage.

The normal dosage of both drugs for a physiologic man per day is: 100 mg of AS and 306.2 mg of AQ. For an average weighted rat of 200 g, the dosages were calculated to be 2.86 mg/kg of AS and 8.75 mg/kg of AQ per day (Walker *et al*., 1984; Okafor, 2013)

Ascorbic acid (Vitamin C)

Ascorbic acid was obtained from Rukayyah Pharmacy, Samaru Zaria, and used for the experiment as standard antioxidant drug. The product is manufactured by Emzor Pharmaceutical Industries Ltd, Nigeria, and was purchased from Rukayya Pharmacy, Samaru Zaria. Each of the blistered tablets contained an equivalent of Vitamin C (100 mg). A dosage solution of the drug was also made by dissolving the weighed tablets in distilled water and administered orally.

3.1.2 Plant Material

Dried *Phoenix dactylifera* fruits were obtained from Samaru Market in Zaria, Kaduna, Nigeria. It was identified, and authenticated in the Herbarium Unit of the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria with the Voucher Specimen Number of 3252.

3.1.3 Experimental Animals

For the purpose of this research, a total of thirty-six (36) apparently healthy adult male Wistar rats weighing between 150 – 180 g were obtained and housed in the Animal House Center of the Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, and they were acclimatized for two weeks prior to the start of the experiment.

All the rats were fed with rat chow (Vital Feeds) and water administered *ad libitum*. They were weighed on the day of commencement of the study (day 1), weekly during the study (days 8, 15, and 22), and on the day of sacrifice (day 29).

3.1.4 Animal Feed

Pelletized Vital Feed was obtained and used to feed the rats throughout the duration of the experiment. The product is manufactured by Grand Cereals and Oil Mills Limited (GCOML), Plateau State, Nigeria.

3.1.5 Set Up for Neurobehavioural Studies

For the purpose of this study, two neurobehavioural set ups were constructed for the test to be conducted. They include:

i. Morris Water Maze (MWM)

ii. Beam Walk Maze (BWM)

3.1.6 Other Materials

Other materials used in the course of the study include: stop watch, ketamine injection, measuring cylinders, digital weighing balance, cages, water bottles, saw dust for bedding, distilled water, microscope, glass slides, cover slips, stains, fixative and bottles for fixing tissues, dissecting set, orogastric tubes, beakers, syringes, etc.

3.2 Methods

3.2.1 Phytochemical Screening of *Phoenix dactylifera*

Phytochemical screening of aqueous and ethanol fruit extracts of *P. dactylifera* was conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The method of Trease and Evans (2002) for phytochemical screening was adopted.

3.2.2 Preparation of Aqueous and Ethanol Extracts of *Phoenix dactylifera* Fruit

Preparation of aqueous and ethanol fruit extracts of *P. dactylifera* was conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The method of maceration as reported by Al-Qarawi *et al.*,(2004); Wahab *et al* (2010) for the preparation of aqueous and ethanol *P. dactylifera* fruit extracts, respectively, was employed. The flesh of the dried *P. dactylifera* fruits were manually separated from the pits and pounded into powder using laboratory mortar and pestle.

For the aqueous extract, about 500 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 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.

In preparing the ethanol extract, about 650 g of the powder was soaked in 1 litre of ethanol in a conical flask. After 24 hours, the solution (a mixture of date palm fruit powder and ethanol) 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 to dryness in an evaporating dish (Royal Worcester, made in England) using H-H Digital Thermometer water bath (McDonald Scientific International - 22050Hz 1.0A) at 40^{o}C.

3.2.3 Dose Preparation of Aqueous and Ethanol Fruit Extract of *Phoenix dactylifera*

100 g of each of both aqueous and ethanol extracts was measured and dissolved in 300ml of distilled water. These made up the working solutions. From the freshly prepared working solutions, little portions were measured out for animal administration. Different doses {10%, 20% and 30% of the LD_{50} (5000 mg/kg bw)} of both the aqueous and ethanol extracts were administered to the rats by oral gavages using orogastric tubes for the experimental period, as reported by Agbon *et al*., (2013)

3.2.4 Dose preparation of Ascorbic Acid

10 g of ascorbic acid (Vitamin C) was dissolved in 300 ml of distilled water to form a stock solution to aid the administration of drug easily (Raghu *et al.*, 2014).

3.2.5 Experimental Design

In this study, a total of thirty-six adult male Wistar rats (36) were obtained and randomly distributed into nine groups of four rats (n=4) per group. Single dosage rate was used, and drugs and extracts administration was carried out once daily. Group I served as the control and was administered distilled water (1ml/kg) while groups II – IX were the treatment groups and were all administered 2.86/8.75 mg/kg of artesunate/amodiaquine (AS/AQ). Group II received only 2.86/8.75 mg/kg of AS/AQ while groups III, IV and V received (in addition to the AS/AQ) 500 mg/kg (10% LD_{50}), 1000 mg/kg (20% LD_{50}) and 1500 mg/kg (30% LD_{50}) of the aqueous extract of *P. dactylifera* respectively. Groups VI, VII and VIII received 500 mg/kg (10% LD_{50}), 1000 mg/kg (20% LD_{50}) and 1500 mg/kg (30% LD_{50}) of the ethanol extract of *P. dactylifera* respectively, while group IX received 100 mg/kg of ascorbic acid (vitamin C) which was used as the standard drug. All administration was done orally and lasted for a period of 28 days.

Table 3.1 Experimental Design

Group	Treatment/Dosage	Duration (Days)
1	Control (distilled water) 1ml/kg	28
2	2.86/8.75 mg/kg AS/AQ	28
3	2.86/8.75 mg/kg AS/AQ + AEPD (500 mg/kg)	28
4	2.86/8.75 mg/kg AS/AQ + AEPD (1000 mg/kg)	28
5	2.86/8.75 mg/kg AS/AQ + AEPD (1500 mg/kg)	28
6	2.86/8.75 mg/kg AS/AQ + EEPD (500 mg/kg)	28
7	2.86/8.75 mg/kg AS/AQ + EEPD (1000 mg/kg)	28
8	2.86/8.75 mg/kg AS/AQ + EEPD (1500 mg/kg)	28
9	2.86/8.75 mg/kg AS/AQ + Ascorbic acid (100 mg/kg)	28

n=4 rats; administration is via oral route.
AEPD = Aqueous extract of *P. dactylifera*; EEPD = Ethanol extract of *P. dactylifera*

3.2.6 Neurobehavioural Study

All experimental procedures were conducted in the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Science, Ahmadu Bello University Zaria. The following tests were carried out:

3.2.6.1 Beam Walk Test

Beam walk test was used to assess motor coordination and balancing in the Wistar rats. Motor coordination and balancing are controlled by the cerebellum (Ghez and Fahn, 1985; Paulin, 1993). Motor coordination and balancing were assessed by measuring the ability of the rats to traverse a narrow beam to reach an enclosed safety platform (Carter *et al*., 1999).

Procedure: the test consisted of elevated platforms connected by a 100 cm long wood beam with a width of 3 cm. the beam was placed horizontally, 50 cm above the bench surface, with one end mounted on a narrow support and the other end attached to a hamster box (20 x 20 x 20cm).

Beam walk ability latency – the time the rat spendsin walking acrossthe narrow beam - until its nose entered the hamster box (up to 60 s)was recorded in seconds, as described by Abou-Donia *et al.*, (2008).

All the rats were trained for seven days with three consecutive trials per day and thereafter tested once weekly throughout the study period. Beam score was based on an average of three trials, each separated by 30 minutes. The beam and the hamster box were cleaned before and after each trial with cotton wool dipped in methylated spirit.

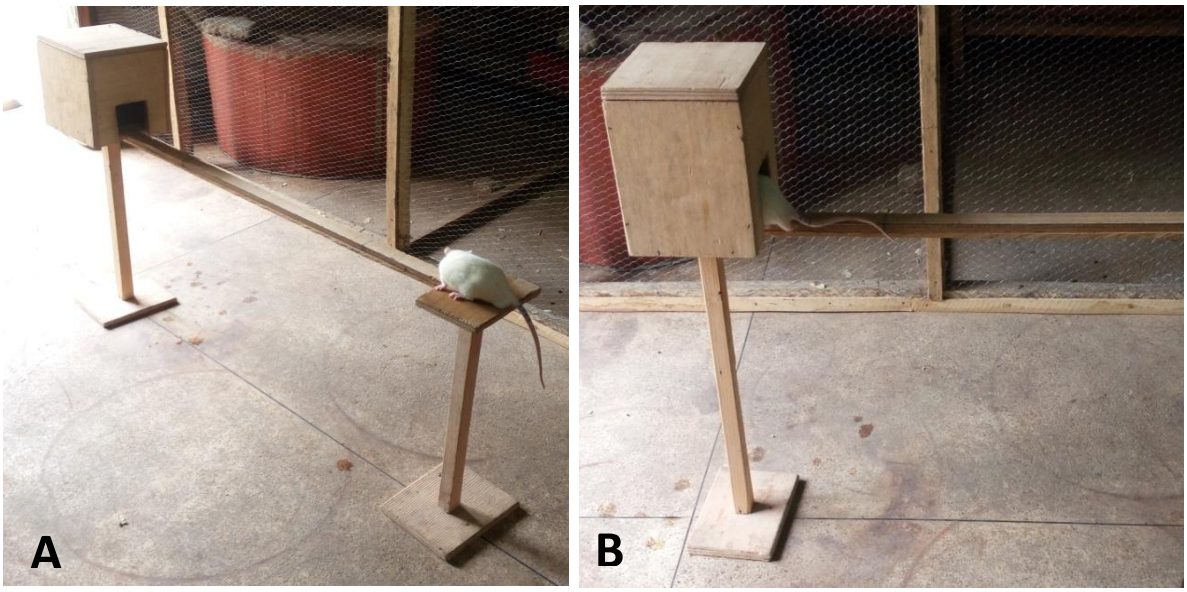


Plate I: The beam walking test setup with (A) rat at the starting point (B) rat traversed the beam to the hamstar box.

3.2.6.2 Morris Water Maze Test

The Morris water maze is one of the most widely used tasks in behavioral neuroscience for studying the psychological processes and neural mechanisms of spatial learning and memory, which are major functions of the hippocampus (Jarrard *et al*., 1984). The water maze was developed by Richard Morris (Morris *et al*., 1982; Morris, 1984) and place navigation in the water maze is now often used as a general assay of cognitive function (Brandeis *et al*., 1989), for example for testing the impact of various disturbances of the nervous system (e.g. animal models of stroke (Nunn *et al*., 1994), aging (Gallagher and Rapp, 1997), neurodegenerative disease (Hsiao *et al*., 1996), or the potential impact of novel therapeutic drugs (D'Hooge and De Deyn, 2001)).

The Morris water maze is not actually a `maze' per se; rather it typically consists of a round aluminium tank filled with water, with a gray coated interior. The tank measured 170 cm in diameter and 50 cm high. It was filled to a depth of 20 cm with water. An escape platform (10 cm in diameter), also made of gray-coloured metal, was submerged 1 cm under the water level.

Procedure: Each rat was trained to swim to the hidden platform that was located just 1 cm beneath the surface of the water. Because the water temperature was maintained at room temperature (24 °C – 25 °C, presumably the rats were highly motivated to find the platform to get out of the water. Because the platform was not visible, the rats had to depend on, and use extra-maze visual cues (permanently positioned distinctive objects such as posters placed outside of the pool) to develop a spatial map of the environment and guide its performance to locate the platform.

The training session consisted of three consecutive trials (with an inter-trial interval of 30 s) during which each rat was randomly placed in one of four quadrants of the pool, and allowed to swim freely to the escape platform, or until 60 s had elapsed. If the rat failed to find the platform within 60 s, it was taken and placed on the safety to stay for 20 s. Each rat that was able to find the platform was allowed to remain on the platform for 10 s and was removed from the pool for 30 s before being placed at the next starting quadrant. For each session, the latency time to find the platform was recorded manually using a stopwatch. This training procedure was repeated for six days, with the starting points (the axis of one imaginary quadrant) varying in a pseudo-randomized manner. The test sessions were subsequently performed once weekly for four consecutive weeks (28 days).

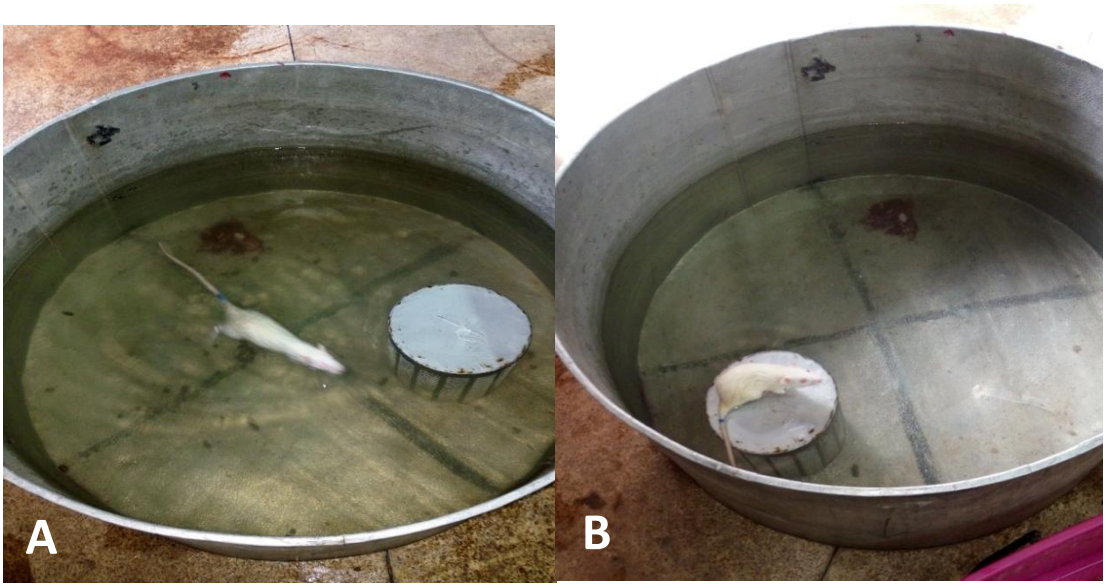


Plate II: The Morris water maze setup (A) rat swimming to locate the safety platform (B) rat on the safety platform.

3.2.7 Animal Sacrifice

On the 29th day, twenty four hours after the last administration (on day 28), all the rats were taken to the dissection section of Human Anatomy Department and humanely sacrificed after being injected with ketamine intraperitoneally. Incisions were made through the scalp of the skulls, the skulls were opened through the mid-sagittal sutures and the brain tissues were harvested and quickly fixed in Bouin's fluid. The tissues were then taken to the Histopathology unit of Ahmadu Bello University Teaching Hospital, Zaria for tissue processing.

3.2.8 Tissue Preparation for Biochemical and Neuro-trace element Processing

The brain tissues were homogenized in Tris buffer (pH 7.4). The tissue homogenate for each rat was made (1g/10ml) and centrifuged at 3000 rpm for 10 minutes. The resulting pellet (P1) consisting of nuclear and cellular materials was discarded. In this study, all biochemical and neuro-trace elements estimations were performed using the supernatant fraction(Bradford,1976) in the Multi-User Laboratory of Chemistry Department, Faculty of Physical Sciences, Ahmadu Bello University Zaria.

3.2.9 Estimation of Oxidative Stress Parameters

Using aliquots of the supernatant after centrifuging the prepared tissue homogenate:
The activity of Superoxide Dismutase (SOD) was determined by a method described by Fridovich (1989), the absorbance was measured every 30 seconds up to 150 seconds at 480 nm from where the SOD activity was calculated

Catalase activity was determined using the method described by Sinha (1972), and the absorbance was read at 570 nm. Standard curve was made by plotting the absorbance obtained at

various levels of the assay. Catalase activity was then obtained from the graph of the standard curve.

Reduced glutathione (GSH) concentration measurement was done according to the method of Ellman (1959) and the absorbance was read at 412 nm.

Lipid peroxidation as evidenced by the reaction of malondialdehyde (MDA) with thiobarbituric acid to produce thiobarbituric acid reactive substances (TBARS) was measured by the method of Niehaus and Samuelson (1968). The absorbance of the pink supernatant was measured at 535 nm against a reference blank using a spectrophotometer.

3.2.10 Neuro-trace Elements Studies

Using aliquots of the supernatant after centrifuging, analysis for metals (Copper, Iron, Manganese, and Zinc) concentration in the tissue (brain) homogenate was conducted using Atomic Absorption Spectrophotometer (AAS – AA240FS, Varian) at the Multi-User Laboratory, Department of Chemistry, Faculty of Physical Sciences, Ahmadu Bello University Zaria. The analytical method for determining metals in biological tissues as reported by Environmental Monitoring Methods Index, EMMI (1997) was adopted.

3.2.11 Histological and Histochemical Studies

The tissue preparation method for the histological studies was as outlined by Culling (1963). The stages of the technique include fixation, tissue processing, sectioning, staining and photomicrography.

Fixation: The cerebellum and hippocampus were carefully removed from the skull and fixed in Bouin's fluid.

Tissue processing: The trimmed cerebellum and hippocampus were processed with the aid of Automated Tissue Processor in the Histopathology unit of Ahmadu Bello University Teaching Hospital, Shika-Zaria.

Sectioning: Sections of the processed tissues were cut using rotary microtome at 8µm.

Staining: Three stains were used: Hematoxylin and Eosin (H and E), Cresyl Fast Violet and Bielschowsky.

Photomicrography: Photomicrographs at magnifications of ×250 and ×400 were carried out with the aid of MD900 Amscope digital camera

3.2.12 Statistical Analysis

All data were presented as mean ± SEM and were statistically analyzed using Statistical Package for Social Science (SPSS) version 20 (IBM, Incorporation, NY) Comparisons between control values and those obtained in treated groups of rats were performed with one-way analysis of variance (ANOVA). A p-value < 0.05 was considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Screening of *P. dactylifera*

Phytochemical analysis of *P. dactylifera* produced positive reaction for each of the following secondary metabolites: Alkaloids, Carbohydrates, Flavonoids, Glycosides, Saponins, and Tannins. However, negative reactions were obtained for Cardiac glycoside in the ethanol extracts (**Table 4.1**).

Table 4.1: Phytochemical Constituents of the Aqueous and Ethanol Fruit Extract of *Phoenix dactylifera*

Constituents	Aqueous extract	Ethanol extract
	Inference	Inference
Alkaloids	+	+
Anthraquinone	+	+
Carbohydrates	+	+
Cardiac glycosides	+	−
Flavonoids	+	+
Glycosides	+	+
Saponins	+	+
Tannins	+	+

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

4.2 Neurobehavioural Studies

4.2.1 Beam Walk Test

The result of the Beam Walk Test performed by the rats within the four weeks of AS–AQ administration showed that the latency time taken to walk across the beam was highest in the 500 mg/kg AEPD-treated group for the first 3 weeks (9.61 ± 2.90 sec, 9.61 ± 2.44 sec and 7.30 ± 1.96 sec respectively) and then followed by the 1500 mg/kg AEPD-treated group (15.12 ± 1.62 sec) in the fourth week. However the latency time lapse spent by the rats of the aqueous and ethanol treatment groups showed no significant differences when compared to the values observed in the control group as shown in **Table 4.2**.

4.2.2 Morris Water Maze

The results of the Morris Water Maze performed on weeks 1 through 4 showed that the highest latency time taken by the rats to swim and locate the platform was observed in the 1000 mg/kg AEPD-treated group for the first three weeks (15.66 ± 3.96 sec, 18.21 ± 1.84 sec and 13.87 ± 5.25 sec respectively) followed by the 1000 mg/kg EEPD-treated group (12.11 ± 3.74 sec) in the fourth week. There were, however, no significant differences in the latency time lapse between the aqueous and ethanol treatments when compared to the control as shown in **Table 4.3**.

Table 4.2: The Beam Walk Test Mean Latencies from Week One to Four

Groups	**Week 1 (sec)**	**Week 2 (sec)**	**Week 3 (sec)**	**Week 4 (sec)**
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Ctrl	3.36 ± 0.96	5.78 ± 1.99	5.44 ± 1.72	4.87 ± 1.31
AS/AQ	5.89 ± 2.22	5.60 ± 2.52	5.55 ± 1.35	14.58 ± 10.39
AAE 500 mg/kg	9.61 ± 2.90	9.61 ± 2.44	7.30 ± 1.96	11.00 ± 3.34
AAE 1000 mg/kg	8.76 ± 2.81	7.42 ± 3.11	4.25 ± 1.05	3.45 ± 0.79
AAE 1500 mg/kg	6.26 ± 2.29	6.43 ± 2.82	5.81 ± 0.85	15.12 ± 1.62
AEE 500 mg/kg	7.32 ± 2.60	5.20 ± 1.26	5.91 ± 0.74	5.95 ± 2.53
AEE 1000 mg/kg	9.26 ± 2.81	4.61 ± 1.22	4.96 ± 1.46	6.81 ± 1.59
AEE 1500 mg/kg	6.14 ± 0.73	6.51 ± 1.58	5.73 ± 1.64	7.13 ± 3.11
AAA	3.94 ± 0.49	3.06 ± 0.80	4.08 ± 0.85	5.21 ± 1.55

n – 4*; Mean ± SEM; One way ANOVA;* ***Ctrl*** *= Control;* ***AS/AQ*** *= Artesunate-amodiaquine treatment;* ***AAE*** *= Artesunate-amodiaquine + Aqueous fruit extract of Phoenix dactylifera;* ***AEE*** *= Artesunate-amodiaquine + ethanol fruit extract of Phoenix dactylifera;* ***AAA*** *= Artesunate-amodiaquine + ascorbic acid.*

Table 4.3: The Morris Water Maze Test Mean Latencies from Week One to Four

Groups	Week 1 (sec)	Week 2 (sec)	Week 3 (sec)	Week 4 (sec)
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Ctrl	5.81 ± 0.47	5.44 ± 0.61	7.03 ± 1.23	7.38 ± 1.41
AS/AQ	6.63 ± 1.61	7.85 ± 1.41	6.68 ± 1.35	5.47 ± 0.63
AAE 500 mg/kg	9.70 ± 0.88	5.94 ± 0.47	6.79 ± 0.89	6.53 ± 0.66
AAE 1000 mg/kg	15.66 ± 3.96	18.21 ± 1.84	13.87 ± 5.25	9.73 ± 1.31
AAE 1500 mg/kg	10.74 ± 2.37	13.30 ± 4.52	5.88 ± 0.98	6.30 ± 1.19
AEE 500 mg/kg	12.20 ± 2.24	10.66 ± 2.29	6.95 ± 1.52	6.88 ± 0.31
AEE 1000 mg/kg	14.32 ± 2.12	17.73 ± 6.61	9.08 ± 5.30	12.11 ± 3.74
AEE 1500 mg/kg	14.08 ± 4.81	14.64 ± 4.23	12.74 ± 7.24	7.20 ± 0.76
AAA 100 mg/kg	13.91 ± 4.15	8.62 ± 1.79	9.26 ± 3.09	6.03 ± 0.58

***n – 4**; Mean ± SEM; One way ANOVA; **Ctrl** = Control; **AS/AQ** = Artesunate-amodiaquine treatment; **AAE** = Artesunate-amodiaquine + Aqueous fruit extract of Phoenix dactylifera; **AEE** = Artesunate-amodiaquine + ethanol fruit extract of Phoenix dactylifera; **AAA** = Artesunate-amodiaquine + ascorbic acid.*

4.4 Oxidative Stress Studies

The results of the oxidative stress markers are as presented in Figures 4.1 to 4.4. The result of CAT concentration (**Fig. 4.1**) showed that the highest CAT concentration was observed in the 1000 mg/kg and 1500 mg/kg AEPD-treated groups while the least values were observed in the 500 mg/kg and 1000 mg/kg EEPD-treated groups. The difference, however, in the CAT concentrations, was not significant when compared to the control ($p \geq 0.05$)

The result of SOD concentrations (**Fig. 4.2**) showed that the SOD concentration in the 1000 mg/kg AEPD-treated group, 1000 mg/kg and 1500 mg/kg EEPD-treated, and ascorbic acid-treated groups were reduced, though not to a degree of significance. The concentrations in AS-AQ, 500 mg/kg AEPD and 500 mg/kg EEPD-treated groups were observed to be relatively the same with that of the control ($p \geq 0.05$).

The result of MDA concentrations in the brain (**Fig. 4.3**) showed that the concentration in the AS-AQ treated group increased significantly when compared to the control ($p < 0.05$) while the other treatment groups showed a non-significant dose-dependent decrease in the concentration when compared to the control.

The GSH concentration (**Fig. 4.4**) across all the treatment groups revealed no increase ($p \geq 0.05$) when compared to the control.

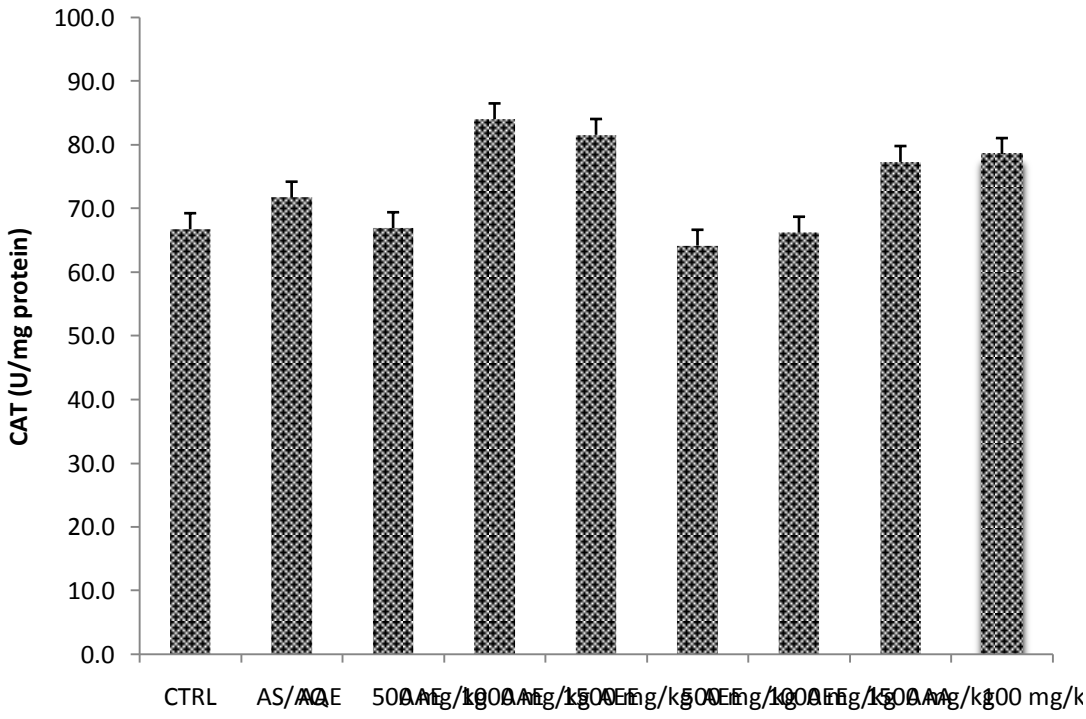


Fig. 4.1: CAT concentration in the brain tissue of Wistar rats after 28 days of treatment.

Values are expressed as Mean ± SEM

CTRL = Control, **AS/AQ =** artesunate + amodiaquine, **AAE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AEE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AAA** = ACT + Amino acid (100 mg/kg)

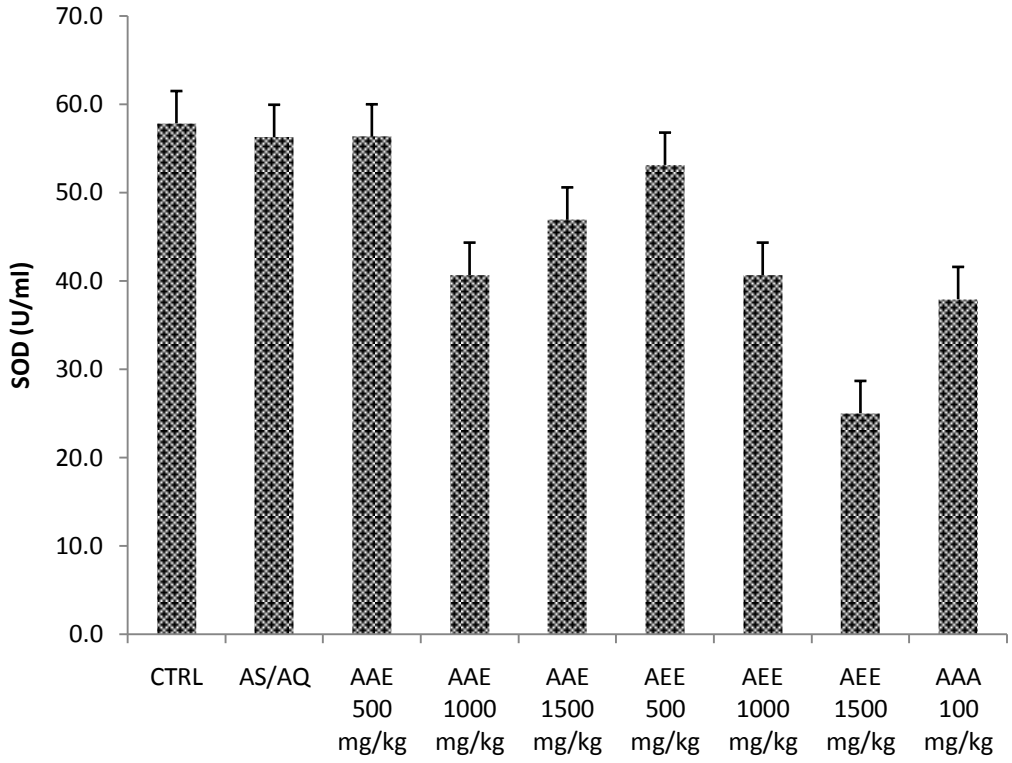


Fig. 4.2: SOD concentration in the brain tissue of Wistar rats after 28 days of treatment.

Values are expressed as Mean ± SEM

CTRL = Control, **AS/AQ** = artesunate + amodiaquine, **AAE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AEE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AAA** = ACT + Amino acid (100 mg/kg)

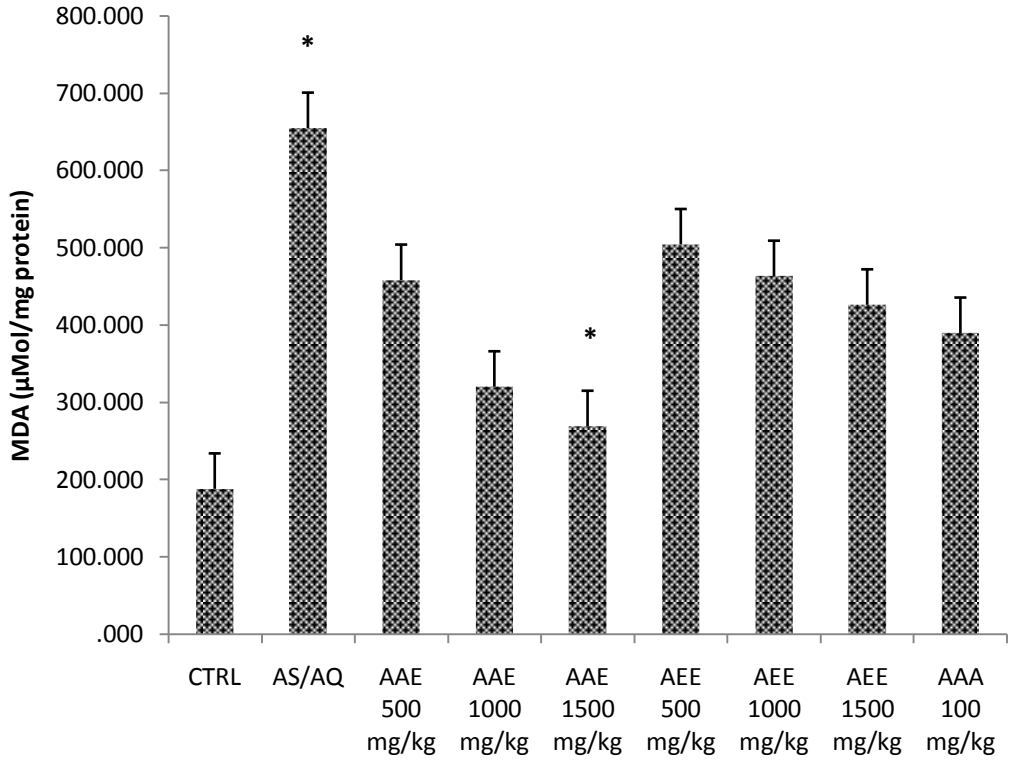


Fig. 4.3: MDA concentration in the brain tissue of Wistar rats after 28 days of treatment.

Values are expressed as Mean ± SEM.
* = significant difference ($p < 0.05$)

CTRL = Control, **AS/AQ** = artesunate + amodiaquine, **AAE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AEE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AAA** = ACT + Amino acid (100 mg/kg)

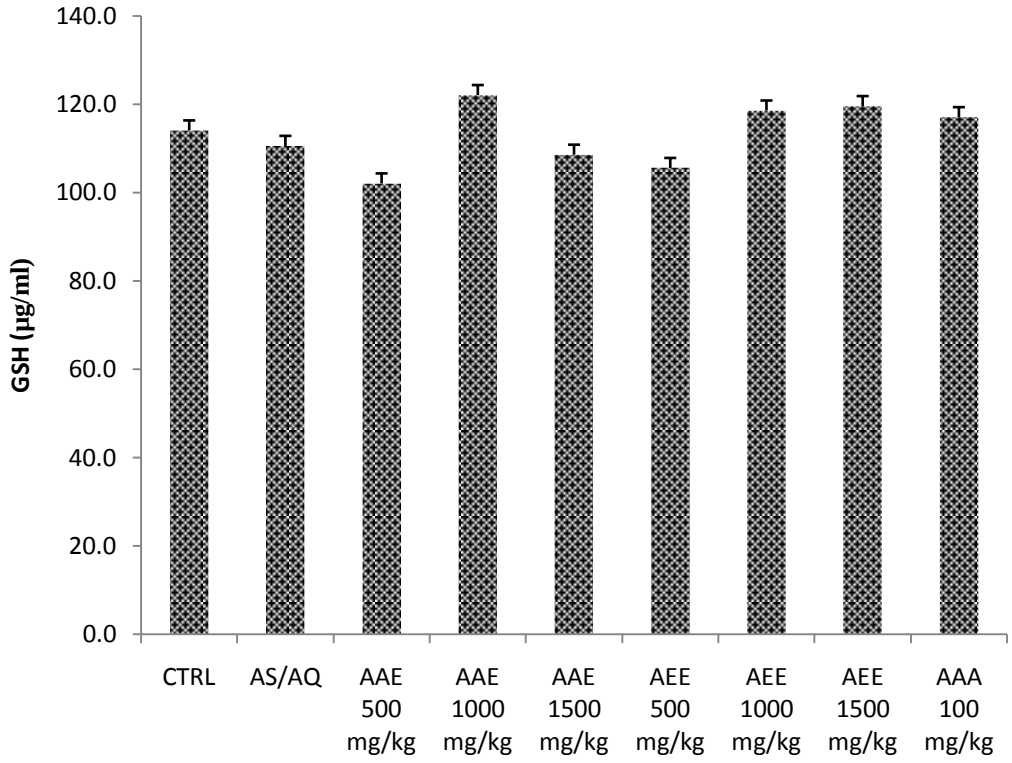


Fig. 4.4: GSH concentration in the brain tissue of Wistar rats after 28 days of treatment.

Values are expressed as Mean ± SEM

CTRL = Control, **AS/AQ** = artesunate + amodiaquine, **AAE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AEE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AAA** = ACT + Amino acid (100 mg/kg)

4.5 Trace Metals Analysis

The results of the concentration of trace metals in the brain of experimental animals are presented in Figures 4.5 to 4.8. The concentration of Zinc (Zn) (**Fig, 4.5**) was observed to be least in the 1500 mg/kg treated groups of both AEPD and EEPD, and AA group, and highest in groups of AS-AQ and 1000 mg/kg AEPD treatment. This concentration difference, however, is not significant when compared to the control.

The concentration of Iron (Fe) (**Fig. 4.6**) was observed to be highest in the Group of AA and least in 500 mg/kg AEPD-treated group. When compared to the control, the AA group differ significantly ($p < 0.05$). The other treatment groups showed no significant difference relative to the control.

Copper (Cu) concentration (**Fig. 4.7**) was observed to be highest in the control as all the treatment groups were all reduced. The difference in concentration between the control and treatment was however, not significant ($p \geq 0.05$)

For Manganese (Mn) concentrations (**Fig. 4.8**), AA group was observed to have the highest while 1500 mg/kg AEPD-treated group has the least concentration with a negative value. When compared to the control, the manganese concentration in the aforementioned groups was observed to differ significantly ($p < 0.05$)

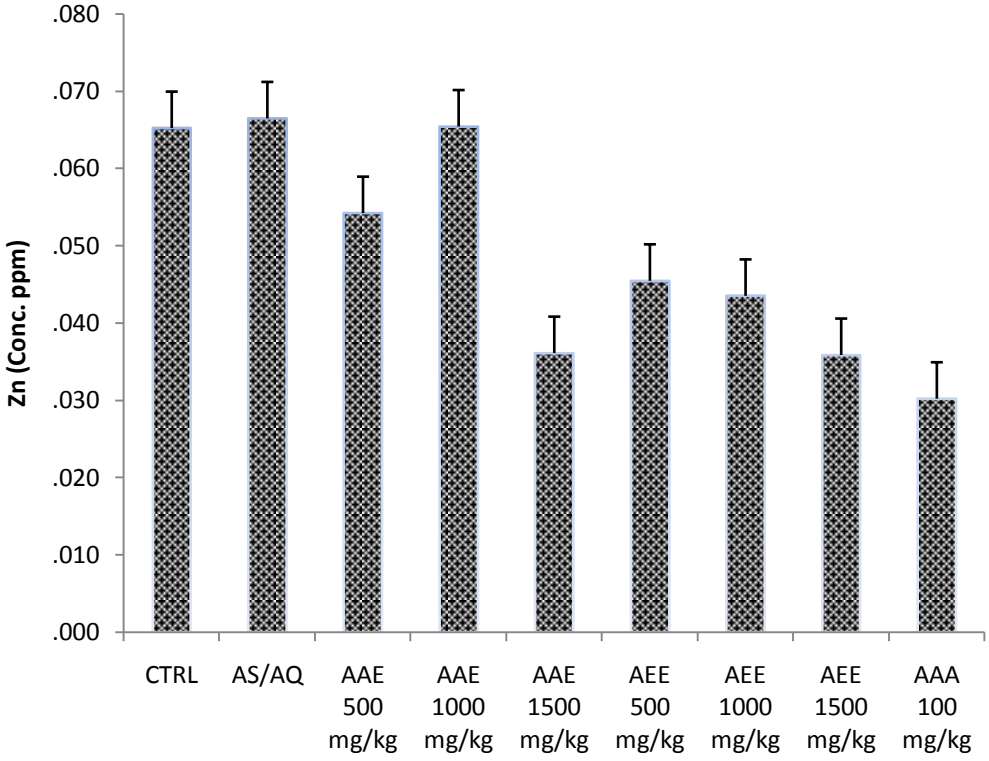


Fig. 4.5: Zinc concentration in the brain tissue of Wistar rats after 28 days oftreatment.

Values are expressed as Mean ± SEM

CTRL = Control, **AS/AQ** = artesunate + amodiaquine, **AAE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AEE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AAA** = ACT + Amino acid (100 mg/kg)

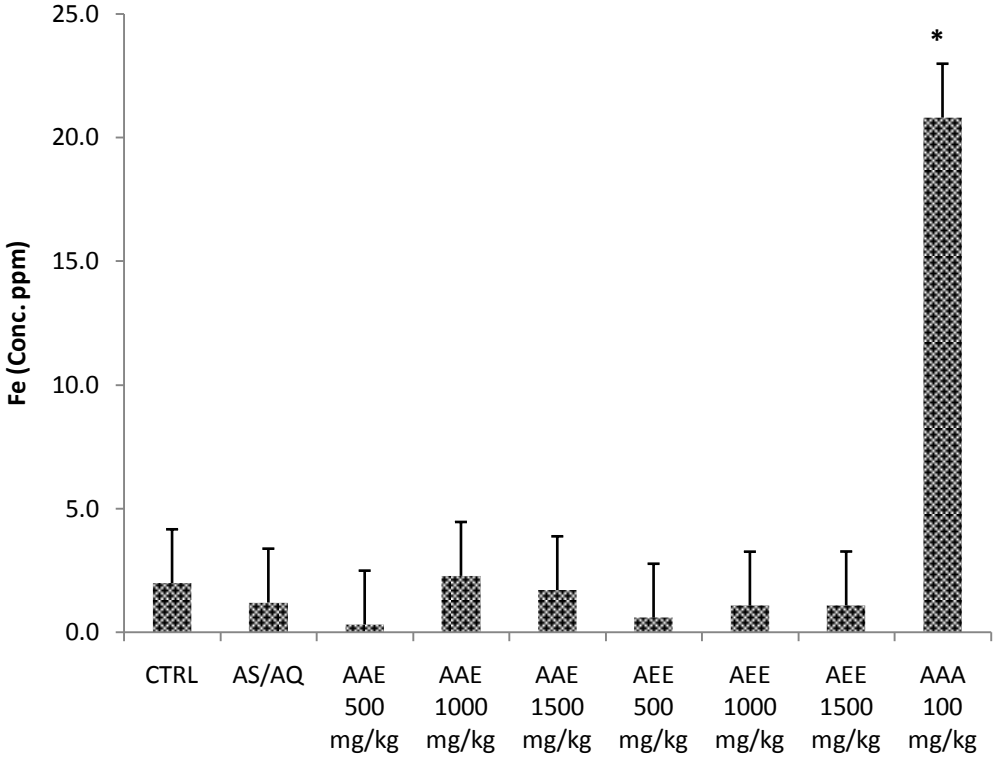


Fig. 4.6: Iron concentration in the brain tissue of Wistar rats after 28 days oftreatment.

Values are expressed as Mean ± SEM
* = significant difference ($p < 0.05$)

CTRL = Control, **AS/AQ** = artesunate + amodiaquine, **AAE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AEE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AAA** = ACT + Amino acid (100 mg/kg)

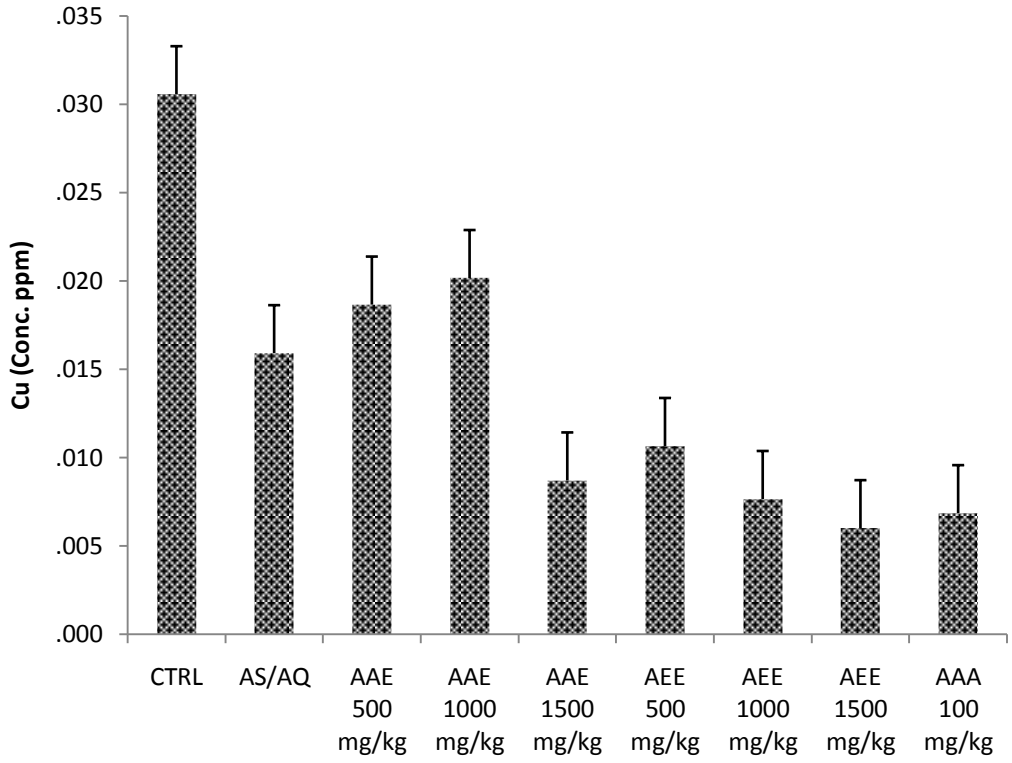


Fig. 4.7: Copper concentration in the brain tissue of Wistar rats after 28 days of treatment.

Values are expressed as Mean ± SEM

CTRL = Control, **AS/AQ** = artesunate + amodiaquine, **AAE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AEE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AAA** = ACT + Amino acid (100 mg/kg)

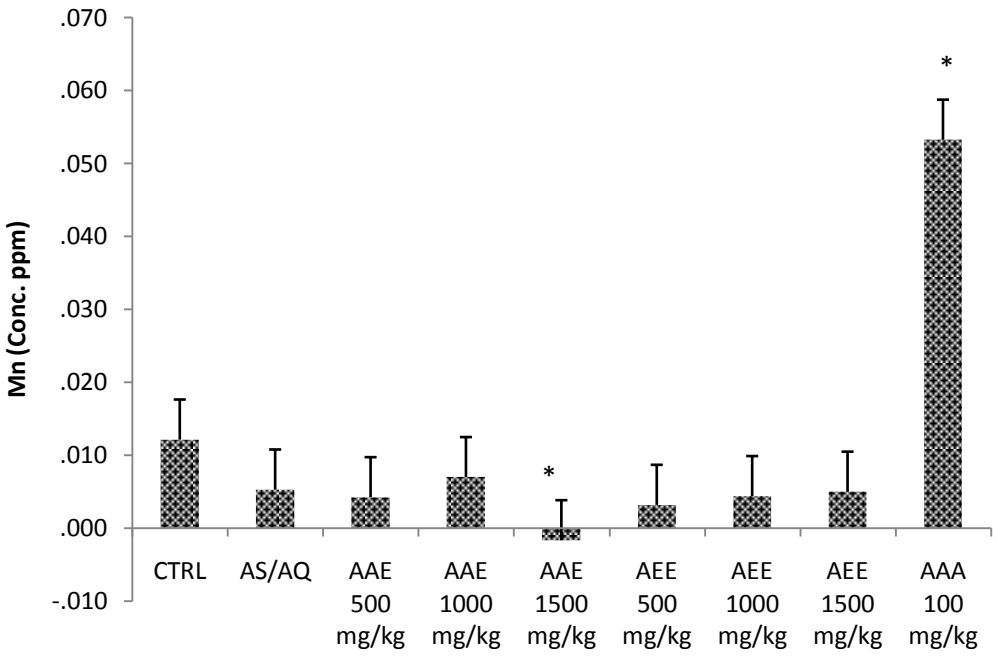


Fig. 4.8: Manganese concentration in the brain tissue of Wistar rats after 28 days oftreatment.

Values are expressed as Mean ± SEM.

CTRL = Control, **AS/AQ** = artesunate + amodiaquine, **AAE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AEE** = ACT + aqueous extract of *P. dactylifera* at Low, Medium and High doses (500 mg/kg, 1000 mg/kg and 1500 mg/kg bw respectively), **AAA** = ACT + Amino acid (100 mg/kg)

4.2 Histological and Histochemical Studies

Plates III, XII and XXI are the histology of the cerebellar cortex of the control group showing the presence of the three distinct layers with normal distribution of neurons: the molecular layer, granule layer, and the Purkinje cell layer with its dendrites. The CFV stain revealed an even distribution of Nissl substance within the neurons while the Bielshowsky stain showed the numerous branches of the Purkinje cell fibres.

The AS-AQ treated group showed histoarchitectural distortion and insults of the cerebellar cortex evidenced by condensed nuclei of Purkinje cells, bright eosinophilic cytoplasm, neuronal and cytoplasmic shrinkage, degeneration of Purkinje neuron dendrites, neuronal vacuolations within the overlying molecular layer, chromatolysis, neuronal cell loss, internal granule necrosis, loss of Nissl substance, cell body shrinkage as well as poorly stained Purkinje cells (Plates IV, XIII and XXII).

Plates V, XIV and XXIII of Group 3 treated with 500 mg/kg of aqueous extract showed poor reparative response of the cerebellum to histoarchitectural insults characterized by Purkinje and granule cells degeneration, pyknosis, neuronal swellings and vacuolations, though with few distinct Purkinje cell dendrites and moderate Nissl substance distribution. The 1000 mg/kg and 1500 mg/kg aqueous extract treated groups show a transition of progressively increased heterogeneous (medium to large sized) neuronal population in the three layers, with moderate to clearly distinct neuron dendrites in the molecular layer indicating no obvious degenerative changes (Plates VI, XV and XXIV for the 1000 mg/kg treatment, and VII, XVI and XXV for the 1500 mg/kg treatment respectively). The photomicrographs of the aqueous extracts treated groups (particularly the AEPD 1000 mg/kg and AEPD 1500 mg/kg treated groups) revealed a

normal appearance of distinct moderate to intensely stained Nissl substance and Purkinje neuronal fibres.

The result of the histological studies of the aqueous extract on the hippocampus revealed a normal histoarchitectural aggregate and staining of the Nissl substance of the pyramidal neurons in the CA3 of the control group (Plates XXX, XXXIV and XLVIII). Plates XXXI, XL and XLIX of AS-AQ treated rats were characterized by neuronal degeneration evidenced by vacuolations, bright eosinophilic neuronal necrosis, indistinct neurons, and distortion of the pyramidal neuronal layer of the CA3 area. Groups 3 and 4 treated with 500 mg/kg and 1000 mg/kg of aqueous extracts respectively showed a reduced distortion of the pyramidal neurons, light traces of eosinophilic cytoplasm, nuclear pyknosis, and vacuolations (Plates XXXII, XLI, and L for the 500 mg/kg treatment, and XXXIII, XLII and LI for the 1000 mg/kg treatment respectively). These light distortions are not evident in Plates XXXIV, XLIII and LII representing 1500 mg/kg of aqueous treatment, as the pyramidal neuronal arrangements were observed to be normal with very distinct neurons and increased staining intensity.

Plates VIII, XVII and XXVI of 500 mg/kg ethanol extract treated group revealed degeneration of Purkinje cell layer evidenced by swellings, vacuolations, pyknosis, loss of dendritic branches and indistinct granule cells. With 1000 mg/kg and 1500 mg/kg ethanol extracts treatment (Plates IX, XVIII and XXVII for the 1000 mg/kg treatment, and X, XIX and XXVIII for the 1500 mg/kg treatment respectively) there was an observed gradual attenuation of histological insults incurred by AS-AQ, very much similar to that of the aqueous groups, though the observed histology components showed the amelioration of the ethanol groups to be poorer than those of the aqueous.

The hippocampal sections of the 500 mg/kg ethanol treatment showed distortion of the pyramidal cells arrangement, chromatolysis, necrosis, vacuolations, and poor staining of the Nissl substance (Plates XXXV, XLIV and LIII). Treatment with 1000 mg/kg ethanol extract still revealed traces of degenerated pyramidal cells, chromatolysis, karyorrhexis, vacuolations and necrosis of granule cells (Plates XXXVI, XLV and LIV). With 1500 mg/kg treatment of ethanol extract, the hippocampal section showed no evidence of distortions owing to AS–AQ treatment. There was normal arrangement of the pyramidal cells, though there was a persistent poor staining of Nissl substance (Plates XXXVII, XLVI and LV).

Plates XI, XX and XXIX of the ascorbic acid treatment revealed little evidence of vacuolations and degeneration of Purkinje cells but with prominent Purkinje fibres in the cerebellum, and with a well arranged pyramidal neuronal layer in the hippocampus (Plates XXXVIII, XLVII and LVI)

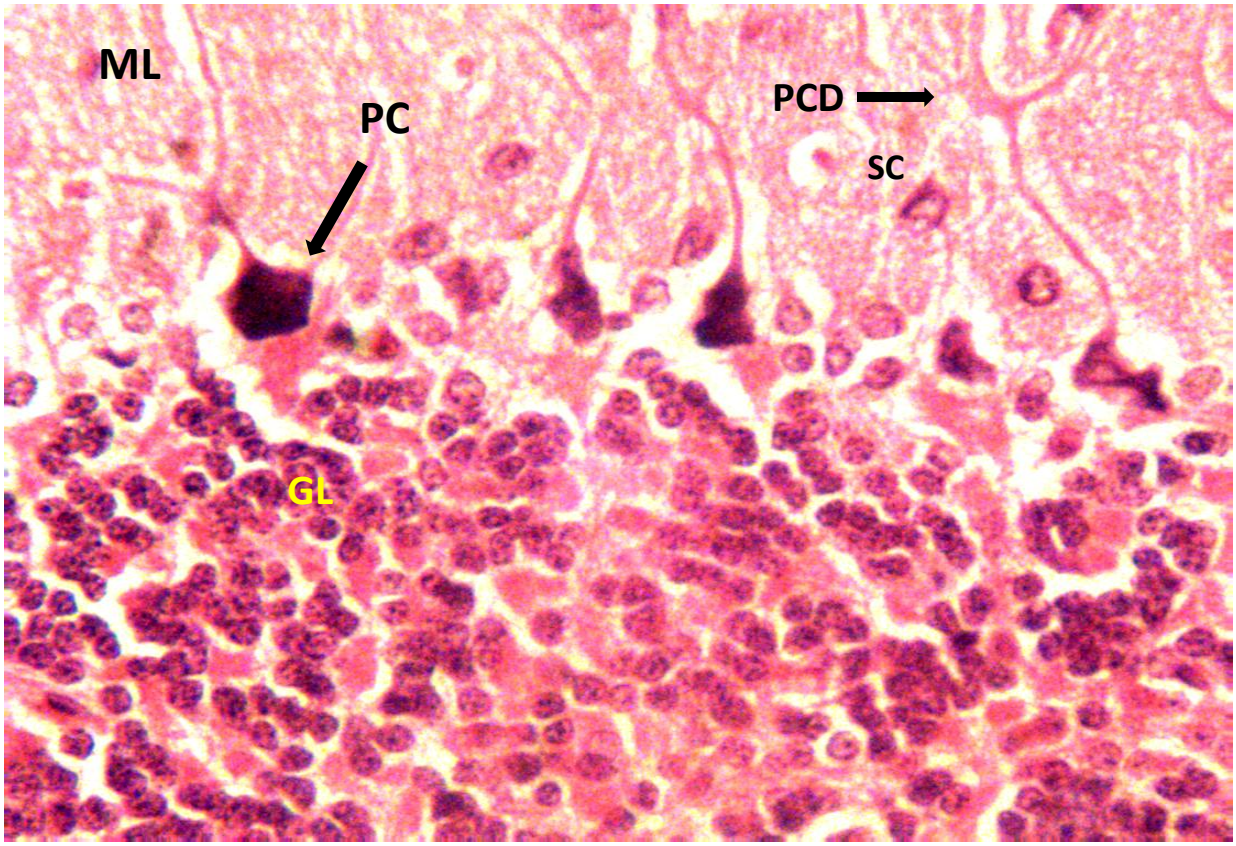


Plate III: A longitudinal section of the cerebellar cortex of the control group showing, normal histoarchitecture of the molecular layer (ML), granular layer (GL) Purkinje cells (PC), the Purkinje cell dendritic branches (PCD) and stellate cells (SC). H and E (×400)

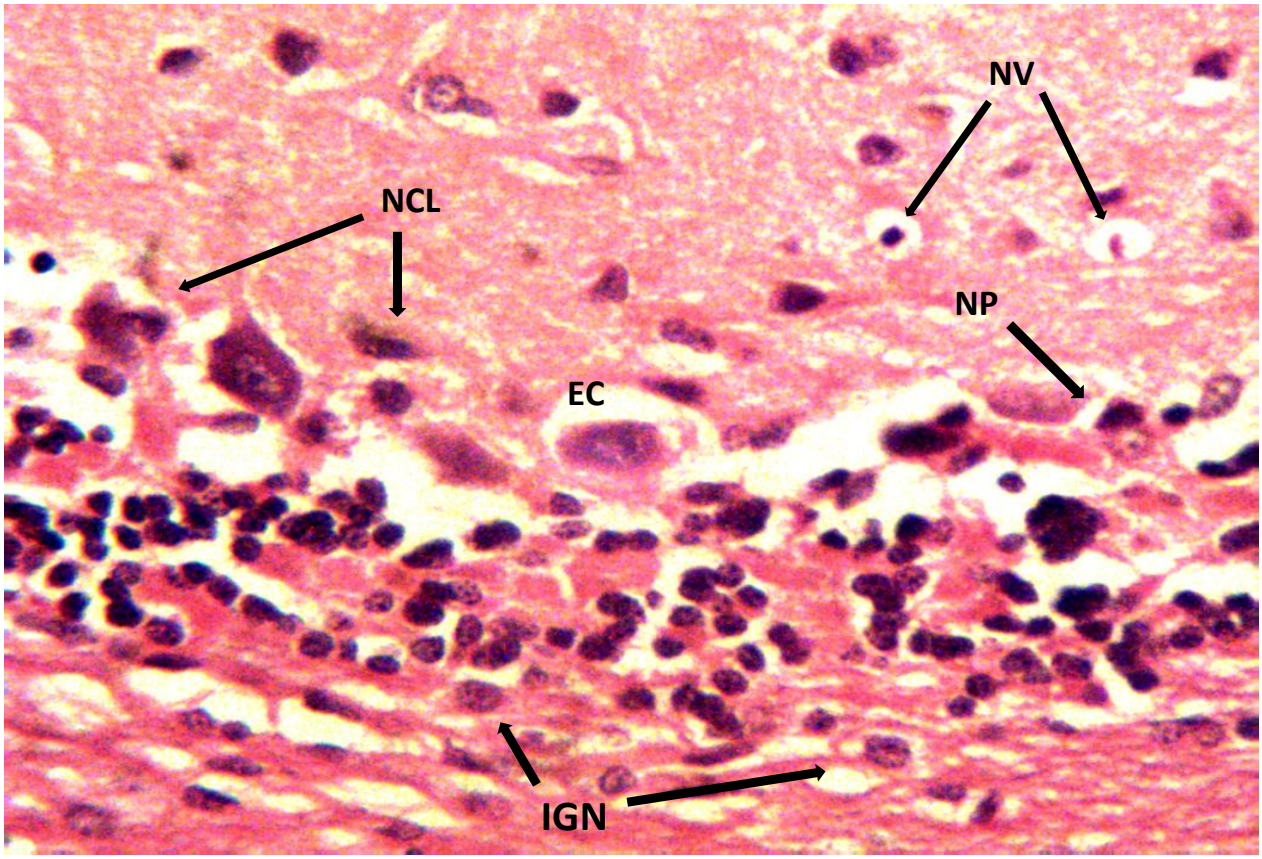


Plate IV: A longitudinal section of the cerebellar cortex of the 2.86/8.75 mg/kg AS-AQ treated group revealing some histologic insults evidenced by neuronal cell loss (NCL), complete loss of Purkinje cell dendrites, neuronal vacuolations (NV), eosinophilic cytoplasm (EC) in the Purkinje cell, nuclear pyknosis (NP) and many indistinct granule neurons (IGN) within the granule cell layer H and E (×400)

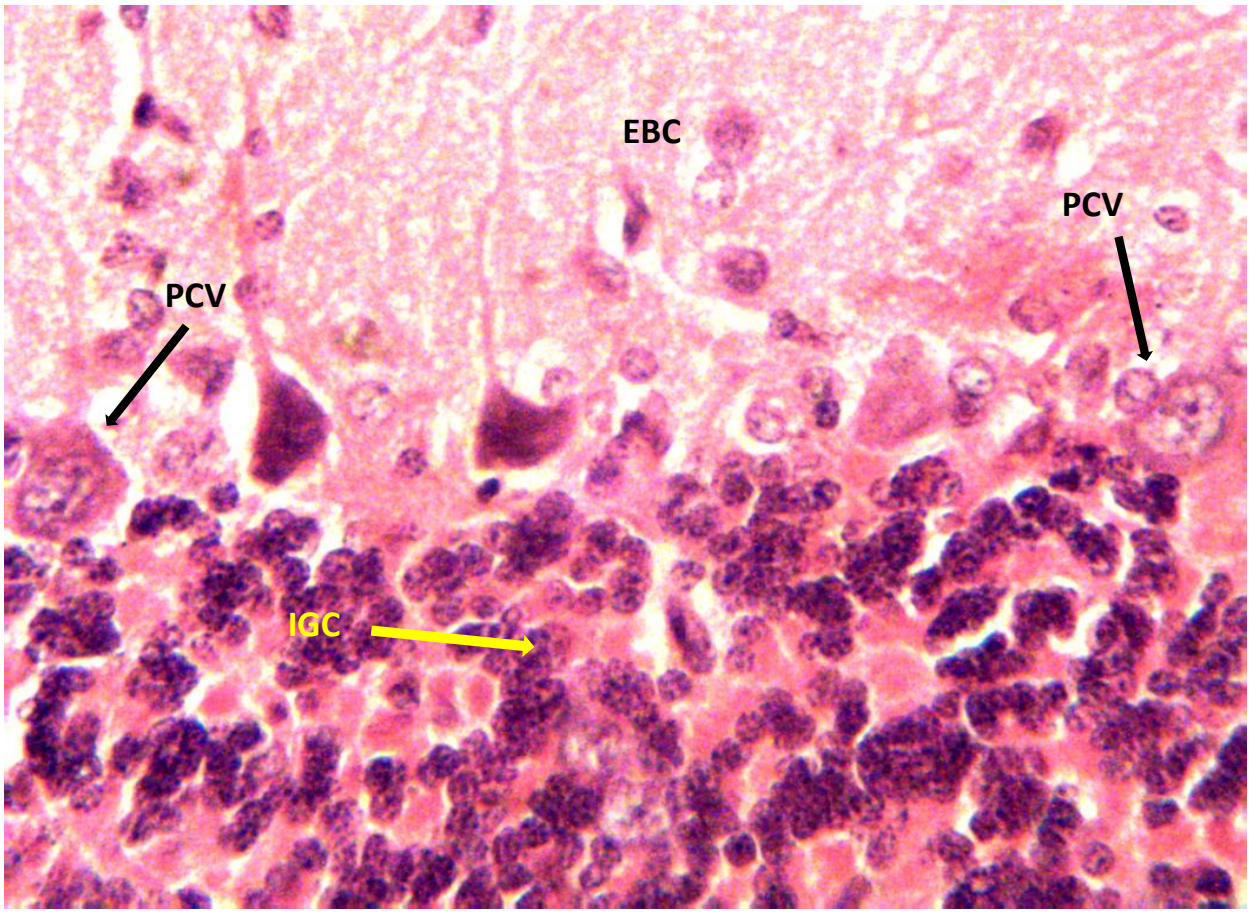


Plate V: A longitudinal section of the cerebellar cortex of Wistar rats treated with 2.86/8.75 mg/kg AS-AQ + 500 mg/kg AEPD. The section revealed diminished network of the Purkinje cell dendrites, Purkinje cell vacuolations (PCV), indistinct granule cells (IGC) and eosinophilic basket cells (EBC). H and E (×400)

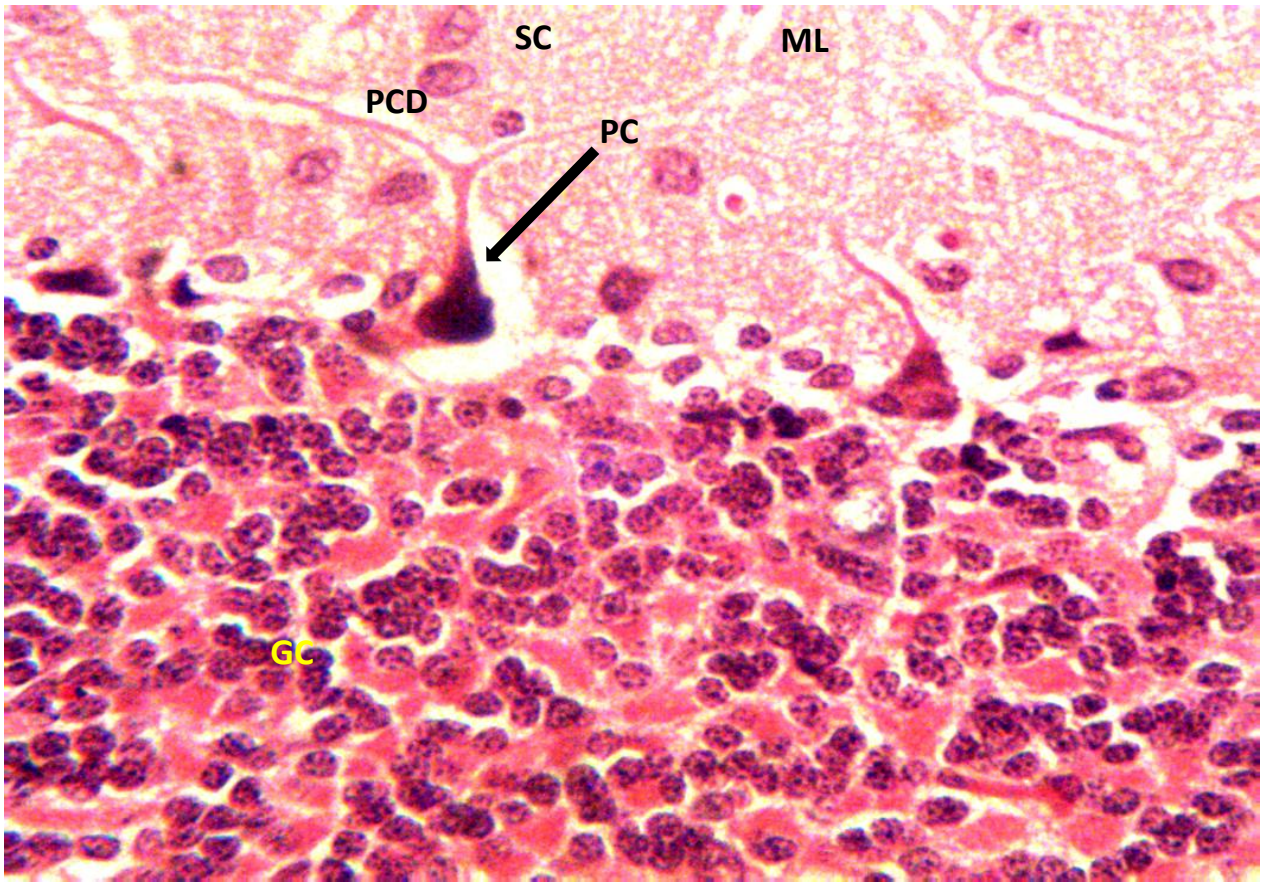


Plate VI: A longitudinal section of the cerebellum of rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg AEPD showing the molecular layer (ML), granule layer (GL), and stellate cells (SC). The Purkinje cells (PC) and their dendrites (PCD) are also visible. H and E (×400)

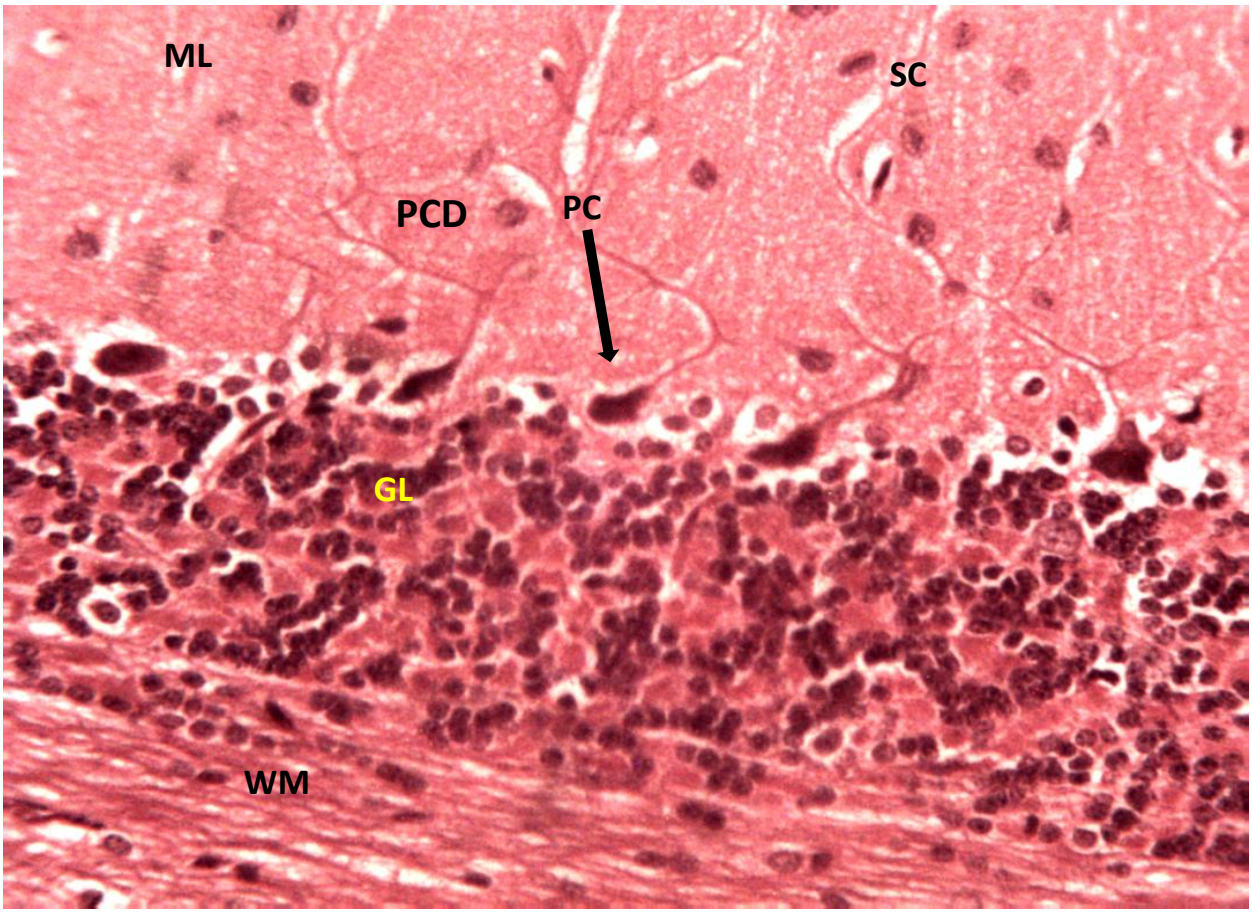


Plate VII: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg AEPD showing the molecular layer (ML), granule layer (GL) and white matter (WM). There is normal distribution of the Purkinje cells (PC), their dendrites (PCD), stellate cells (SC) and numerous granule cells within the granular cell layer. H and E (×400)

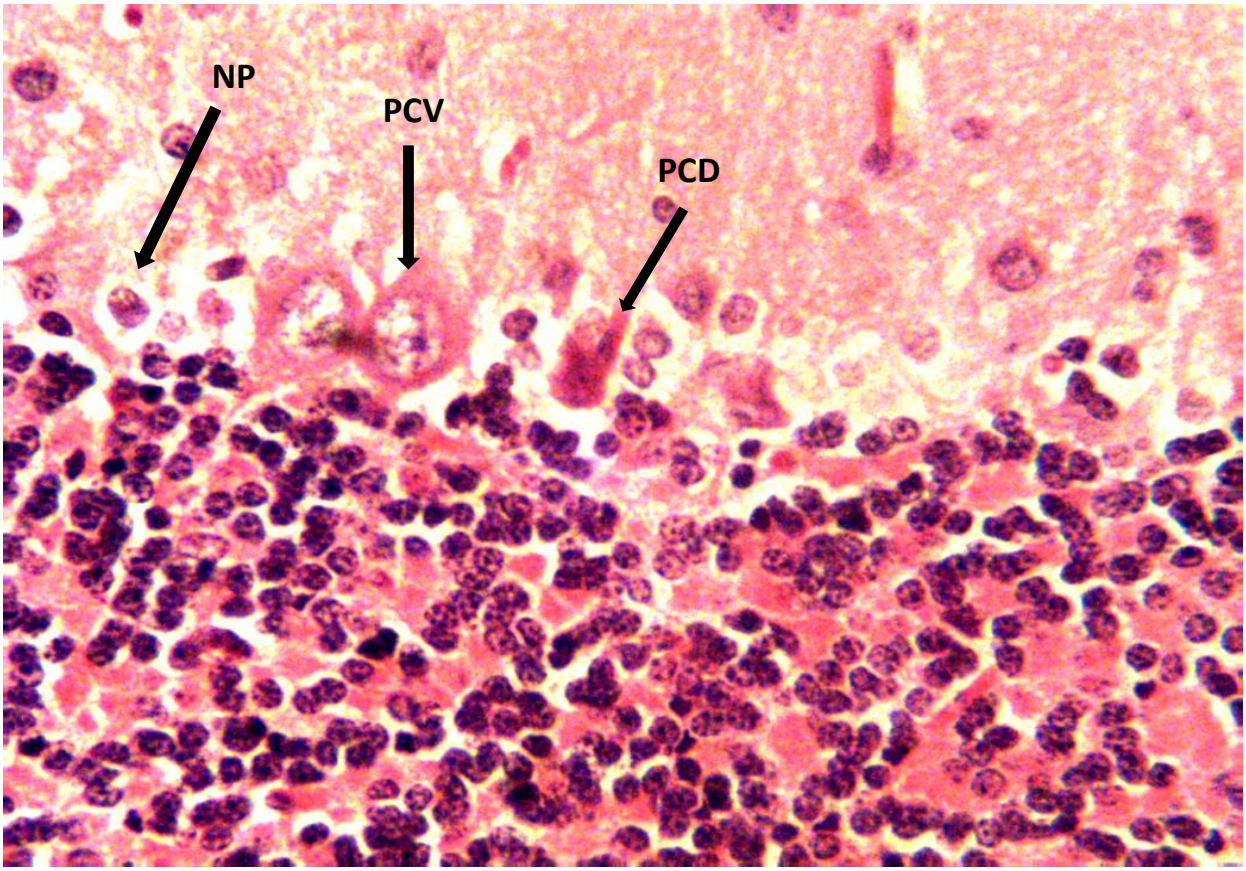


Plate VIII: A longitudinal section of the cerebellum of the rats treated with 2.86/8.75 mg/kg AS/AQ + 500 mg/kg EEPD showing evidence of Purkinje cells swelling and vacuolations (PCV), neuronal pyknosis (NP), Purkinje cell degeneration (PCD), disappearance of Purkinje cell dendrites, H and E (×400)

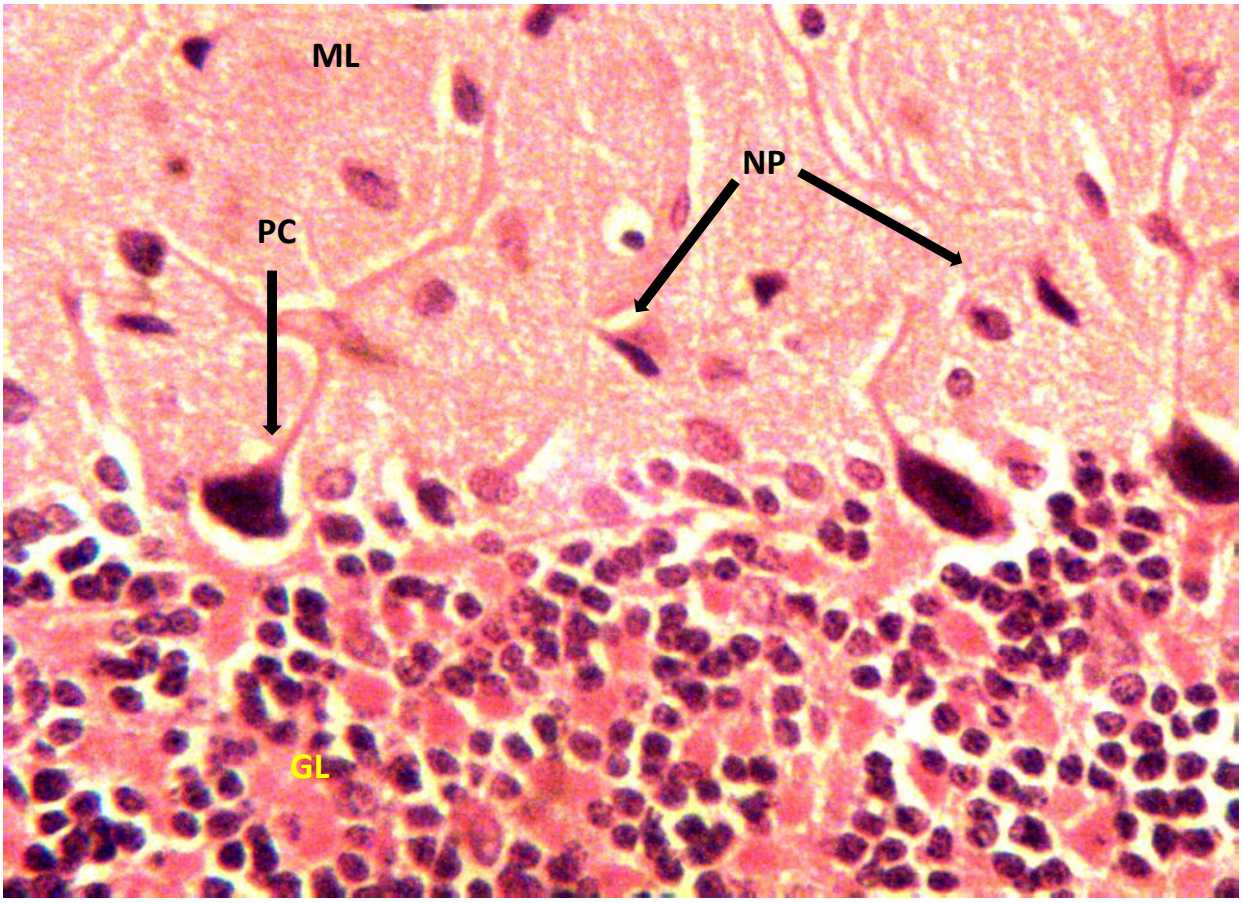


Plate IX: A longitudinal section of the cerebellum of rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg EEPD showing the molecular layer (ML), granule layer (GL), Purkinje cells (PC) with their dendritic branches. There is also evidence of a few nuclear pyknosis (NP). H and E (×400)

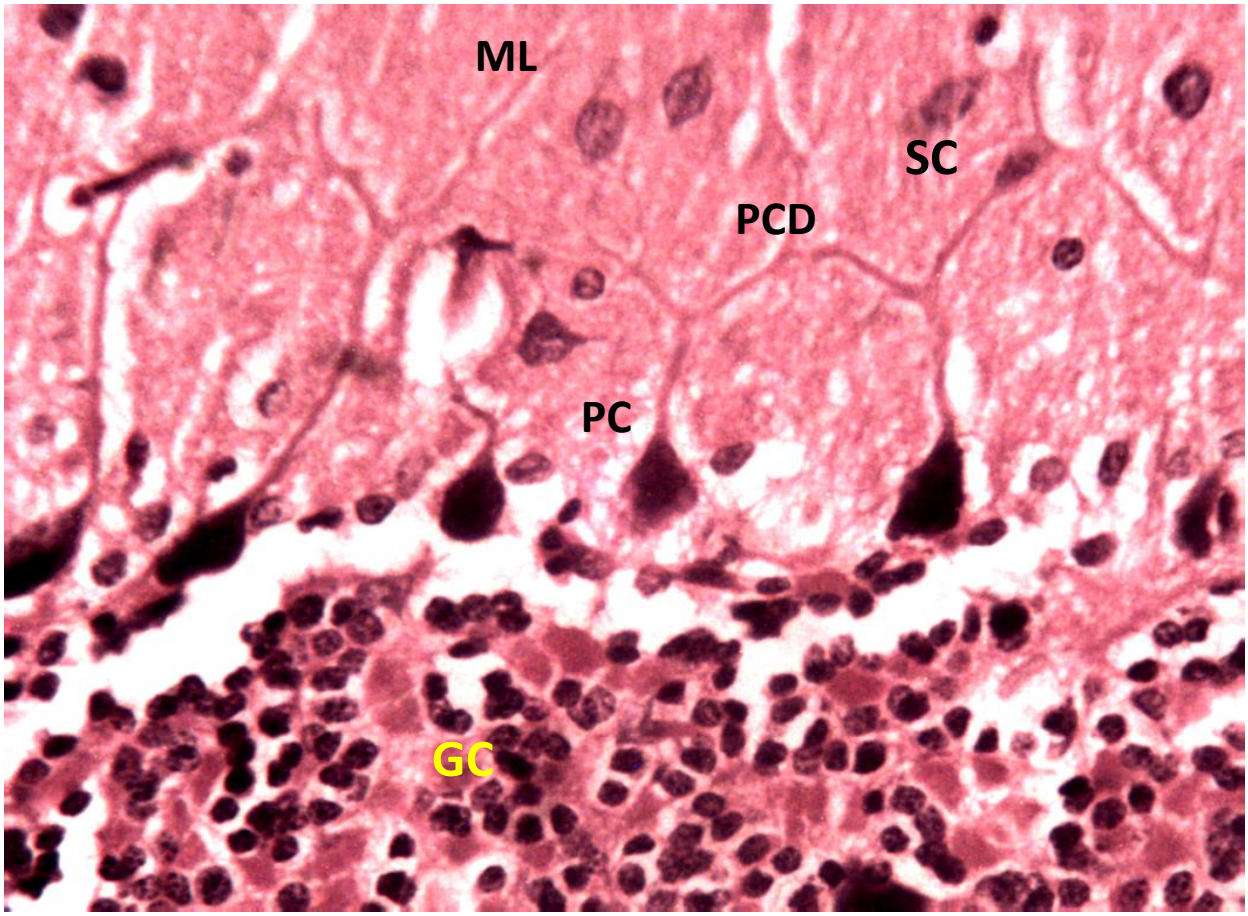


Plate X: A longitudinal section of the cerebellar cortex of rats treated with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg EEPD showing the molecular layer (ML), granule cell layer (GC), and stellate cells (SC). It also shows normal Purkinje cells (PC) with their dendrites (PCD). H and E (×400)

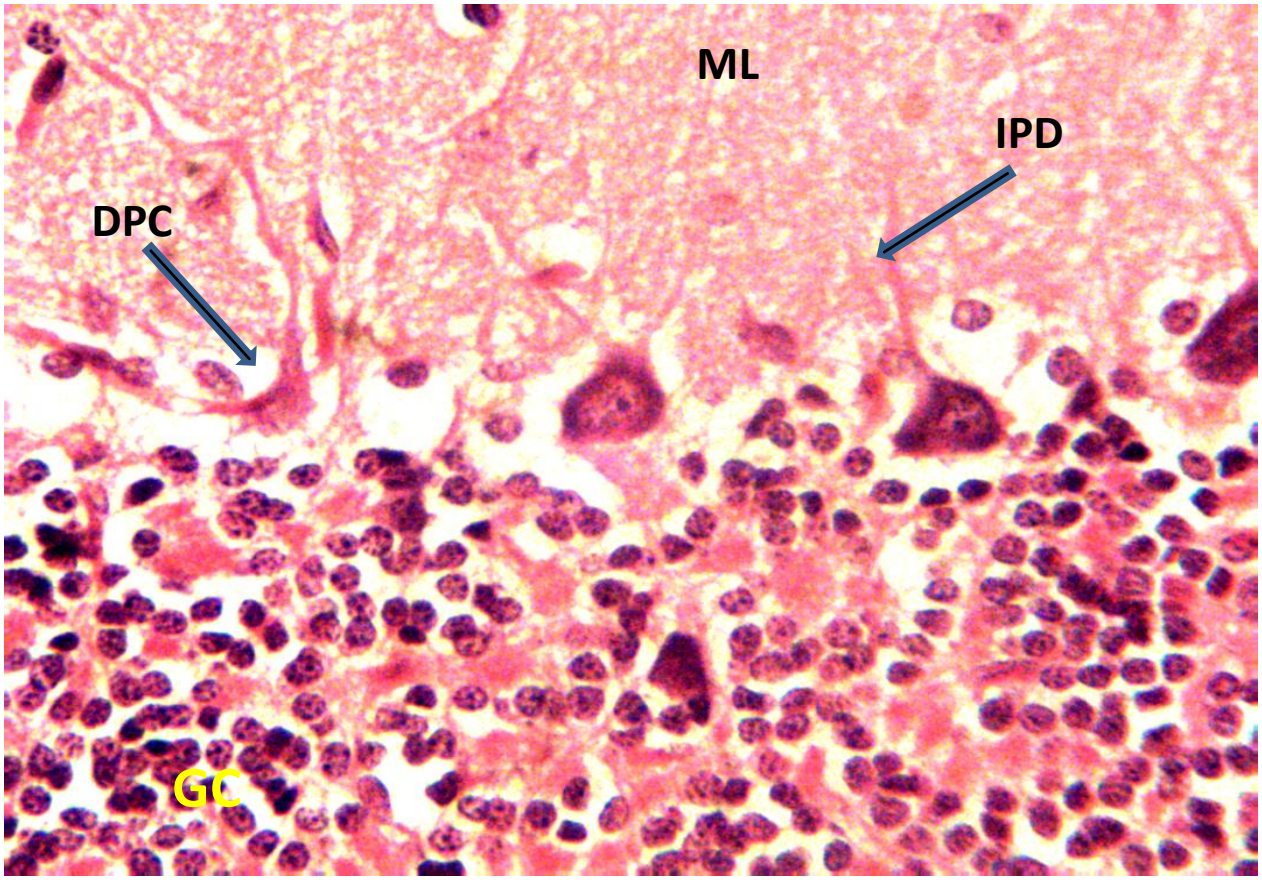


Plate XI: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 100 mg/kg ascorbic acid showing the molecular layer (ML) and granule cell layer (GC). It also shows evidence of vacuolated and degenerated Purkinje cells (DPC) with indistinct dendrites (IPD). H and E (×400)

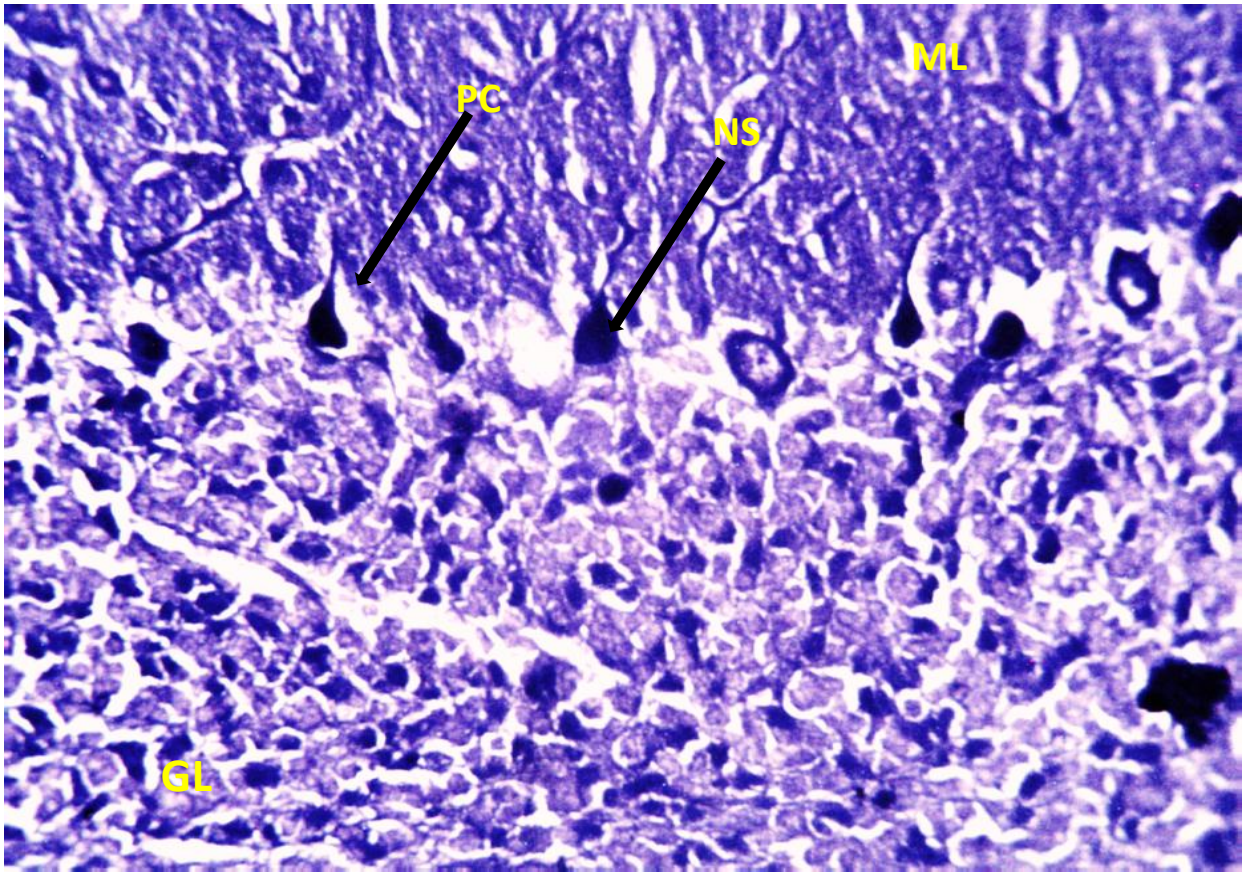


Plate XII: A longitudinal section of the cerebellar cortex of the control group showing normal histoarchitecture of the molecular layer (ML), granule cell layer (GL), normal distribution of the Purkinje cells (PC) and Nissl substance (NS). CFV (×250)

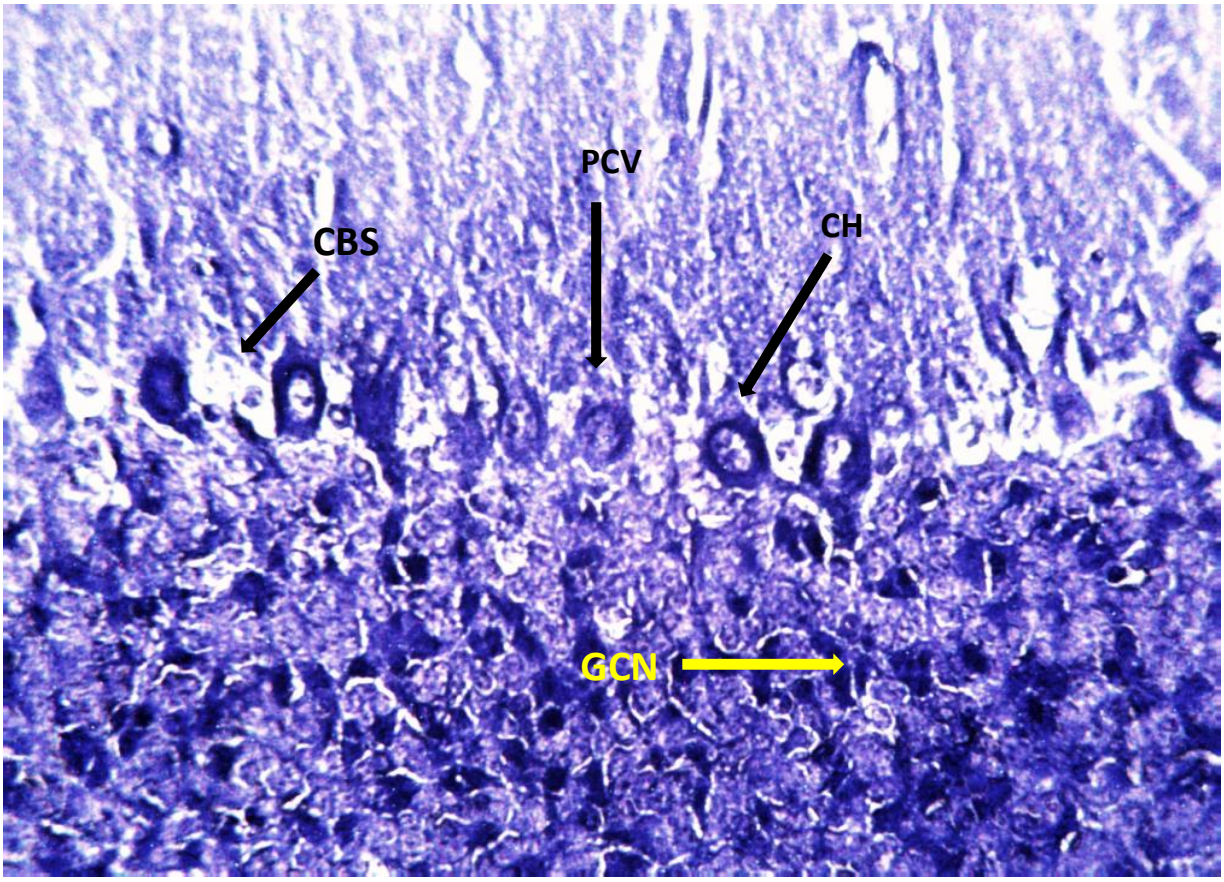


Plate XIII: A longitudinal section of the cerebellar cortex of the 2.86/8.75 mg/kg AS-AQ treated group. There are observed histologic degeneration: reduced Nissl substances from the Purkinje neurons, cell body shrinkage (CBS), chromatolysis (CH), Purkinje cell vacuolations (PCV) and granule cell necrosis (GCN), CFV (×250)

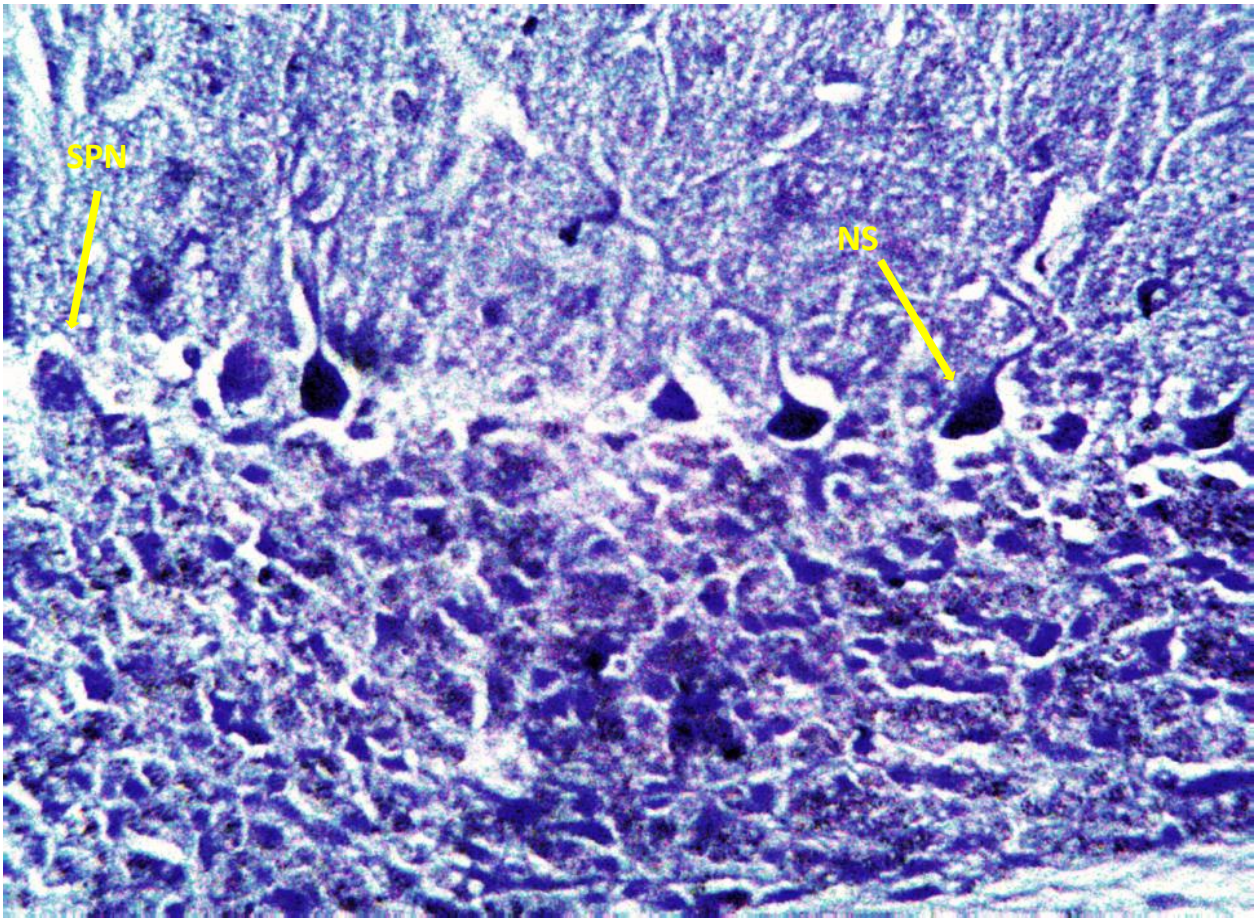


Plate XIV: A longitudinal section of the cerebellar cortex of Wistar rats treated with 2.86/8.75 mg/kg AS-AQ + 500 mg/kg AEPD, showing a near normal distribution of Nissl substance (NS), with a few shrunken Purkinje neurons (SPN). CFV (×250)

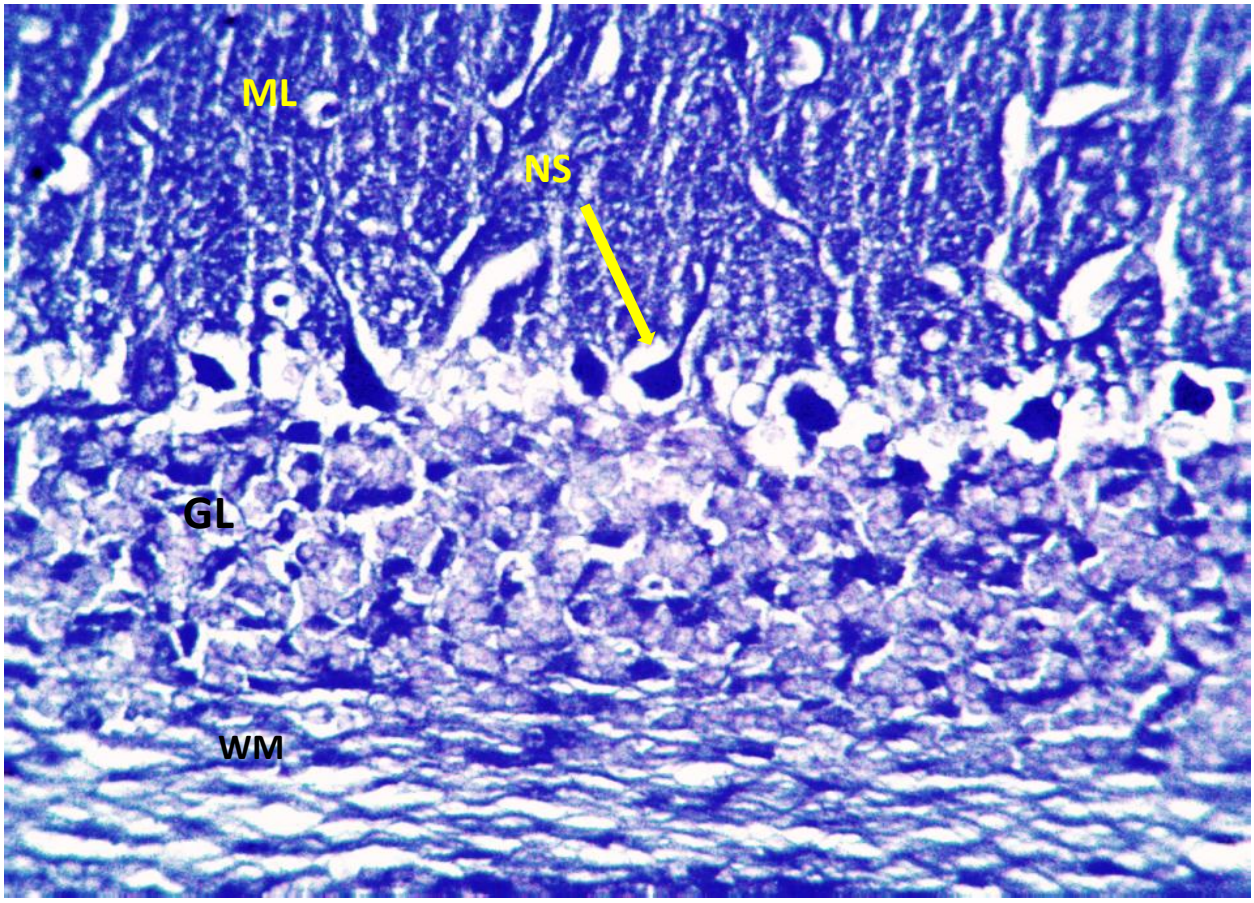


Plate XV: A longitudinal section of the cerebellum of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg AEPD showing the molecular layer (ML), granule layer (GL), white matter (WM) and a normal distribution of Nissl substances (NS) in the Purkinje cells. CFV (×250)

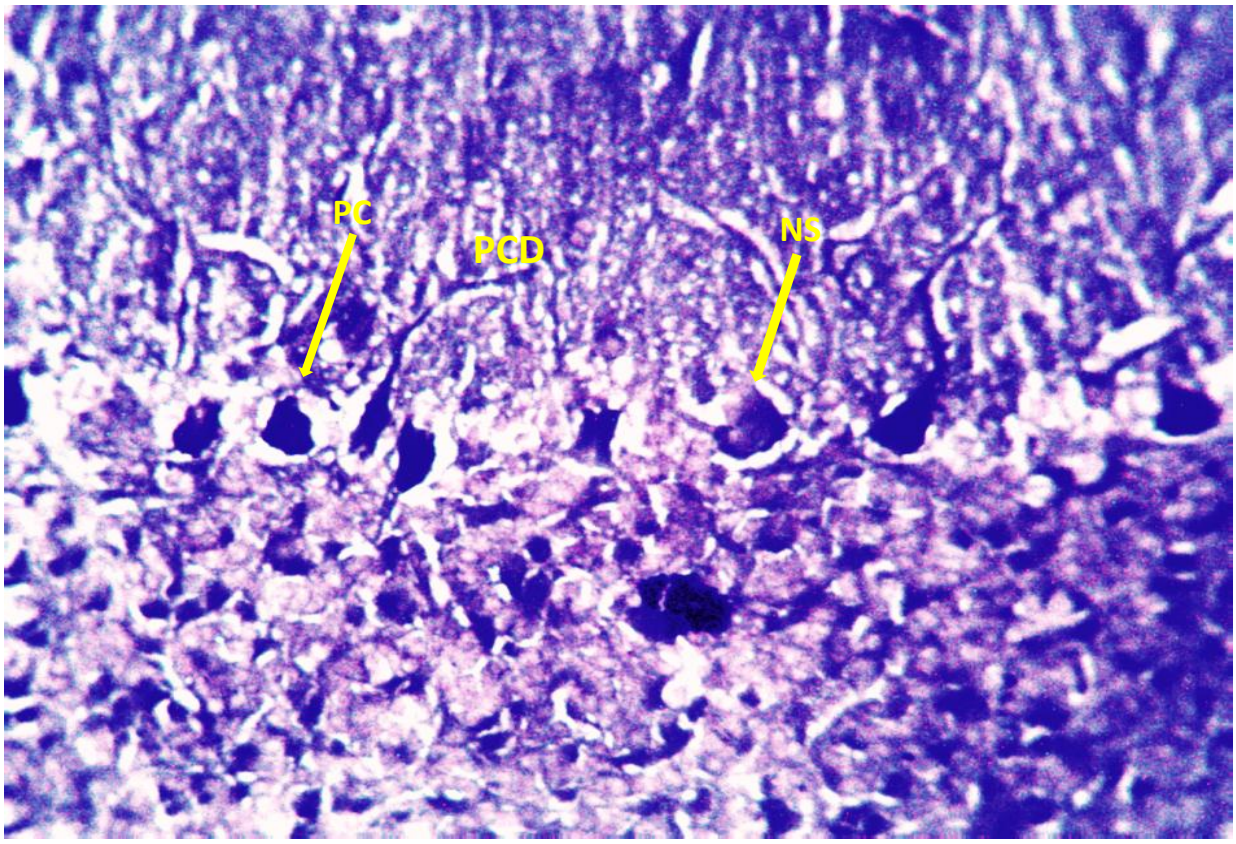


Plate XVI: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg AEPD showing staining of the Nissl substance (NS) within the Purkinje cell neurons (PC) and granule cells. The dendritic branches of the Purkinje cells (PCD) are also visible. CFV (×250)

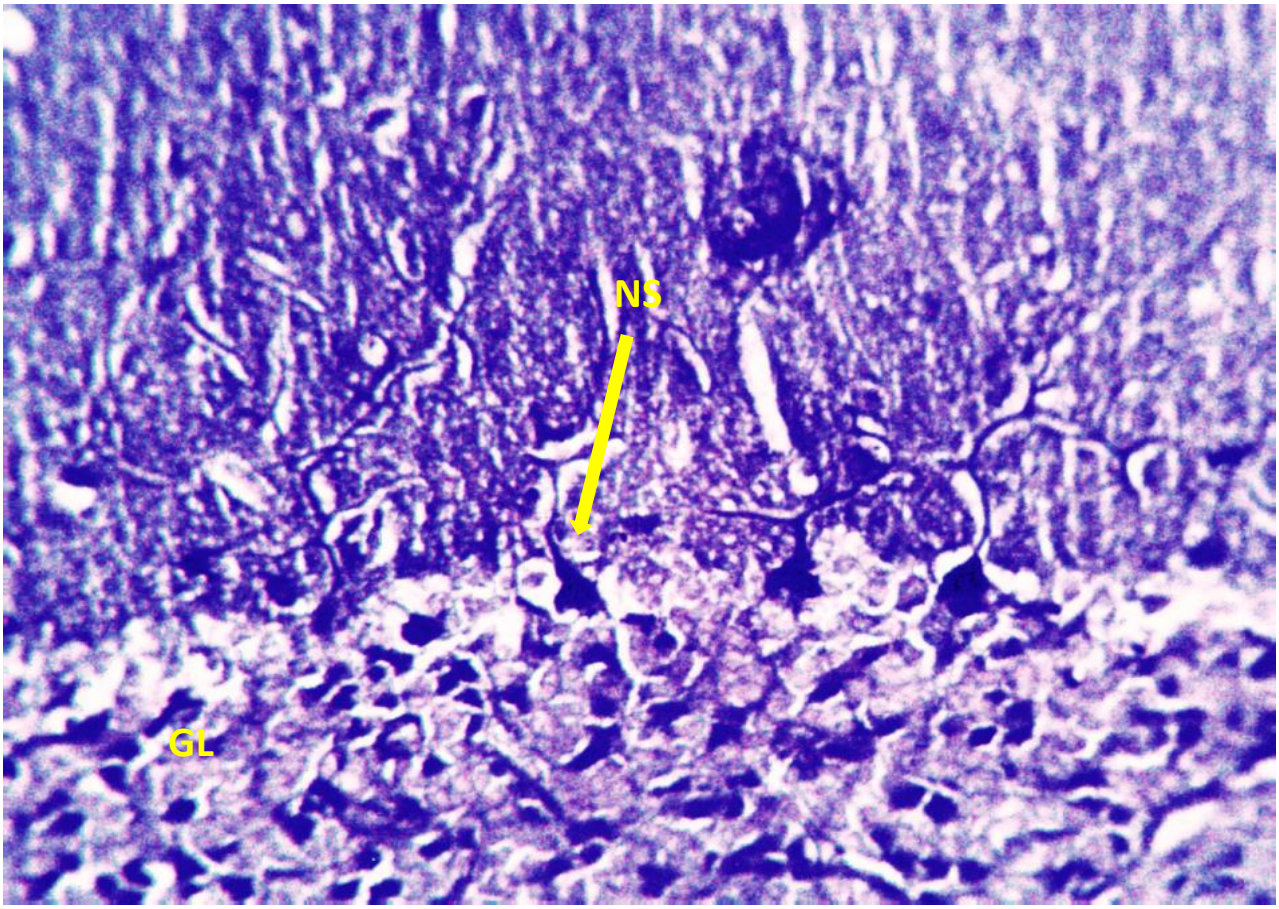


Plate XVII: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 500 mg/kg EEPD showing a poor staining of the Nissl substance (NS) in the Purkinje cells and in the granule cell layer (GL). CFV (×250)

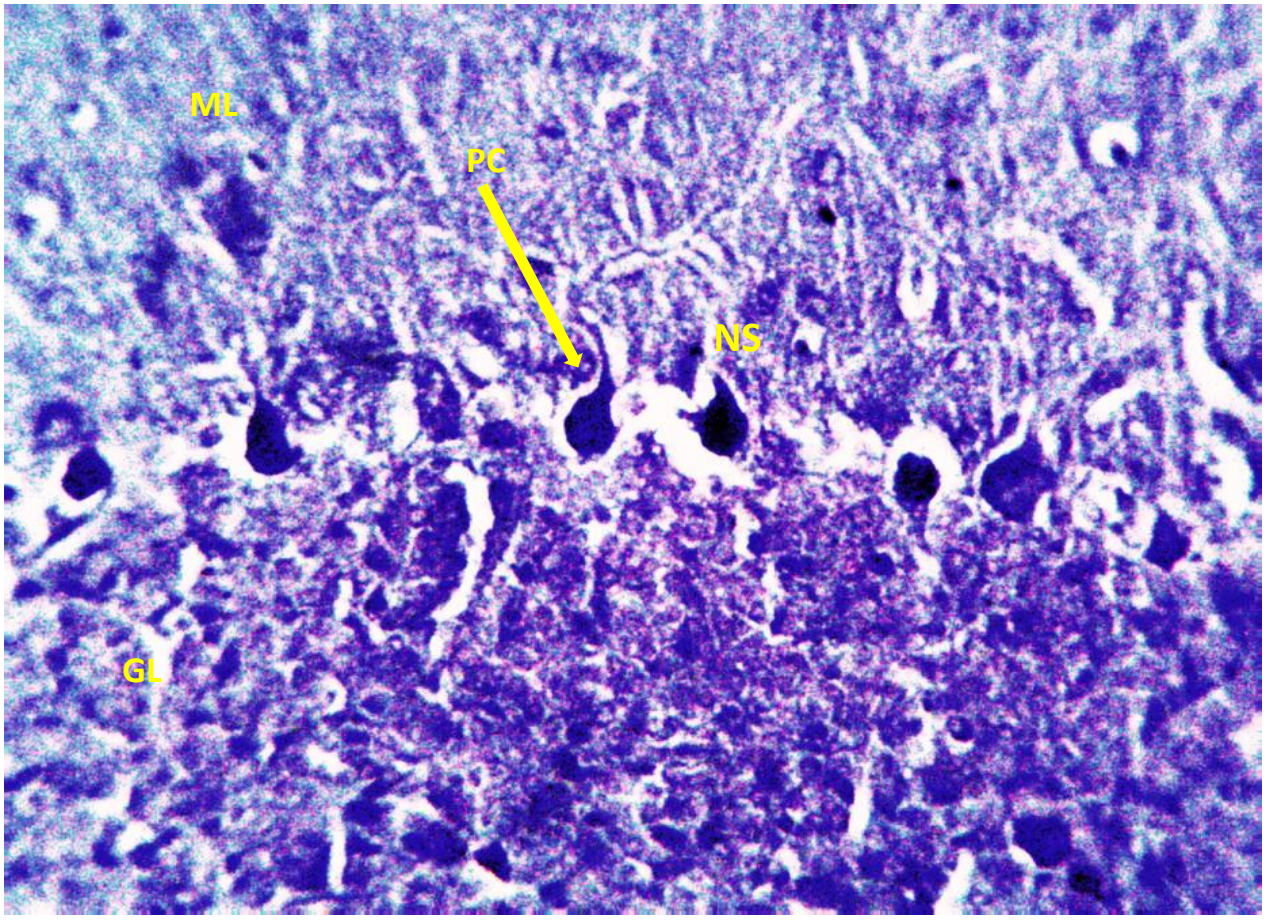


Plate XVIII: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg EEPD showing the molecular layer (ML) and granule layer (GL). There is moderate staining of the Nissl substance (NS) in the Purkinje cells (PC), but not in the granule cells. CFV (×250)

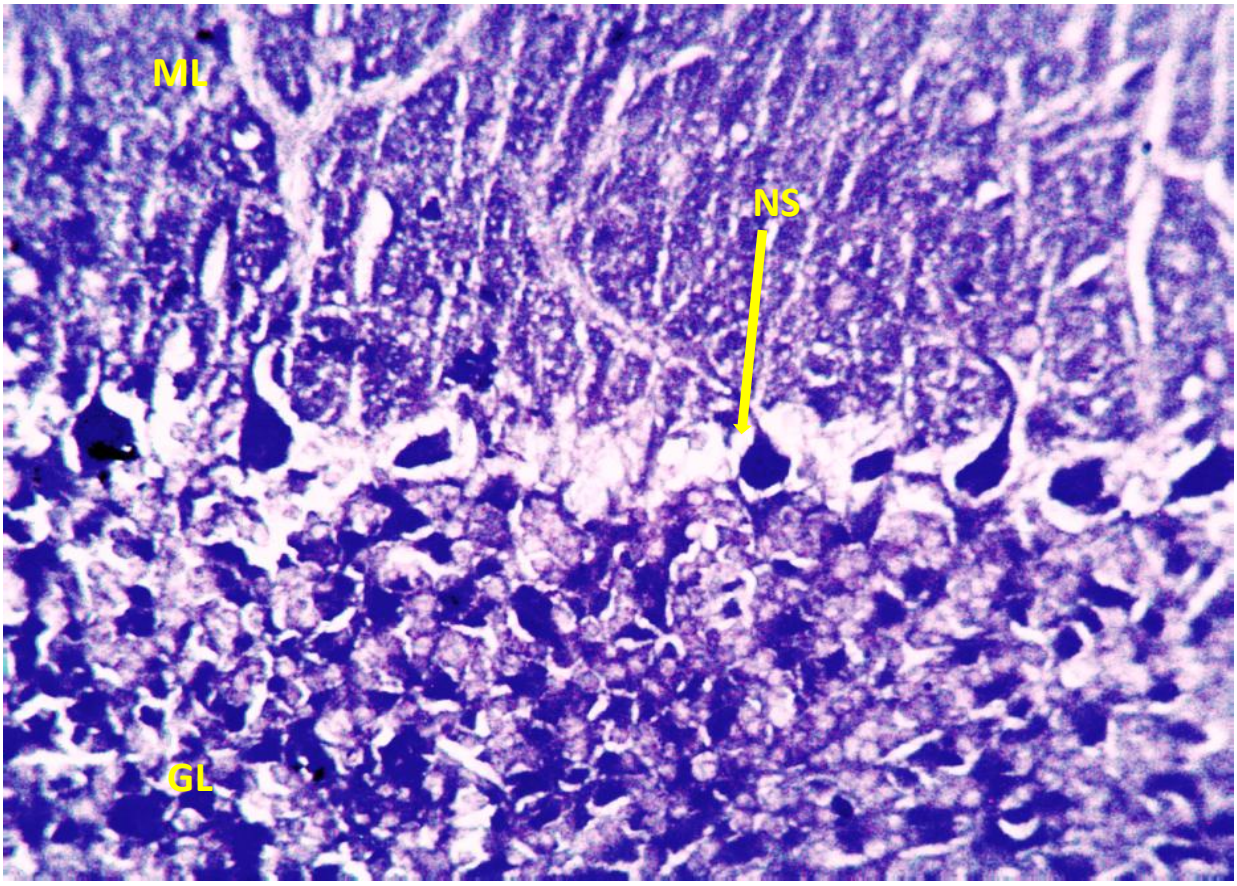


Plate XIX: A longitudinal section of the cerebellum of rats with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg EEPD showing the molecular layer (ML), granule cells (GC) with moderate staining of the Nissl substance (NS). CFV (×250)

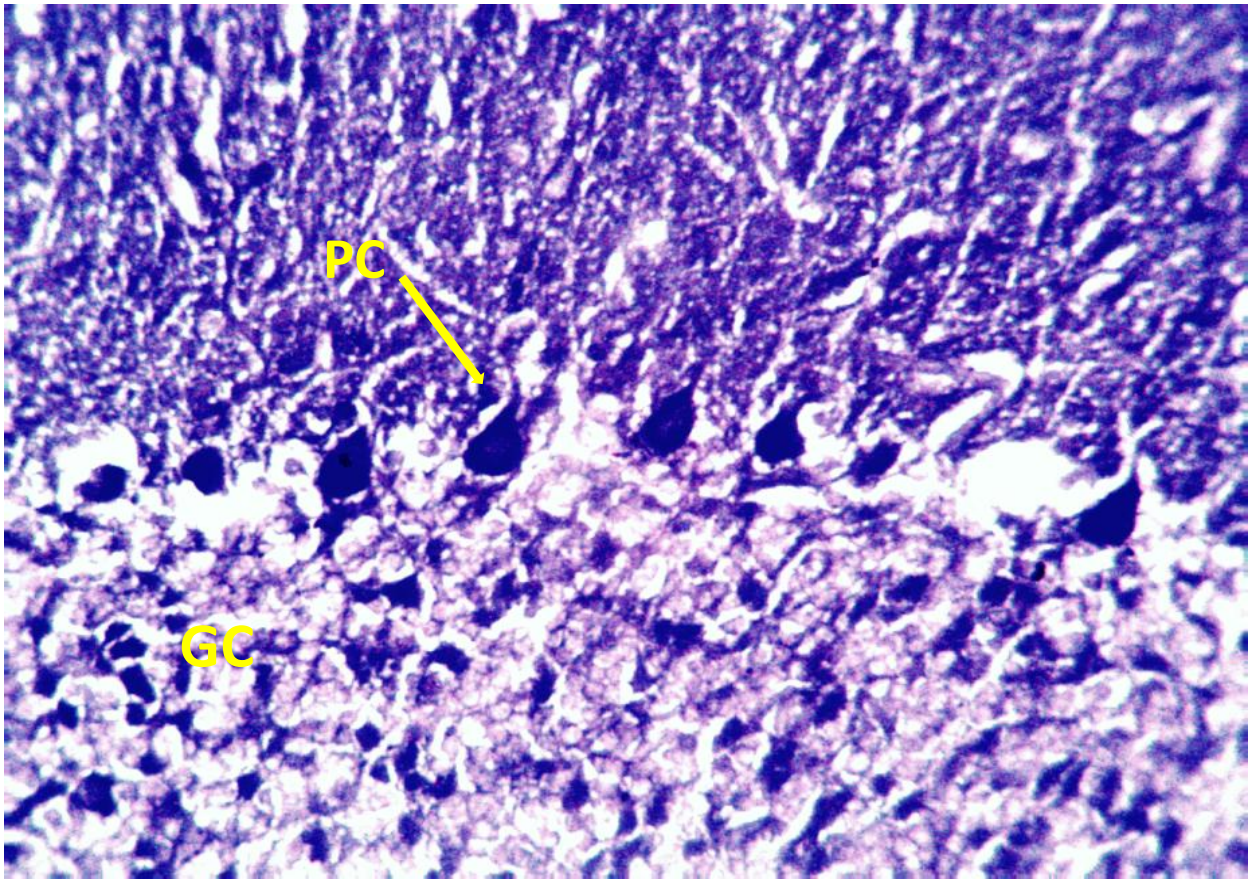


Plate XX: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 100 mg/kg ascorbic acid, showing a moderate staining of the Nissl substance and Purkinje cells (PC) and granule cells (GC). CFV (×250)

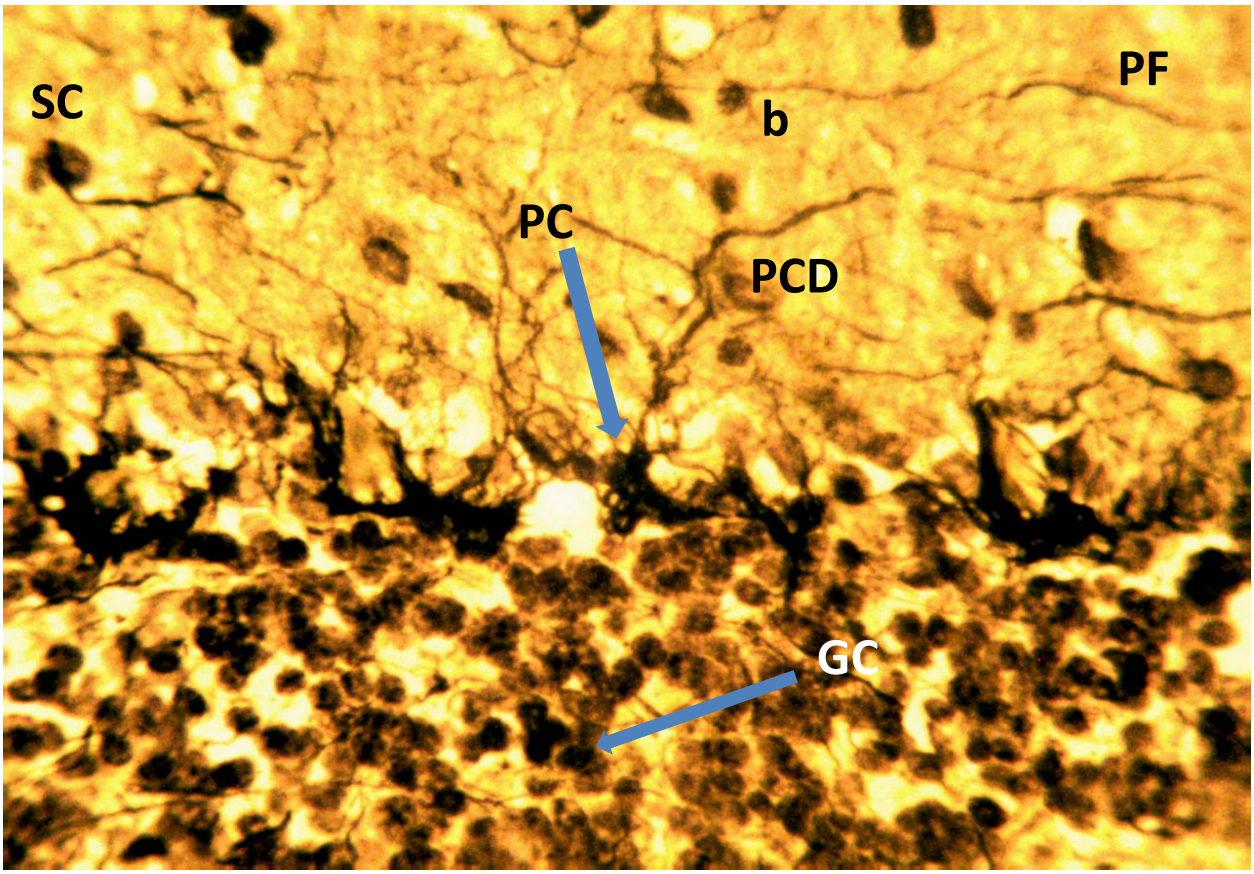


Plate XXI: A longitudinal section of the cerebellar cortex of the control group showing, normal histoarchitecture of the Purkinje cells (PC) and their dendritic branches (PCD), granule cells (GC), basket cells (b), and stellate cells (SC) with their parallel fibres (PF). Bielschowsky (×400)

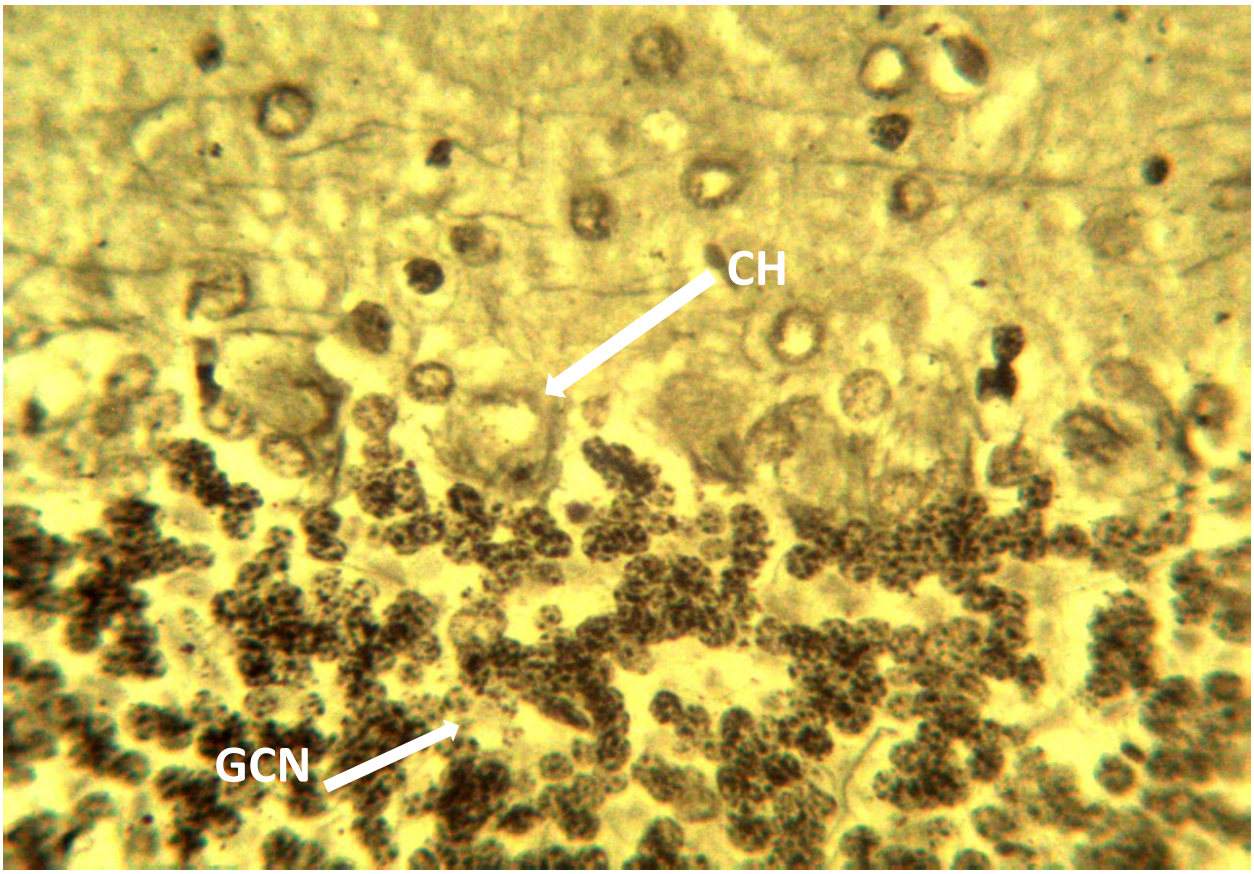


Plate XXII: A longitudinal section of the cerebellar cortex of the 2.86/8.75 mg/kg AS-AQ treated group. There are observed histologic degeneration: chromatolysis (CH) of the Purkinje neurons, eroded dendritic fibres and parallel fibres network, and granule cell necrosis (GCN). Bielschowsky (×400)

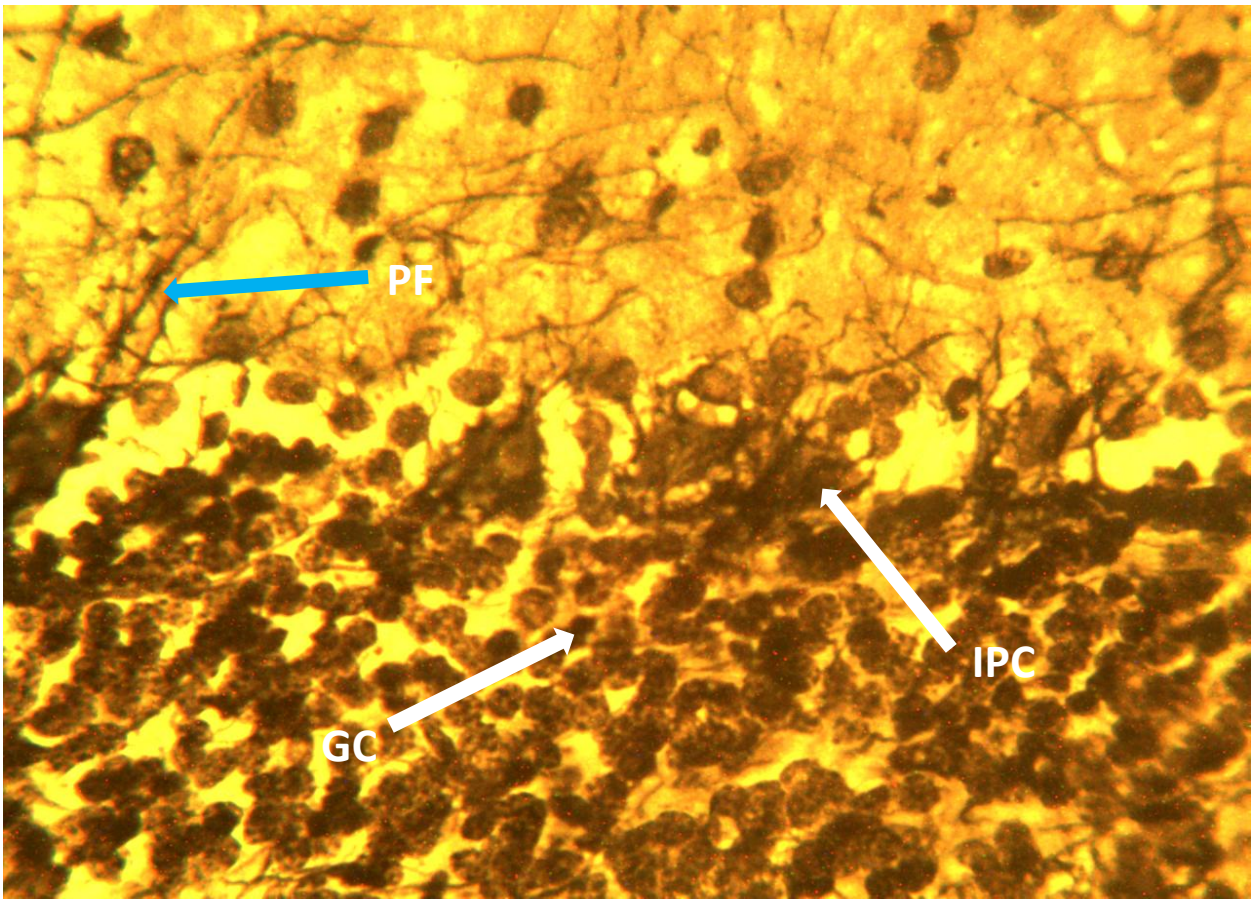


Plate XXIII: A longitudinal section of the cerebellar cortex of Wistar rats treated with 2.86/8.75 mg/kg AS-AQ + 500 mg/kg AEPD, showing indistinct Purkinje cells (IPC) a normal distribution of granule cells (GC), with a distinct Purkinje and parallel fibres (PF) network. Bielschowsky (×400)

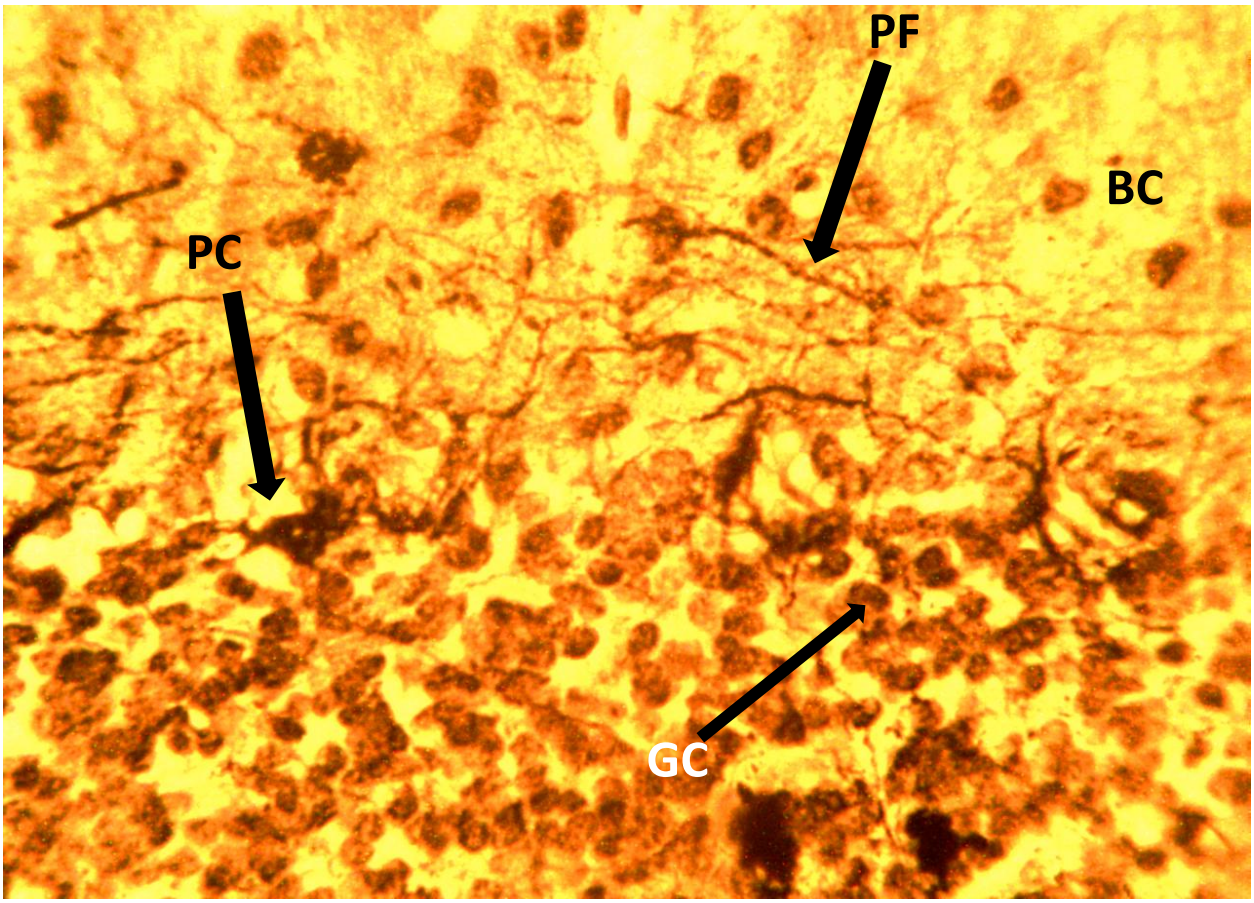


Plate XXIV: A longitudinal section of the cerebellum of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg AEPD showing a normal distribution of granule cells (GC), Purkinje cells (PC), their dendritic branches and parallel fibres (PF) of basket cells (BC). Bielschowsky (×400)

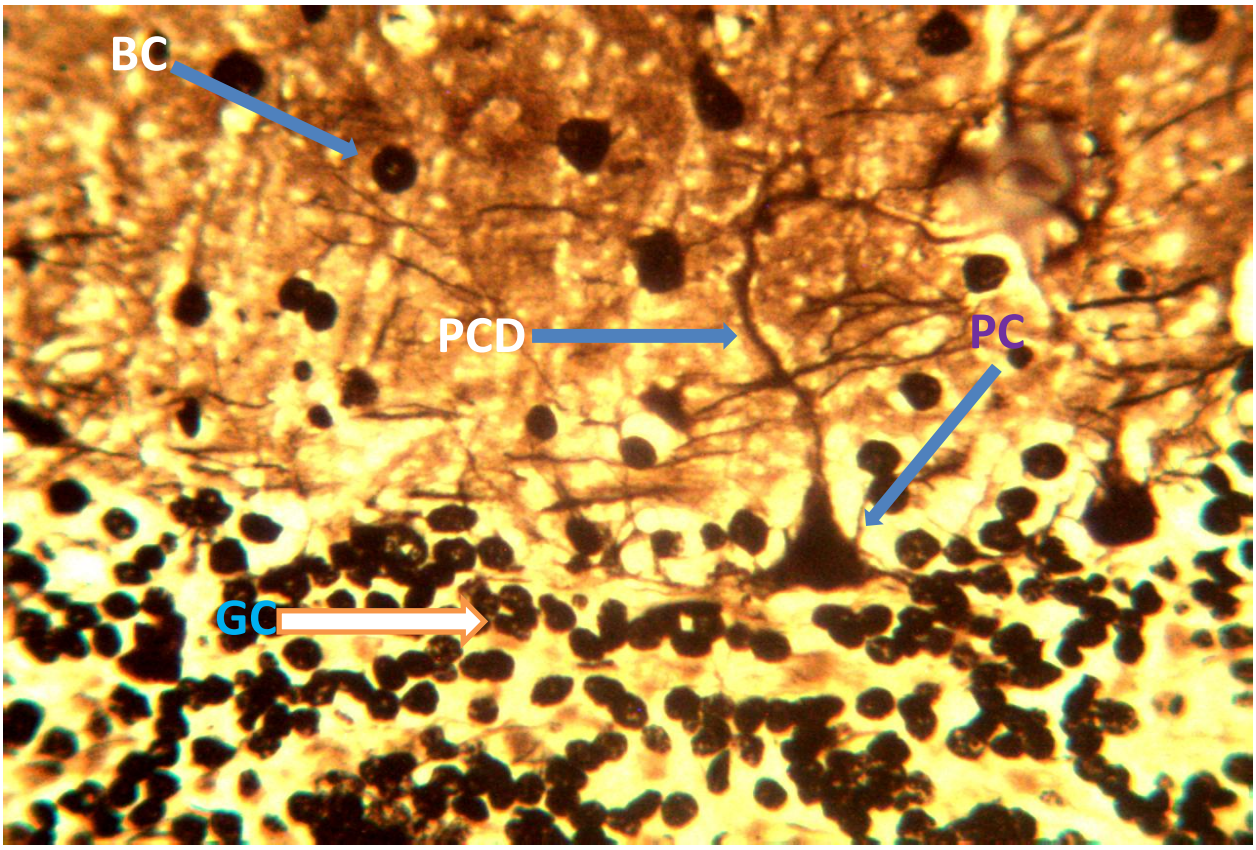


Plate XXV: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg AEPD showing the Purkinje cell neurons (PC), their dendritic branches (PCD), basket cells (BC) with their parallel fibres, and granule cells (GC). Bielschowsky (×400)

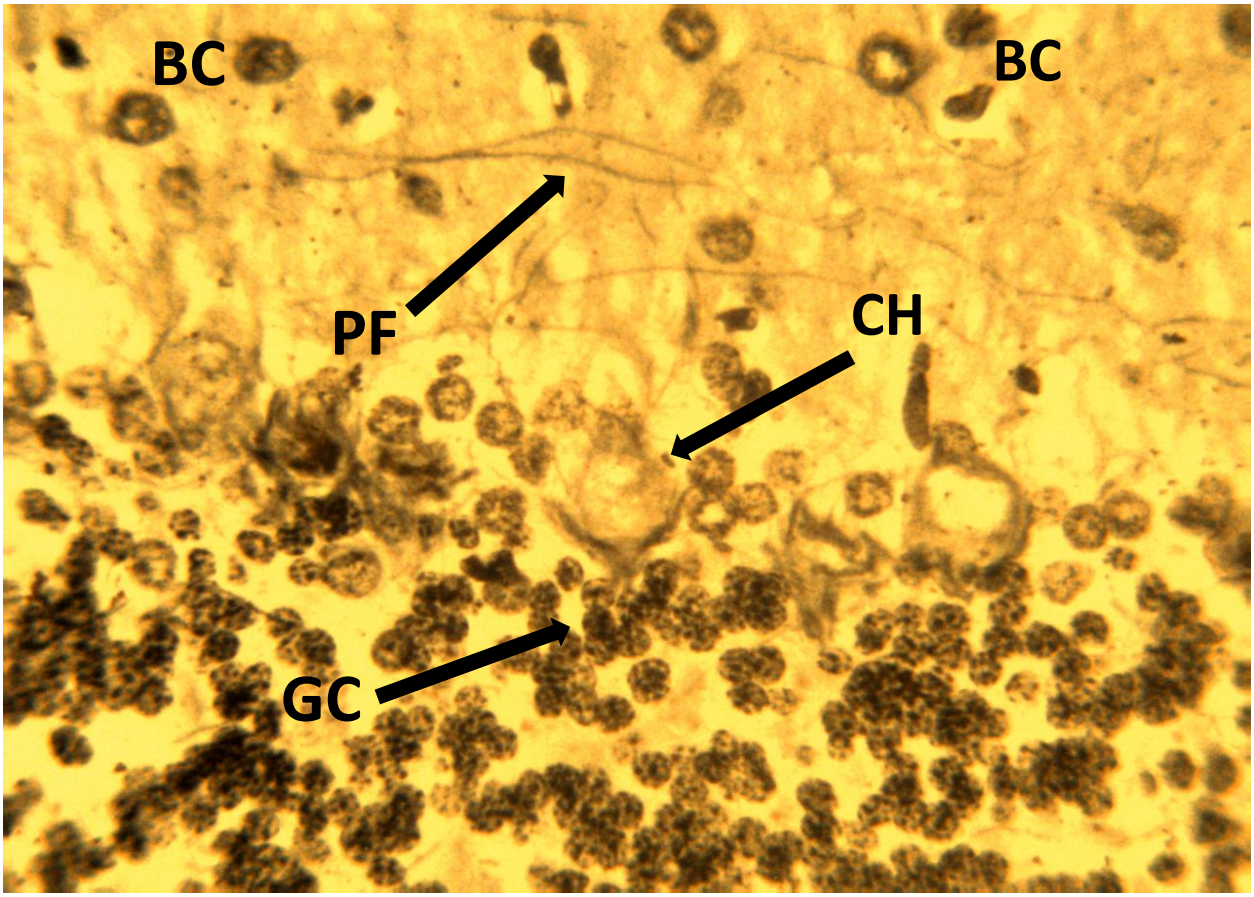


Plate XXVI: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 500 mg/kg EEPD showing chromatolysis (CH) of the Purkinje cells, eroded Purkinje cell dendrites, basket cells (BC) with their parallel fibres (PF), and the granule cells (GC). Bielschowsky (×400)

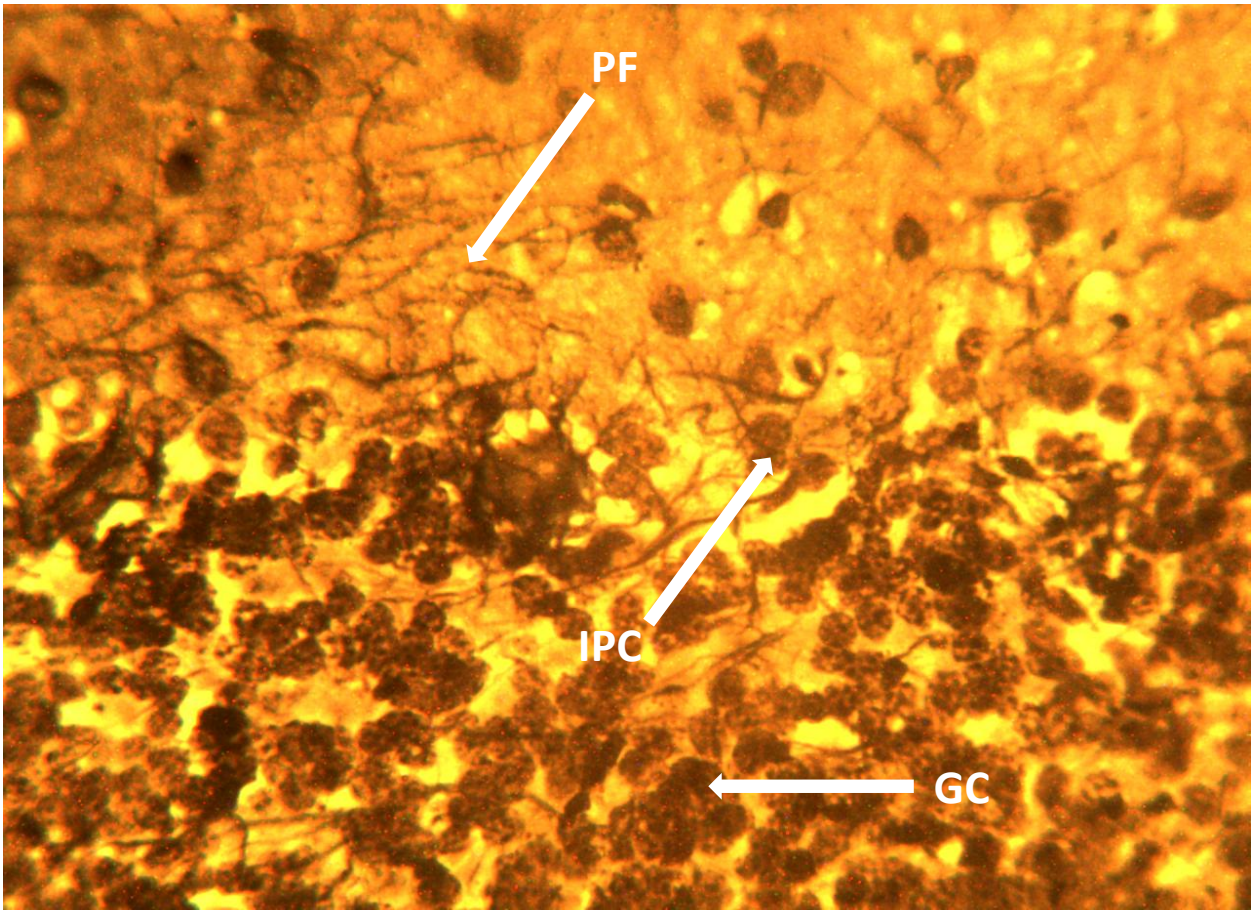


Plate XXVI1: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg EEPD showing indistinct Purkinje cells (IPC), prominent Purkinje cell dendrites, parallel fibres (PF) of basket cells, a normal distribution of granule cells (GC). Bielschowsky (×400)

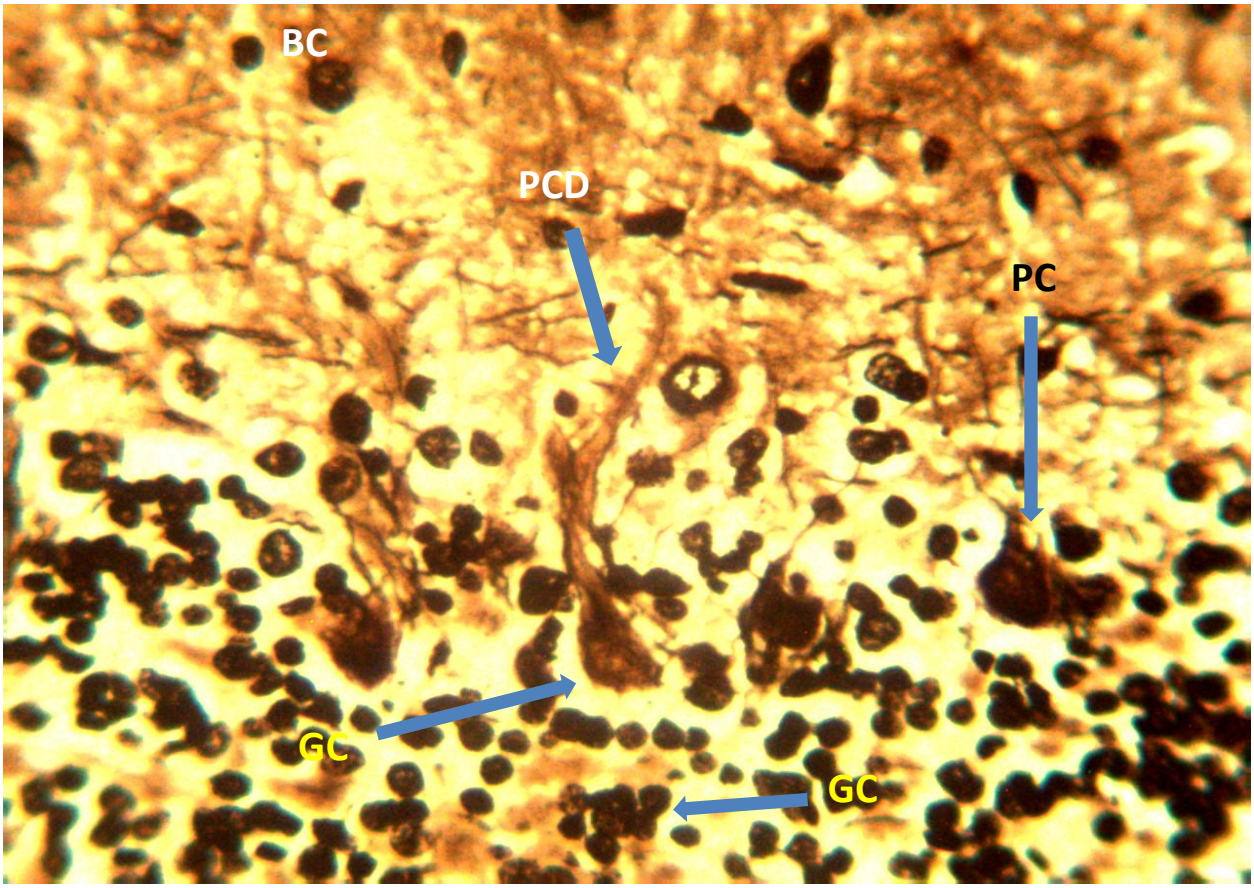


Plate XXVIII: A longitudinal section of the cerebellum of rats with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg EEPD showing distinct Purkinje cells (PC), their dendrites (PCD), basket cells (BC) and granule cells (GC). Bielschowsky (×400)

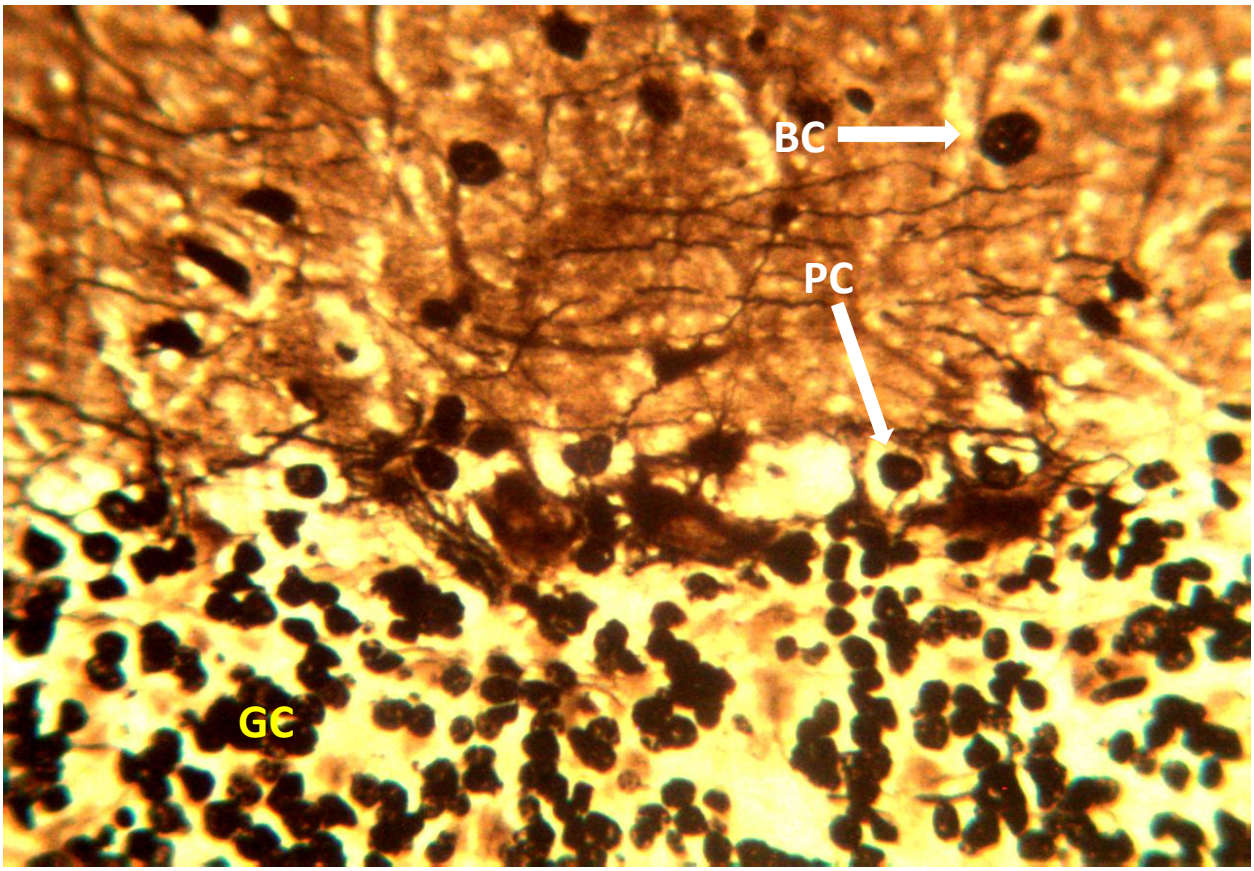


Plate XXIX: A longitudinal section of the cerebellar cortex of the rats treated with 2.86/8.75 mg/kg AS/AQ + 100 mg/kg ascorbic acid, showing a normal histoarchitecture of Purkinje cells (PC), the network of their fibres with those of basket cells (BC), and granule cells (GC). Bielschowsky (×400)

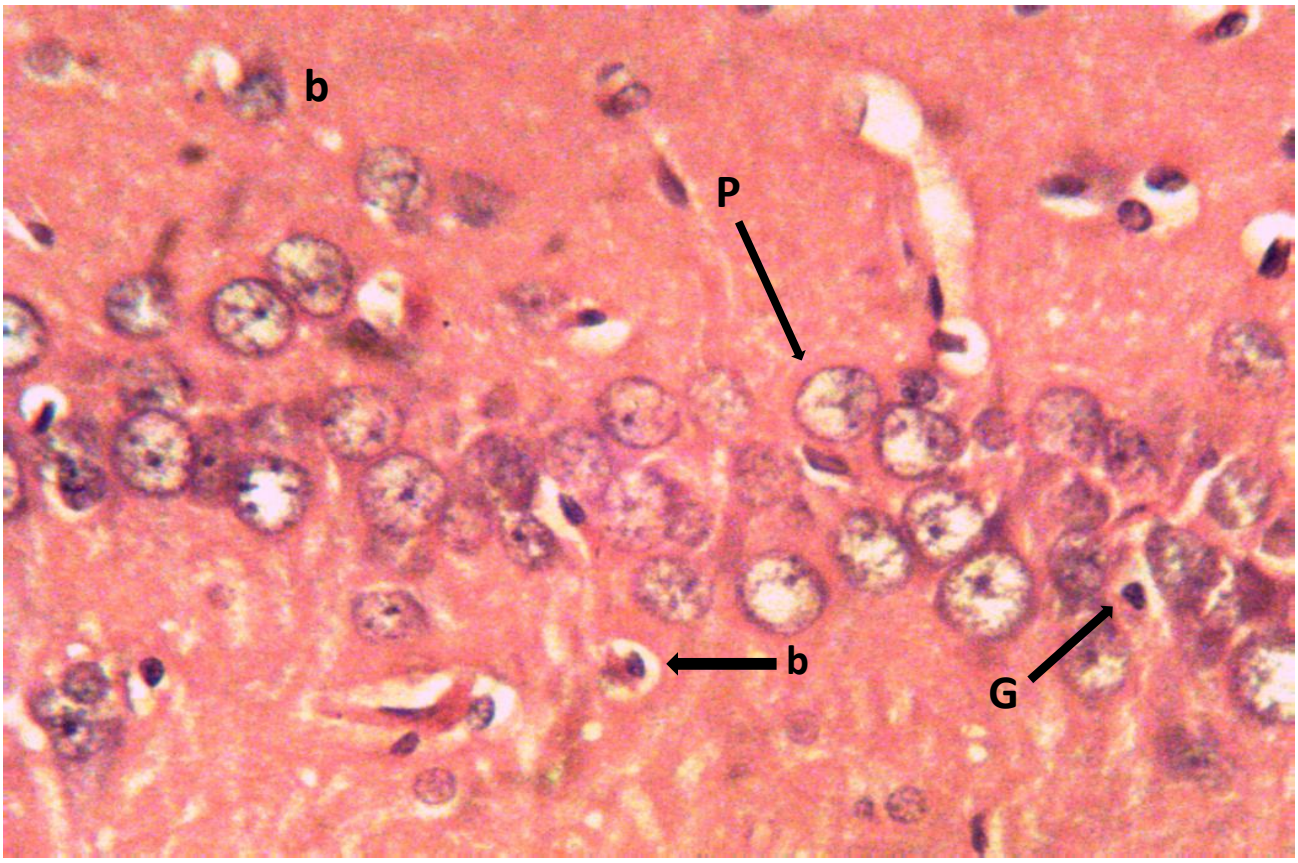


Plate XXX: A longitudinal section of CA3 region of the hippocampus of the control group showing normal arrangement and distribution of Pyramidal cells (P), interspersed with granule cells (G) and basket cells (b). H and E (×400)

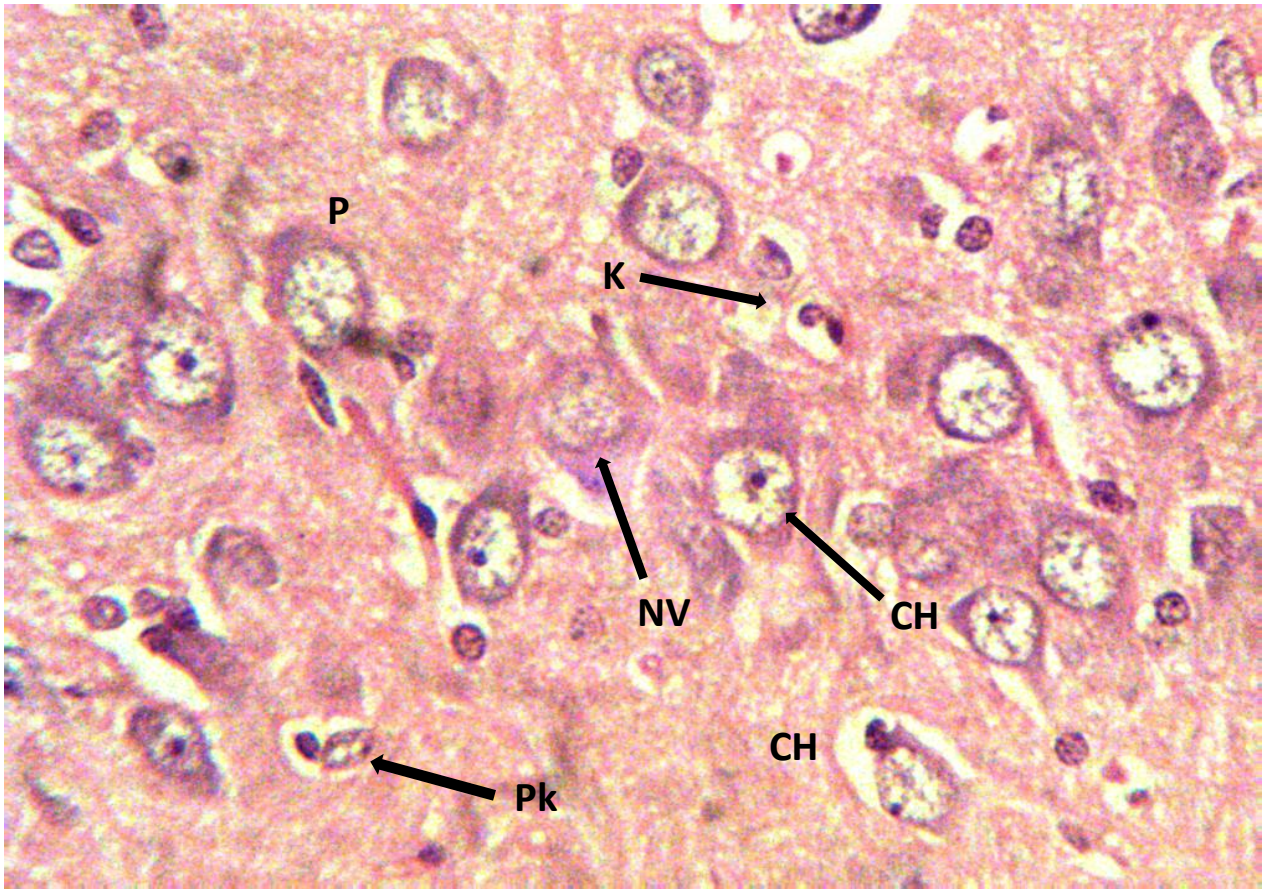


Plate XXXI: A longitudinal section of the CA3 region of the hippocampus of 2.86/8.75 mg/kg AS-AQ treated Wistar rats showing distortions of the compact arrangement of the pyramidal cell layer (P), neuronal vacuolations (NV), chromatolysis (CH), pyknosis (Pk) and karyorrhexis (K). H and E (×400)

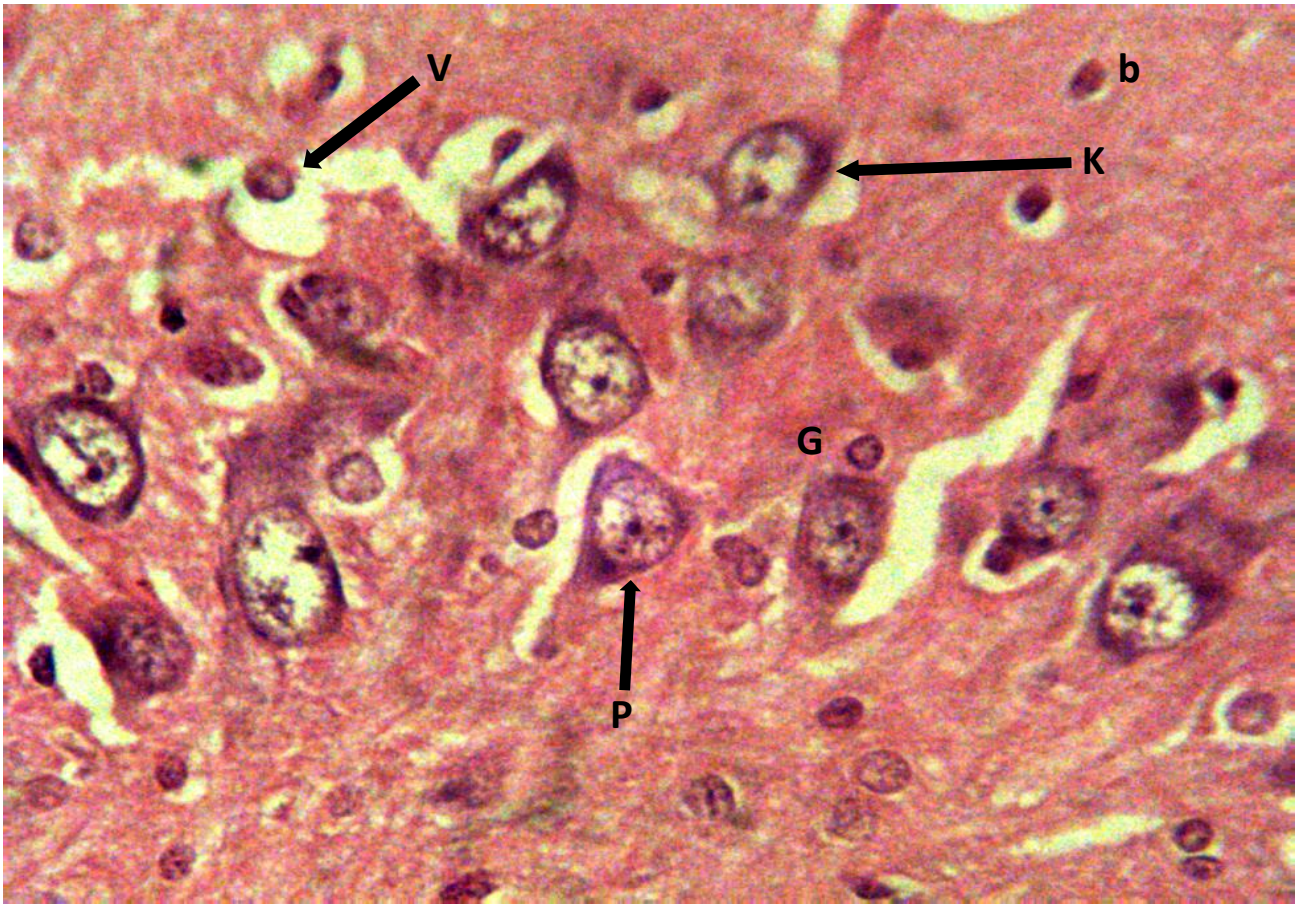


Plate XXXII: A longitudinal section of the CA3 hippocampal region of rats treated with 2.86/8.75 mg/kg AS-AQ + 500 mg/kg AEPD. It shows the evidence of Pyramidal cell vacuolations (V), karyorrhexis (K). Some few basket cells (b) and granule cells (G) are also visible. H and E (×400)

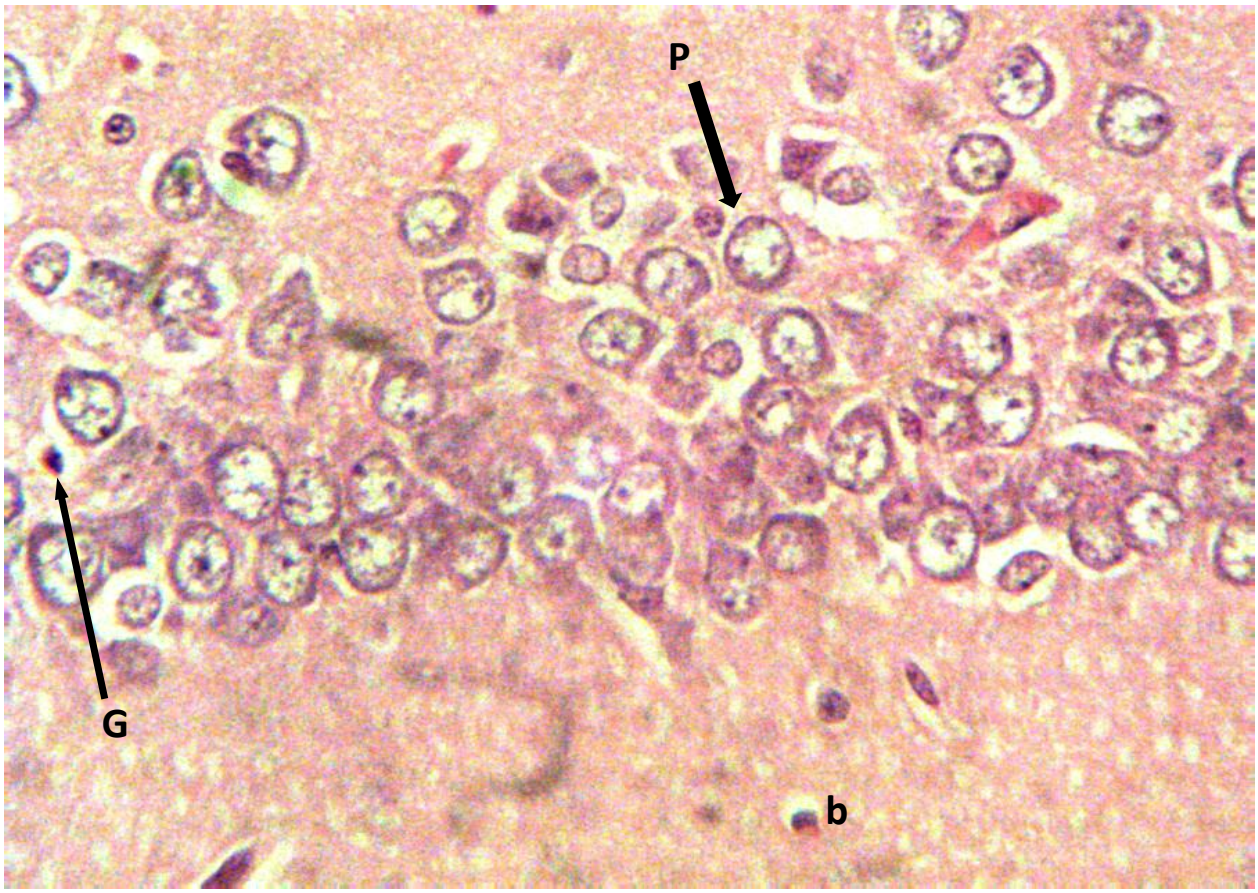


Plate XXXIII: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg AEPD showing the pyramidal cells (P), granule (G) and basket (b) cells. There is a distortion of the pyramidal cell organization. H and E (×400)

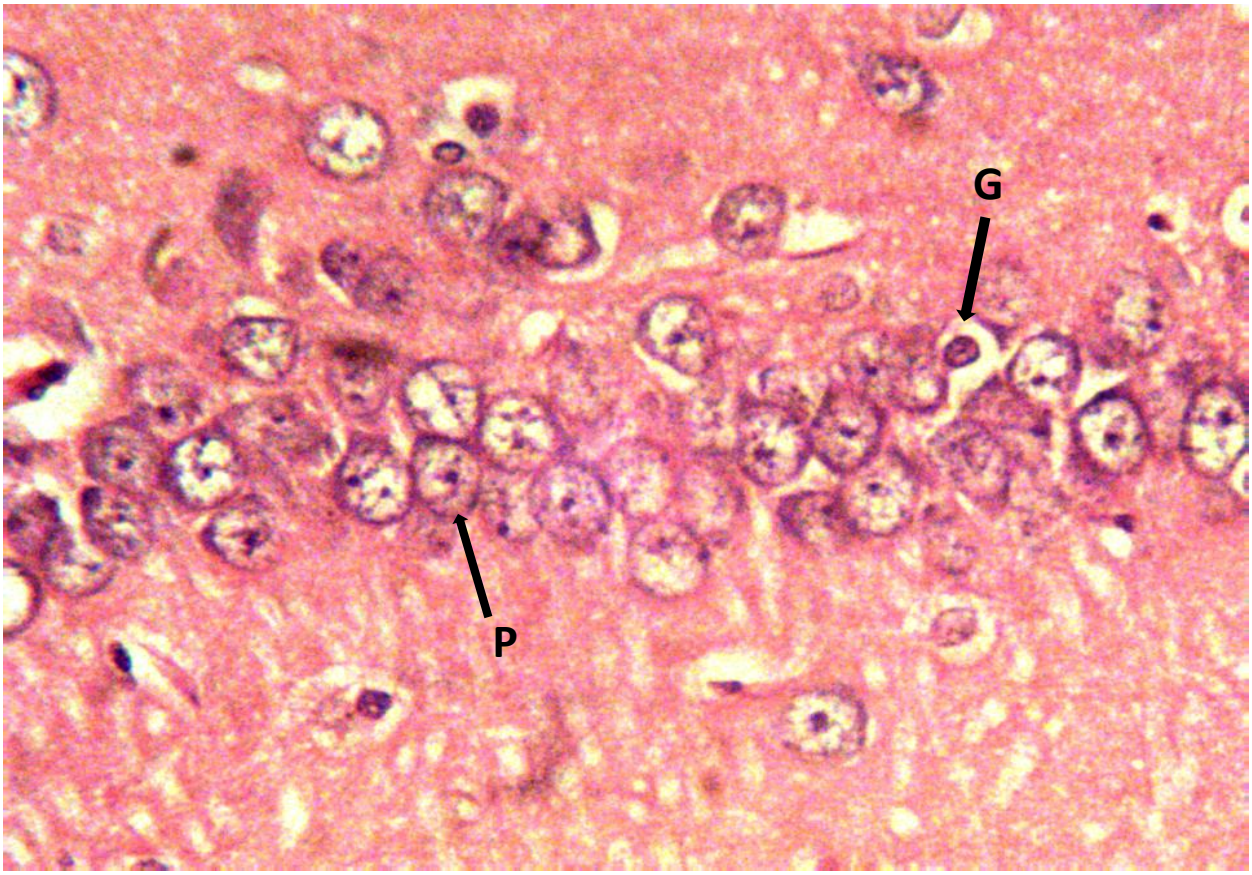


Plate XXXIV: A longitudinal section of the CA3 hippocampal regions of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg AEPD showing a normal histoarchitectural organization of the pyramidal cells (P) interspersed with a few granular (G). H and E (×400)

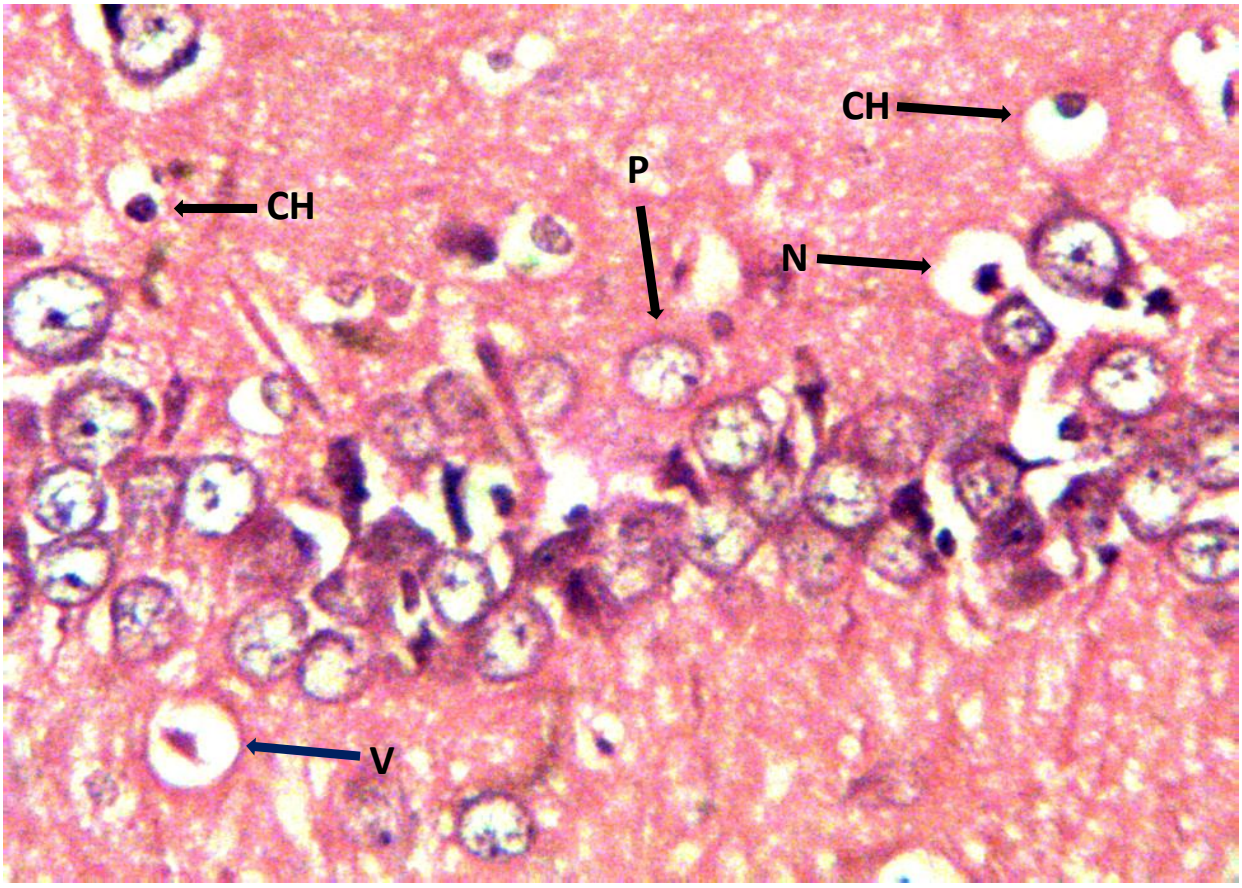


Plate XXXV: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 500 mg/kg EEPD showing a distortion of the pyramidal cell (P) layer organization, chromatolysis (CH), necrosis (N), and vacuolations (V). H and E (×400)

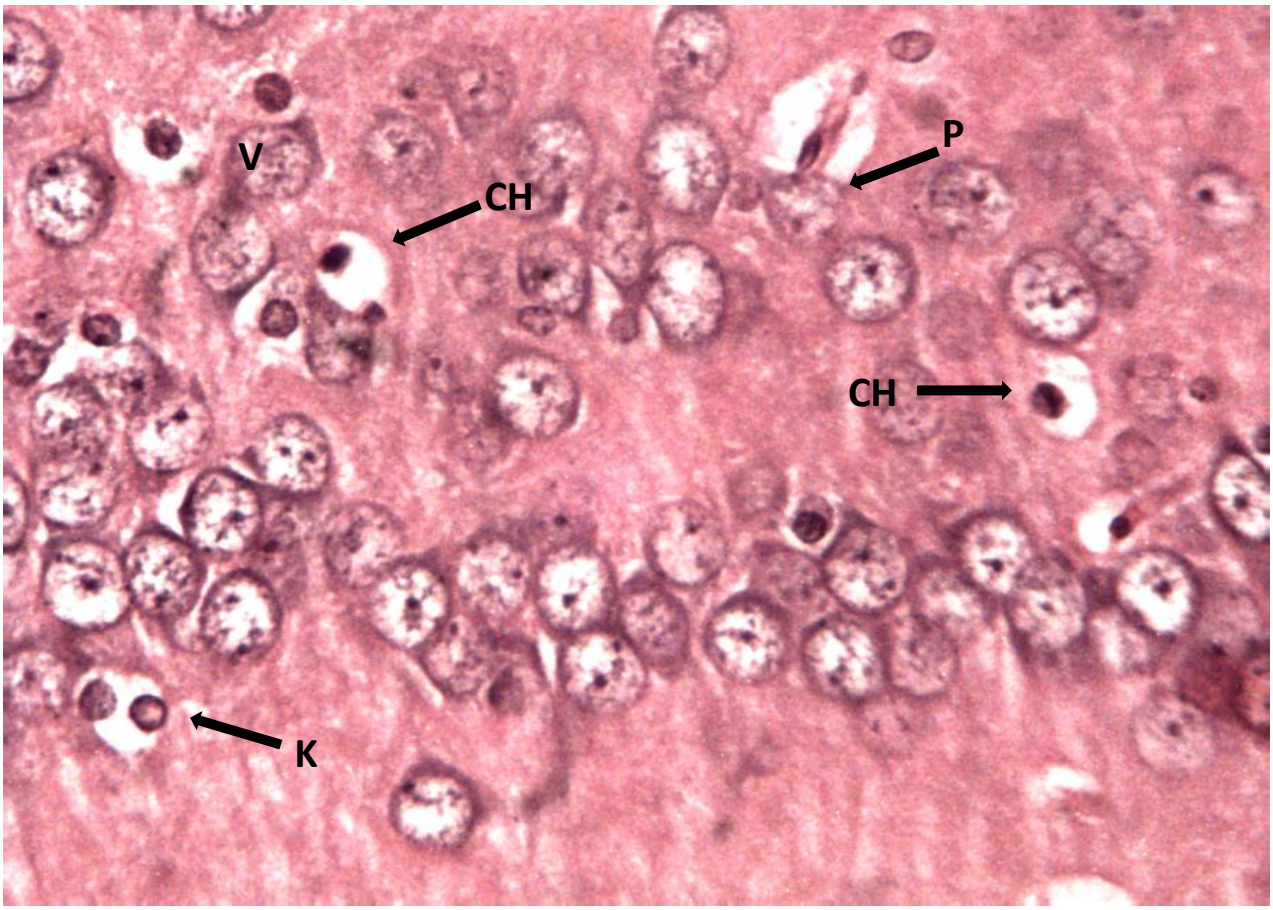


Plate XXXVI: A longitudinal section of the CA3 region of the hippocampus of rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg EEPD showing distortions and degeneration of the pyramidal cells (P) organization with evidence of chromatolysis (CH), karyorrhexis (K). H and E (×400)

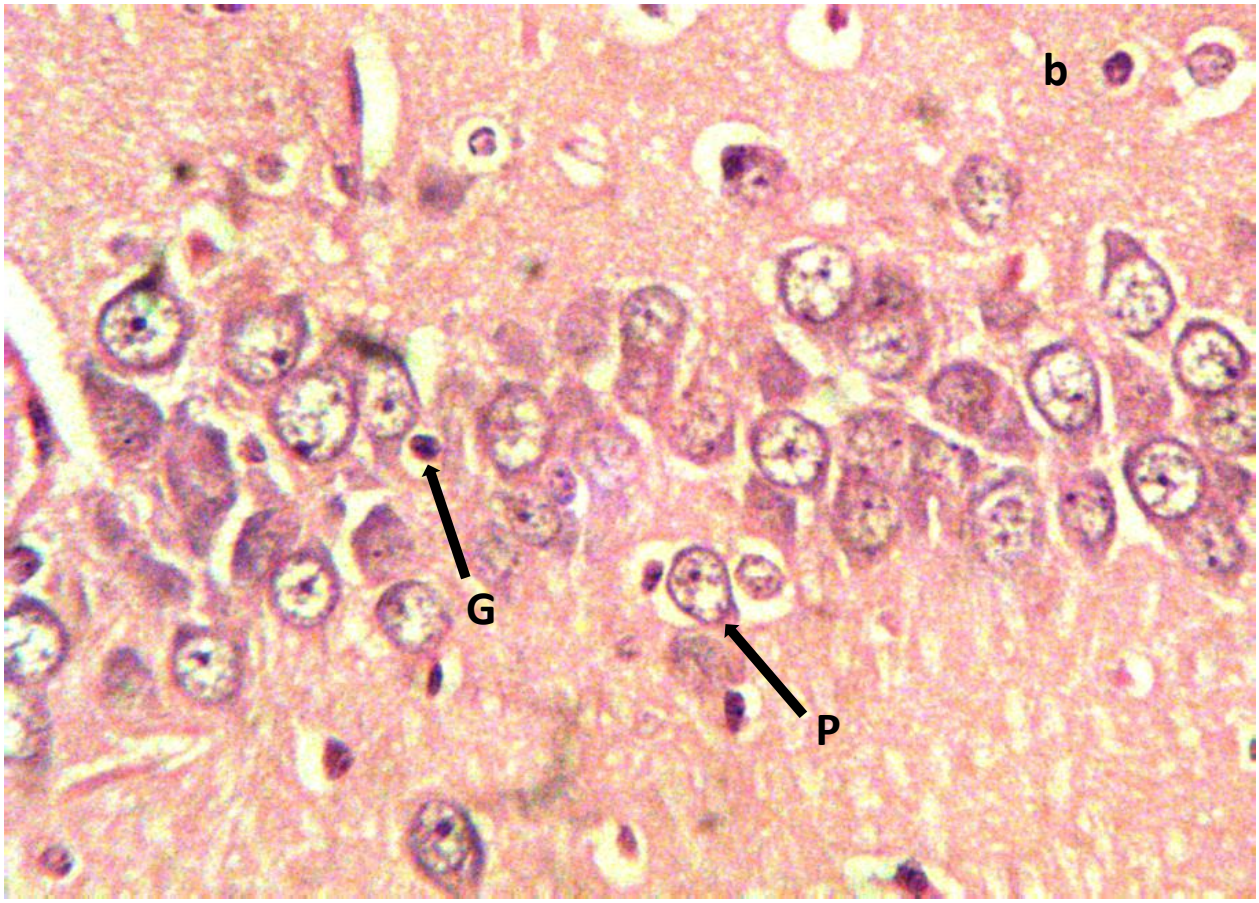


Plate XXXVII: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS-AQ + 1500 mg/kg EEPD showing lost compaction of the pyramidal cells (P) arrangement, interspersed with granule (G) and basket cells (b). There is, however, no visible associated degeneration. H and E (×400)

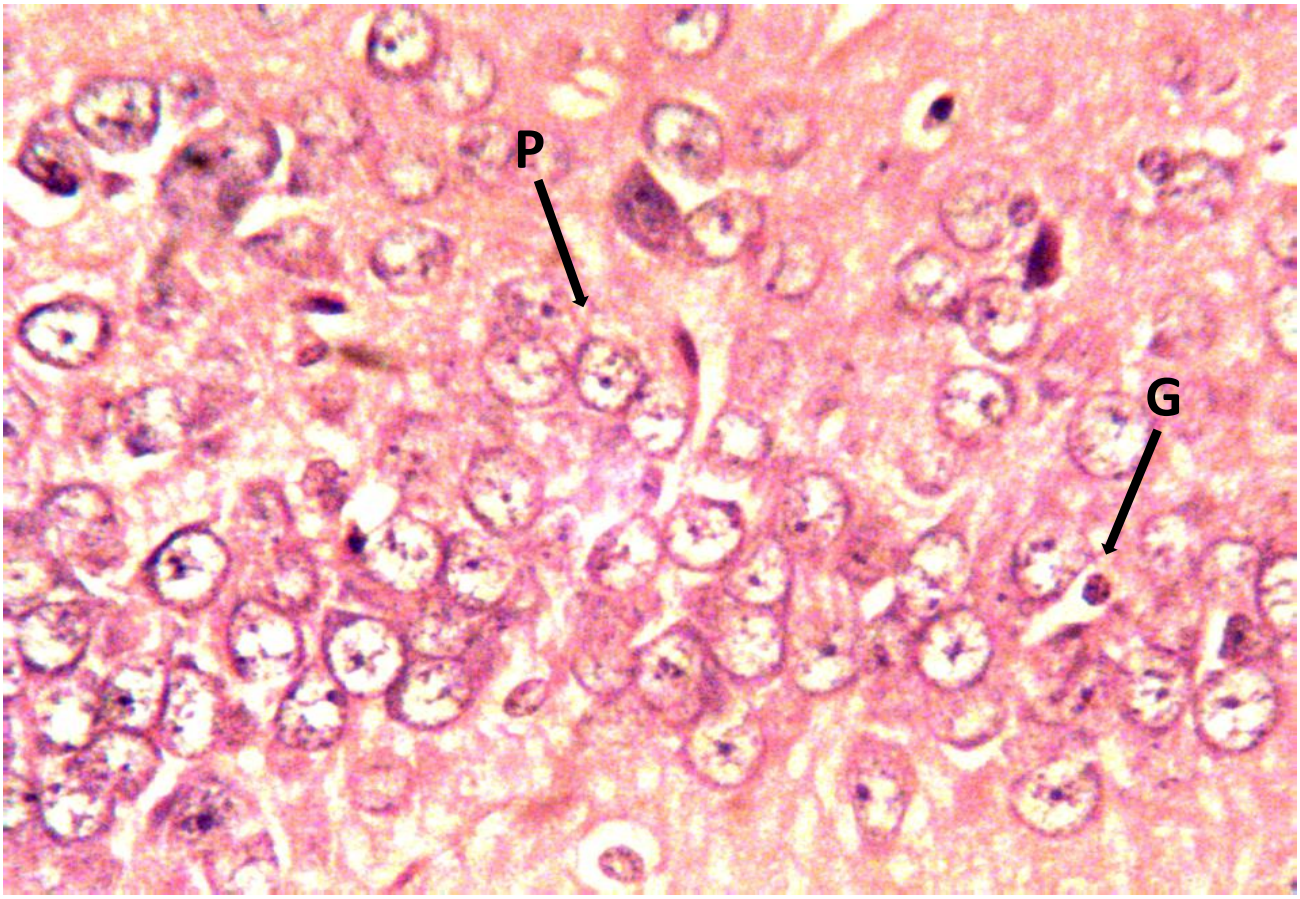


Plate XXXVIII: A longitudinal section of the CA3 hippocampal region of rats treated with 2.86/8.75 mg/kg AS/AQ + 100 mg/kg ascorbic acid (standard drug) showing a normal compact organization of the pyramidal cells (P), interspersed with few granule cells (G). There is no visible evidence of histoarchitectural insults/degeneration. H and E (×400)

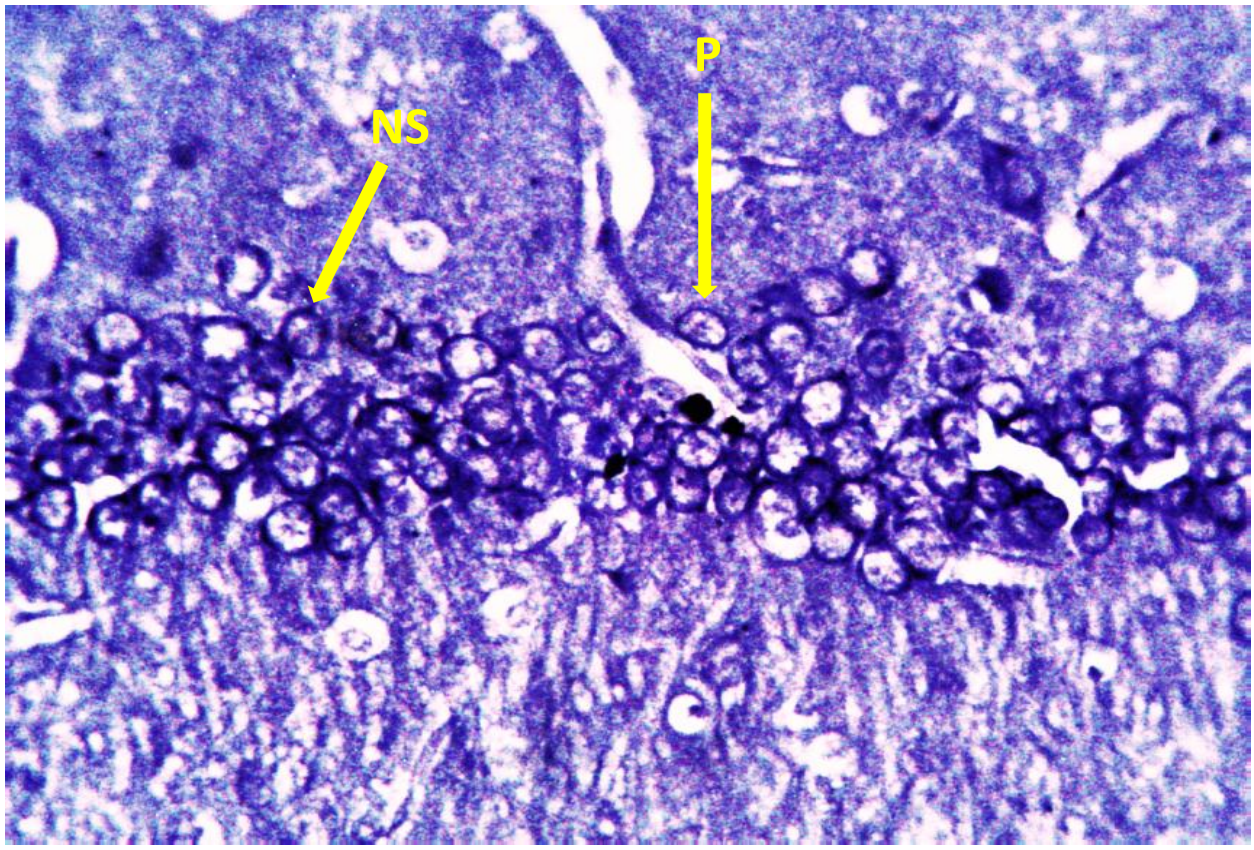


Plate XXXIX: A longitudinal section of the CA3 region of the control group showing a normal arrangement of, and distribution of Nissl substance (NS) in the pyramidal cells (P). CFV (×250)

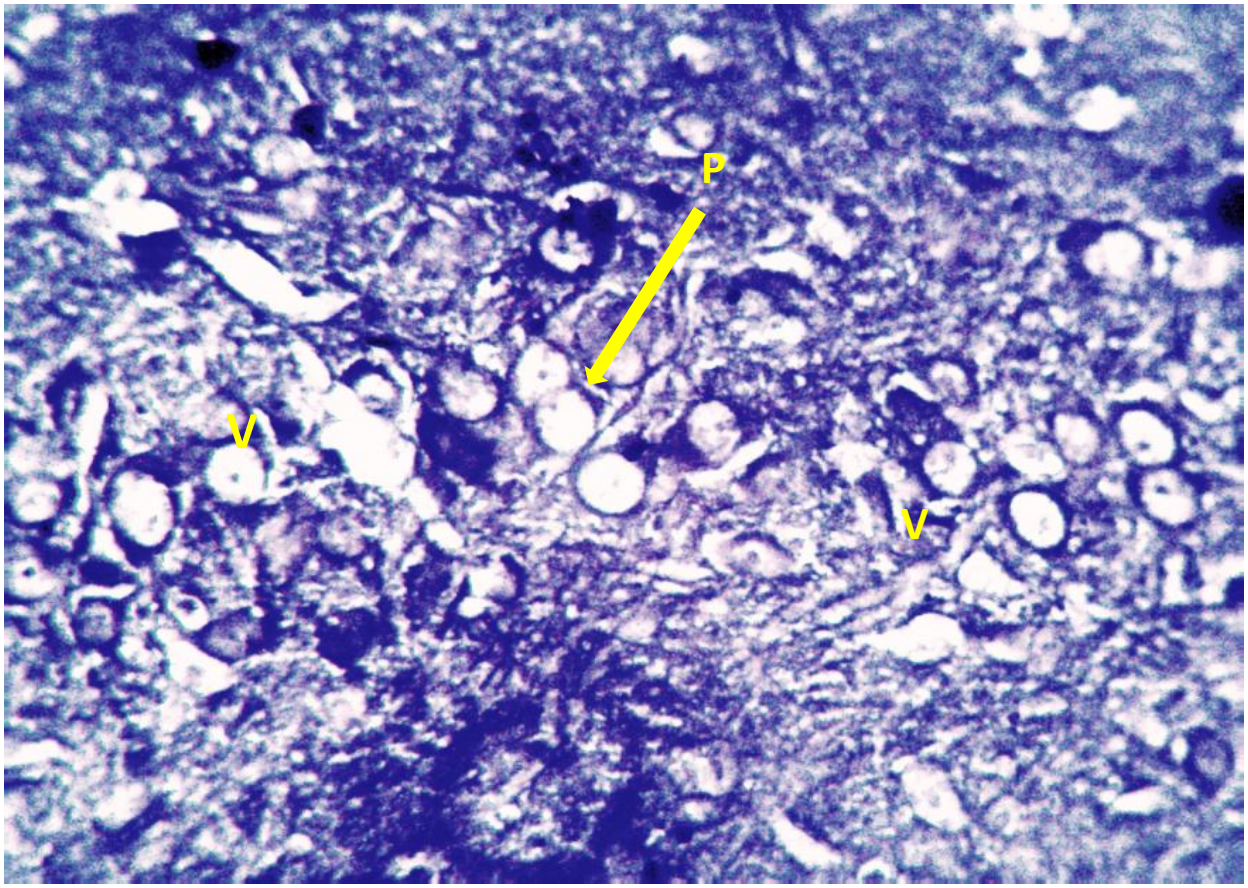


Plate XL: A longitudinal section of the CA3 region of the hippocampus of 2.86/8.75 mg/kg AS-AQ treated group revealing no staining of Nissl substance, distortion of the pyramidal cell (P) layer and cellular vacuolations (V). CFV (×250)

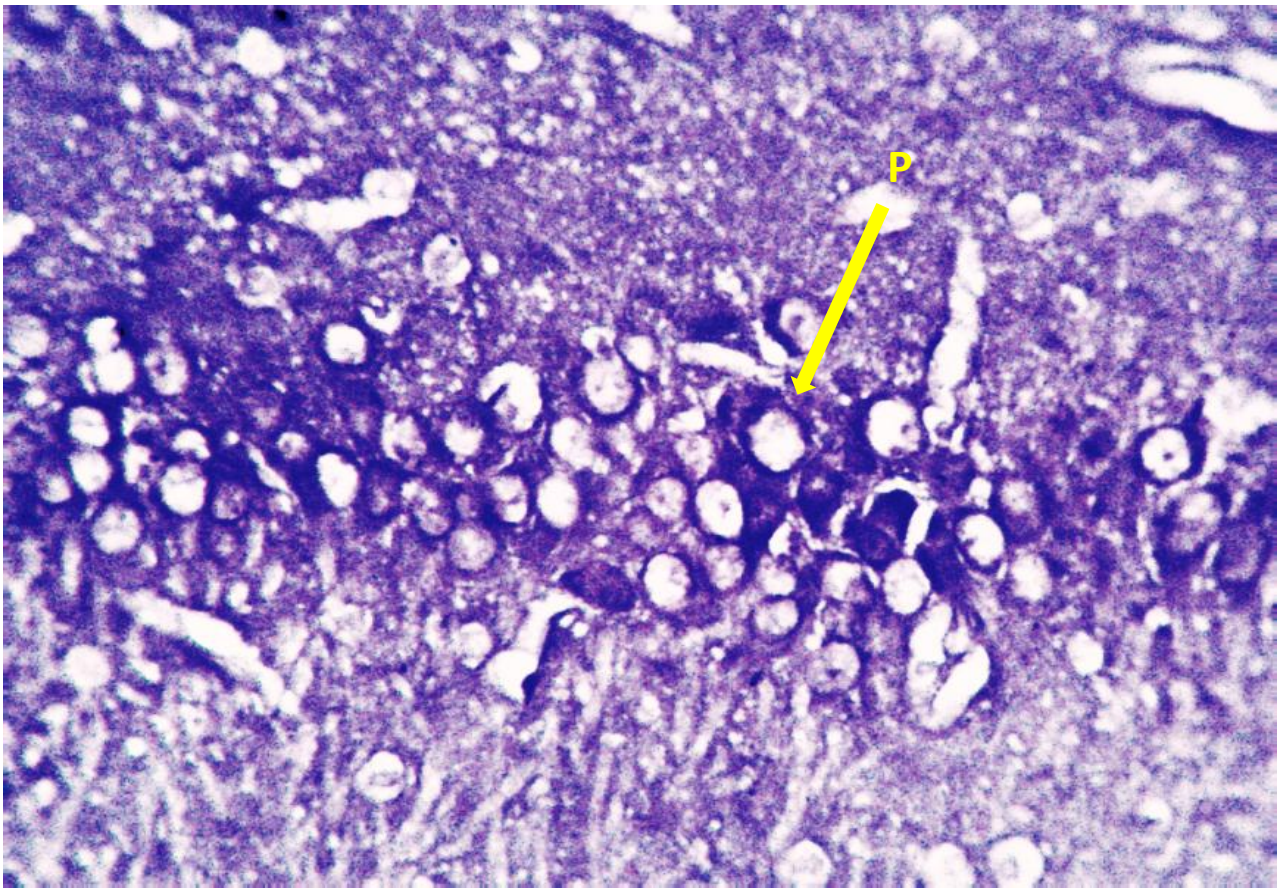


Plate XLI: A longitudinal section of the CA3 hippocampal region of rats treated with 2.86/8.75 mg/kg AS/AQ + 500 mg/kg AEPD, showing a near normal compaction of the pyramidal cells (P) but with disappearance of Nissl substances. CFV (×250)

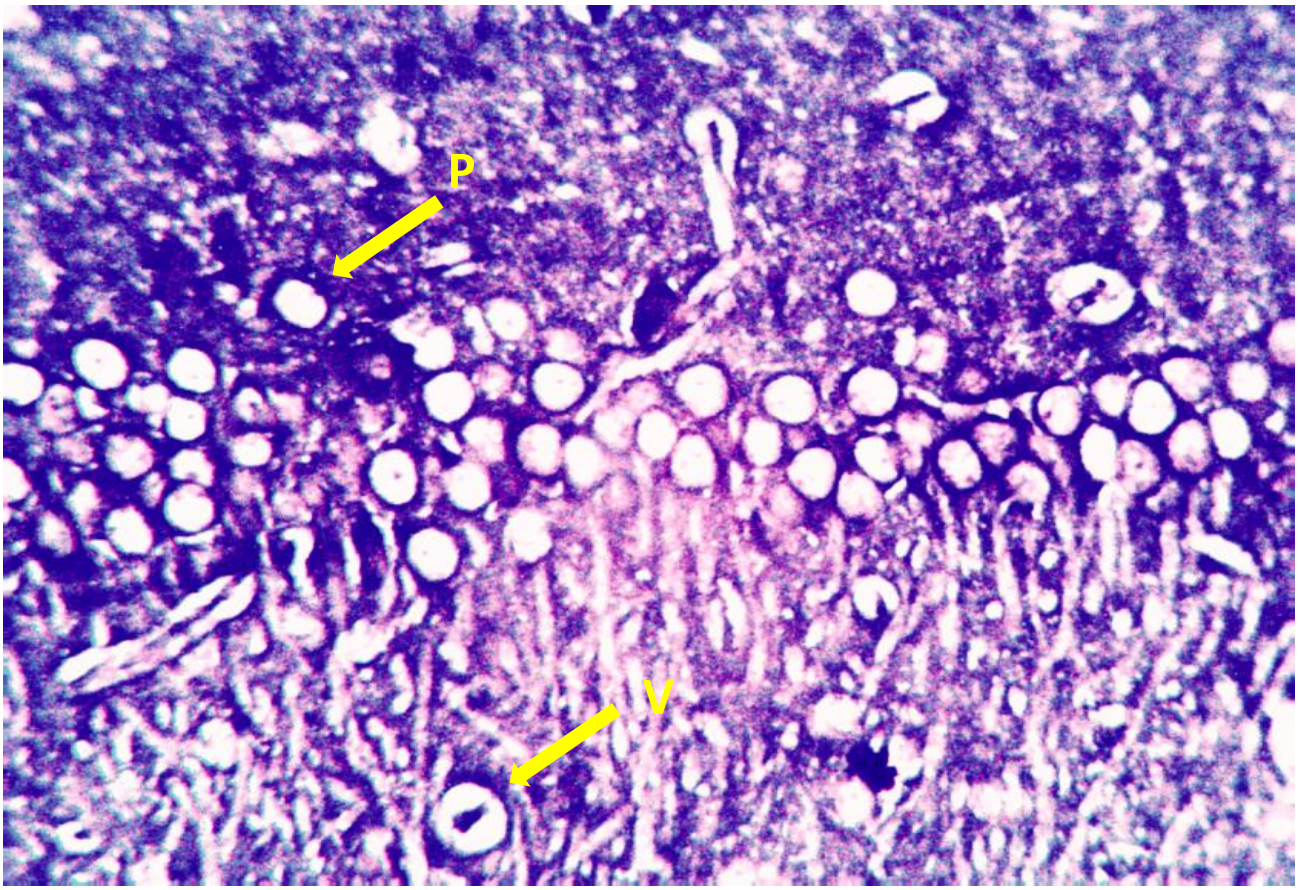


Plate XLII: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg AEPD showing a normal organization of the pyramidal cells (P) but devoid of Nissl substance, and having a few neuropil vacuolations (V). CFV (×250)

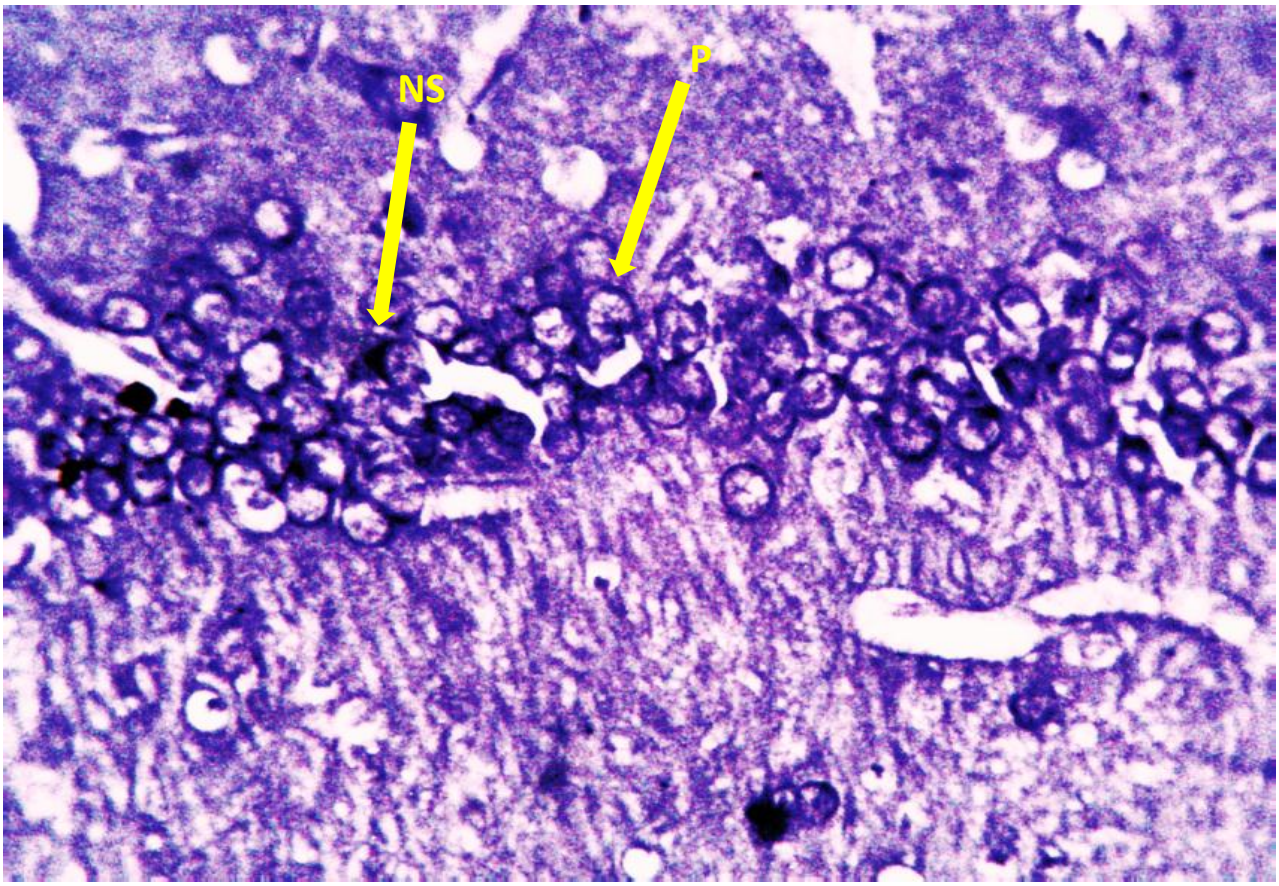


Plate XLII1: A longitudinal section of the CA3 hippocampal regions of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg AEPD showing a normal histoarchitecture of the pyramidal cells aggregates (P) and Nissl substances (NS). CFV (×250)

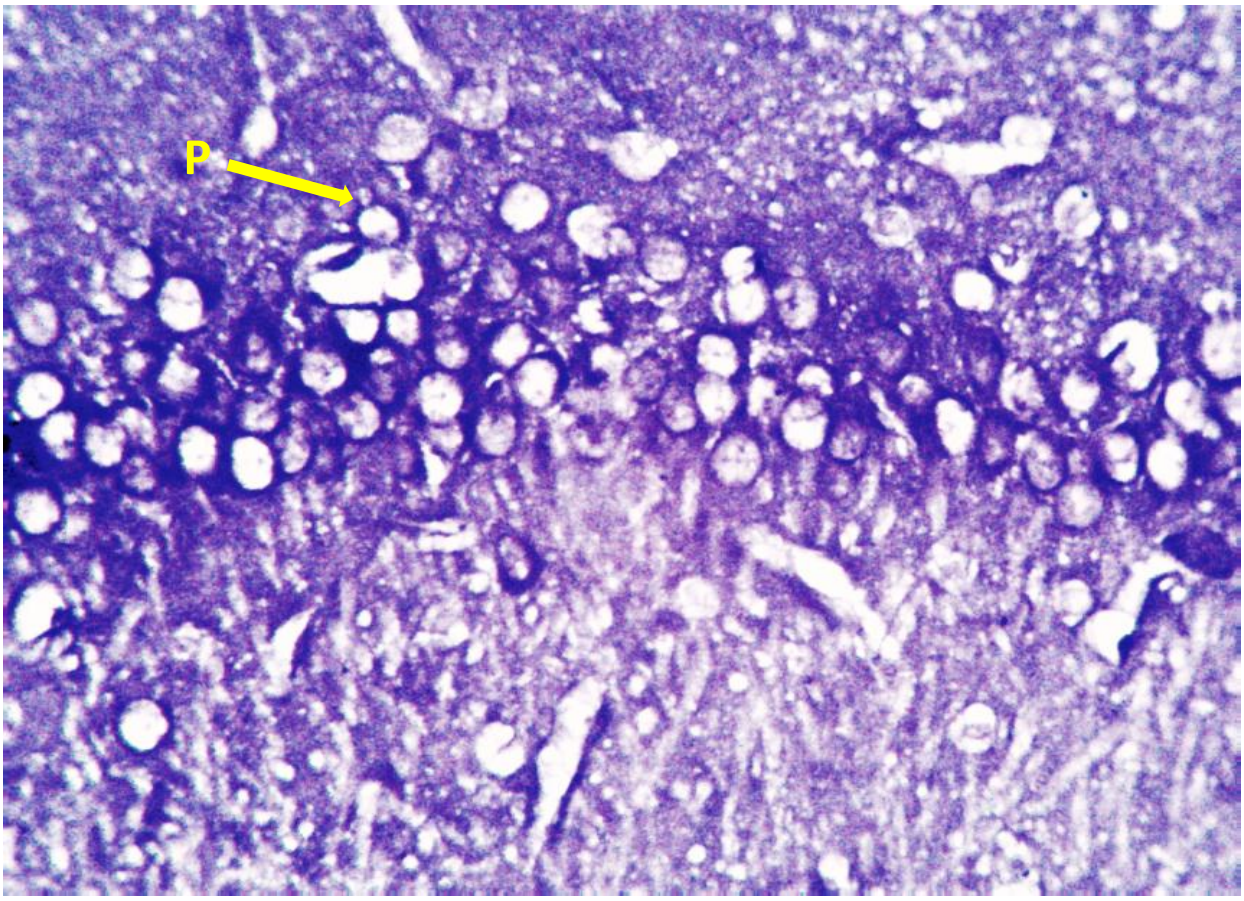


Plate XLIV: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 500 mg/kg EEPD showing a distortion of the pyramidal cell layer (P) with poor staining of the Nissl substance. CFV (×250)

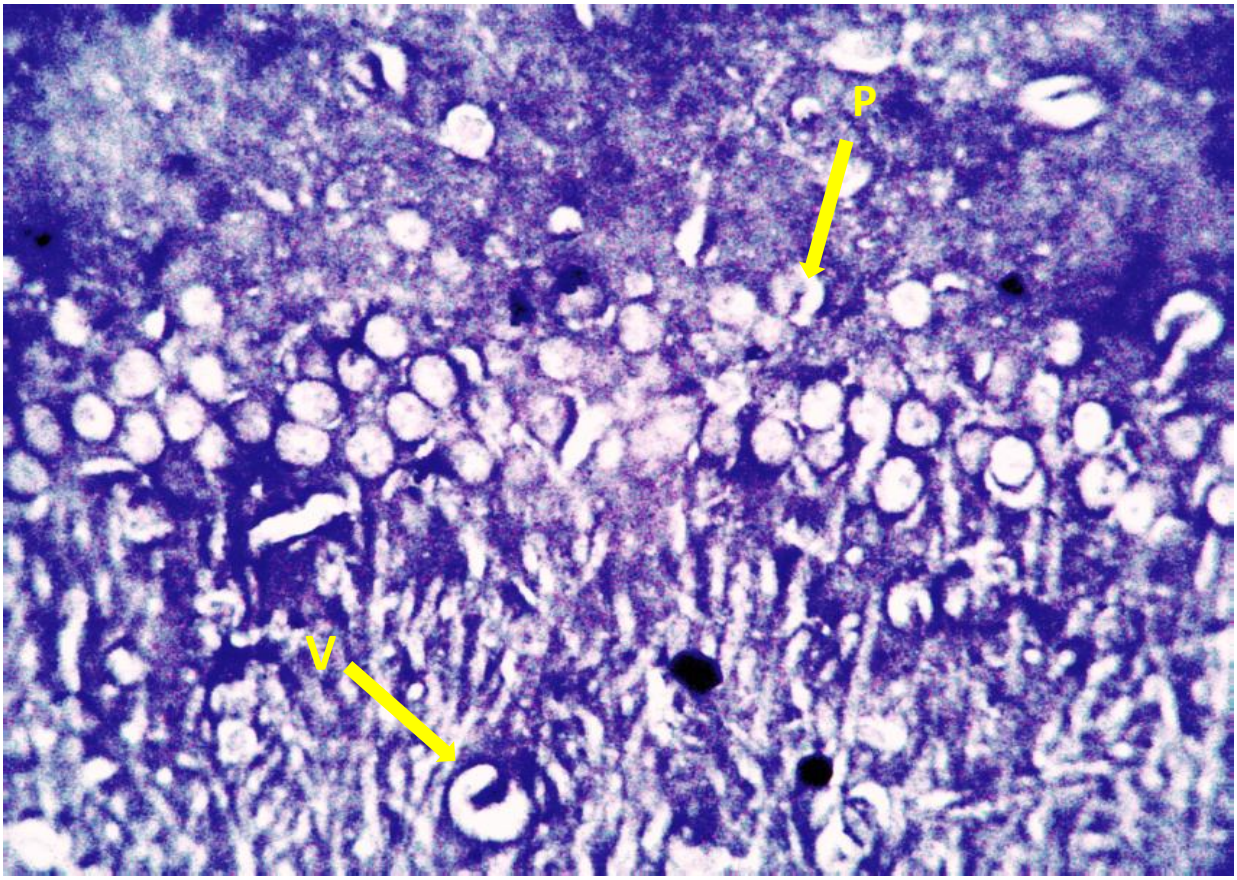


Plate XLV: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg EEPD showing a distortion of the pyramidal cell (P) aggregates and poor staining of the Nissl substance. There is also presence of vacuolations (V) of granule cells. CFV (×250)

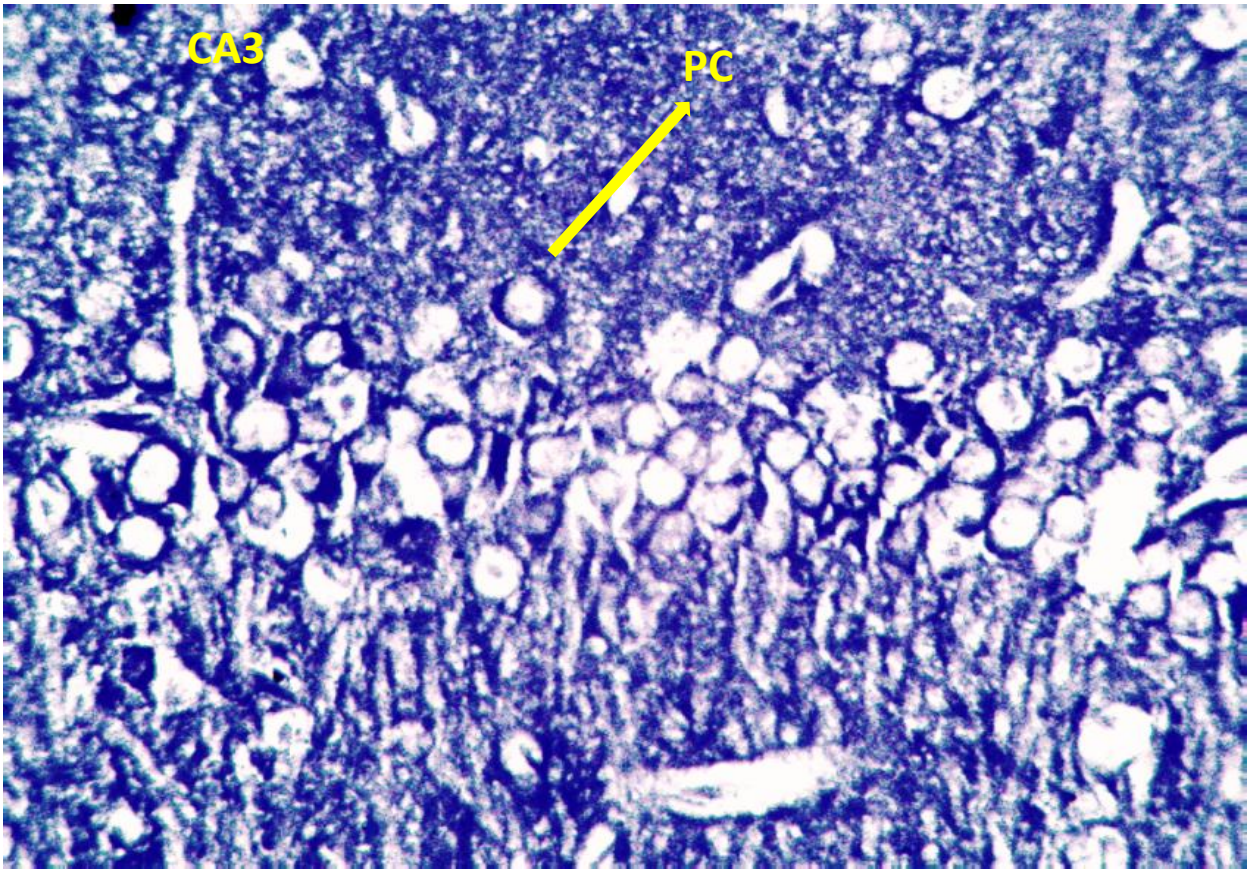


Plate XLVI: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg EEPD showing a near normal compaction of the pyramidal cells (PC) but with very poor staining intensity of the Nissl substance. CFV (×250)

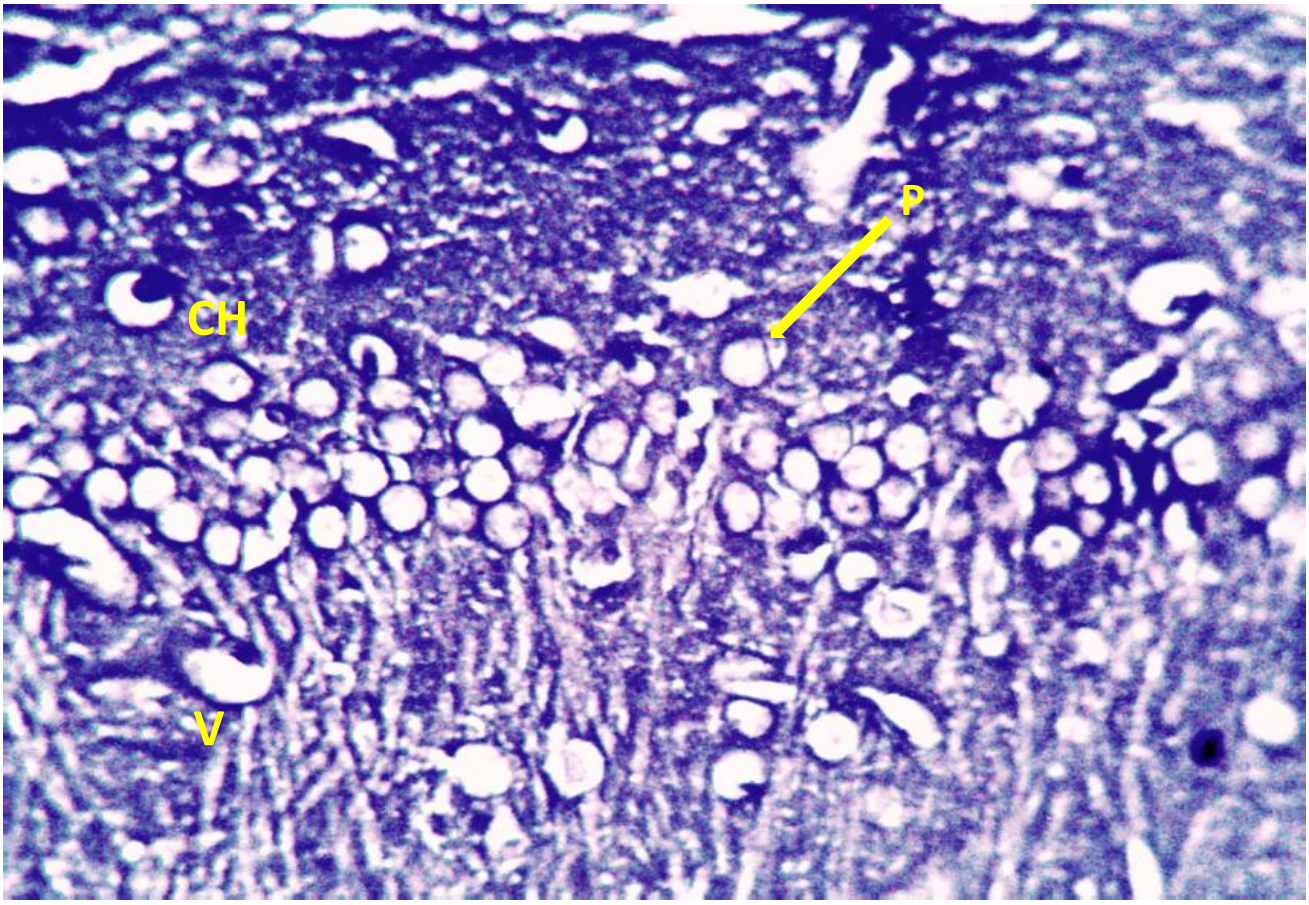


Plate XLVII: A longitudinal section of the CA3 hippocampal regions of rats treated with 2.86/8.75 mg/kg AS/AQ + 100 mg/kg ascorbic acid showing, a normal histoarchitecture of the pyramidal cells (P) with poor staining of the Nissl substance. There is also evidence of vacuolations (V) and chromatolysis (CH). CFV (×250)

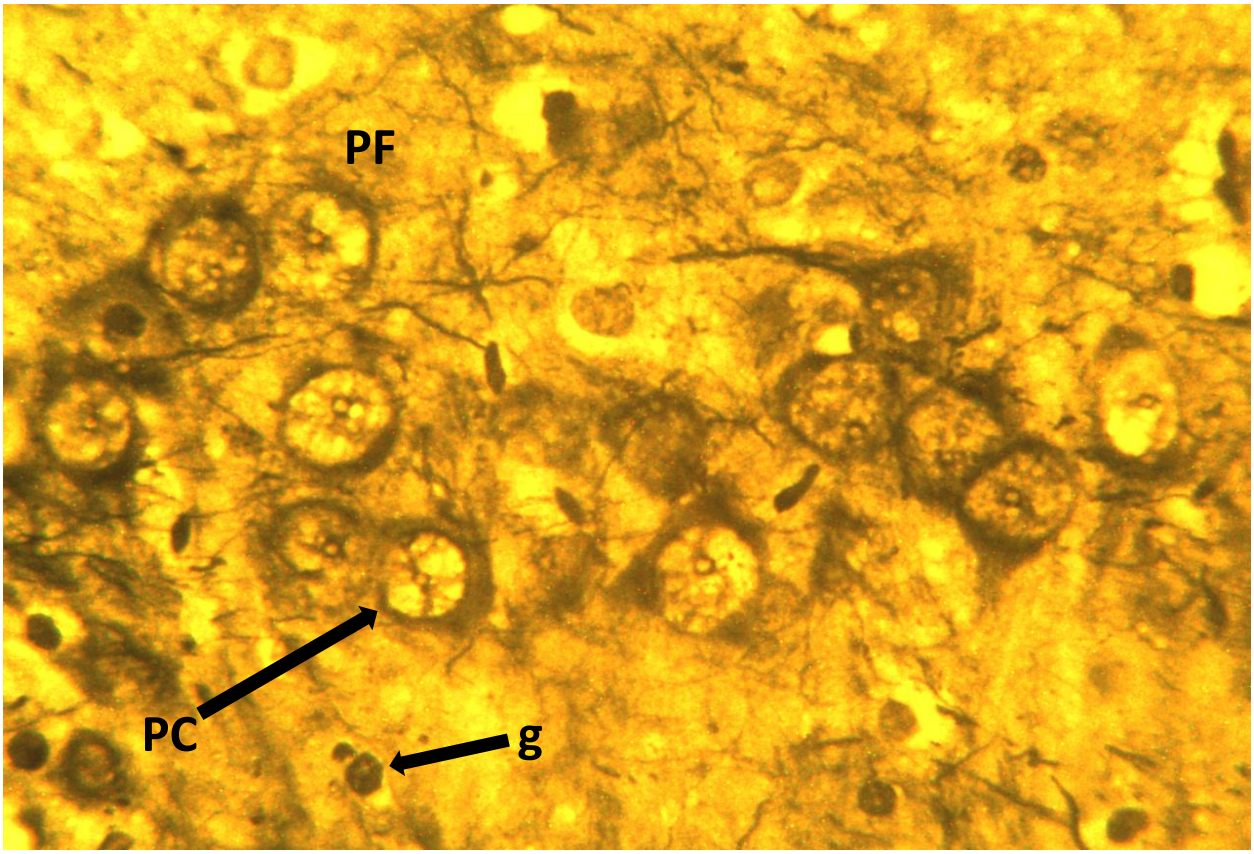


Plate XLVIII: A longitudinal section of the CA3 region of the hippocampus of the control group showing a normal arrangement of, and distribution of pyramidal cells (PC), network of parallel fibres (PF) of stellate cells, and sparsely distributed granule cells (g). Bielschowsky (×400)

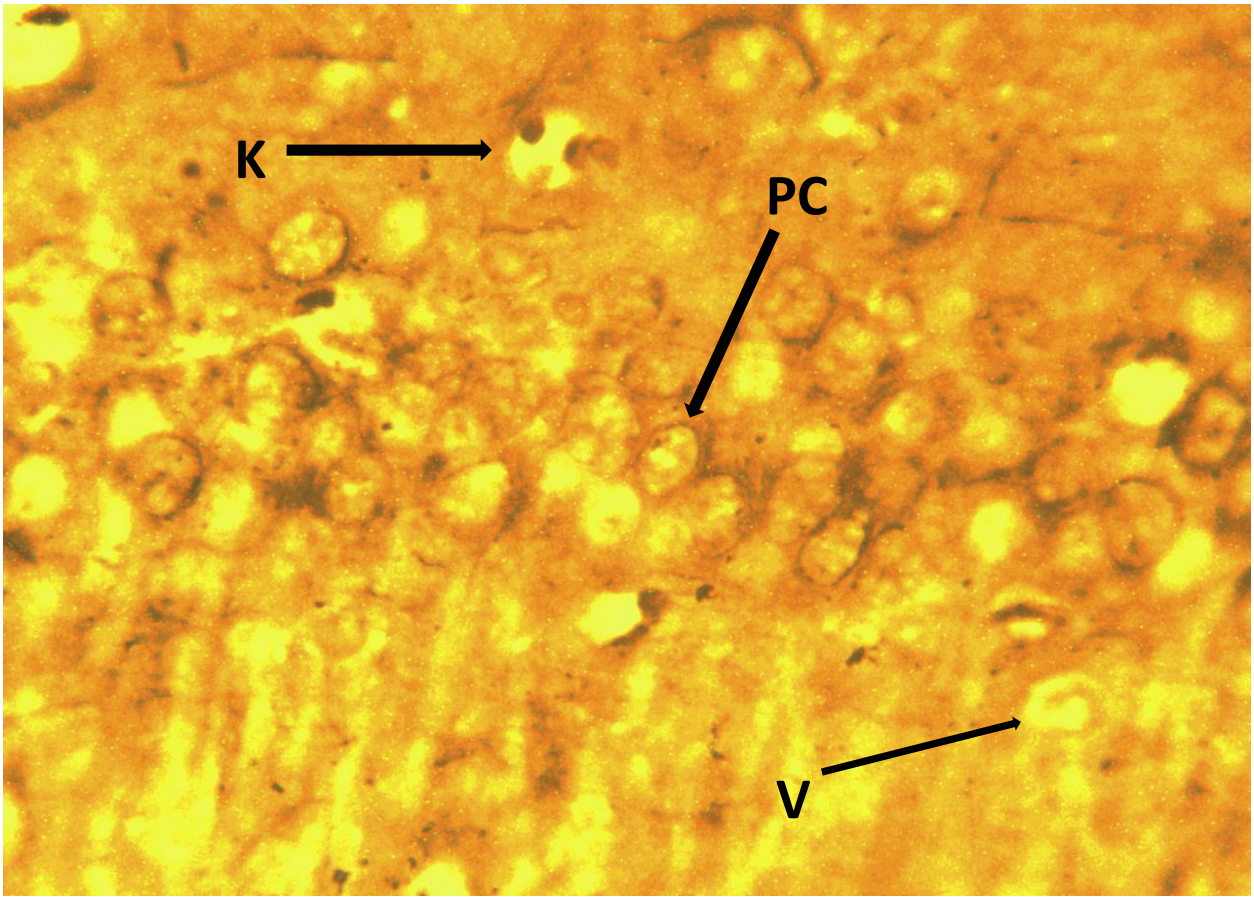


Plate XLIX: A longitudinal section of the CA3 region of the hippocampus of 2.86/8.75 mg/kg AS-AQ treated group revealing karyorrhexis (K) of the pyramidal cell (PC) and cellular vacuolations (V) and disappearance of the neuronal fibre network. Bielschowsky (×400)

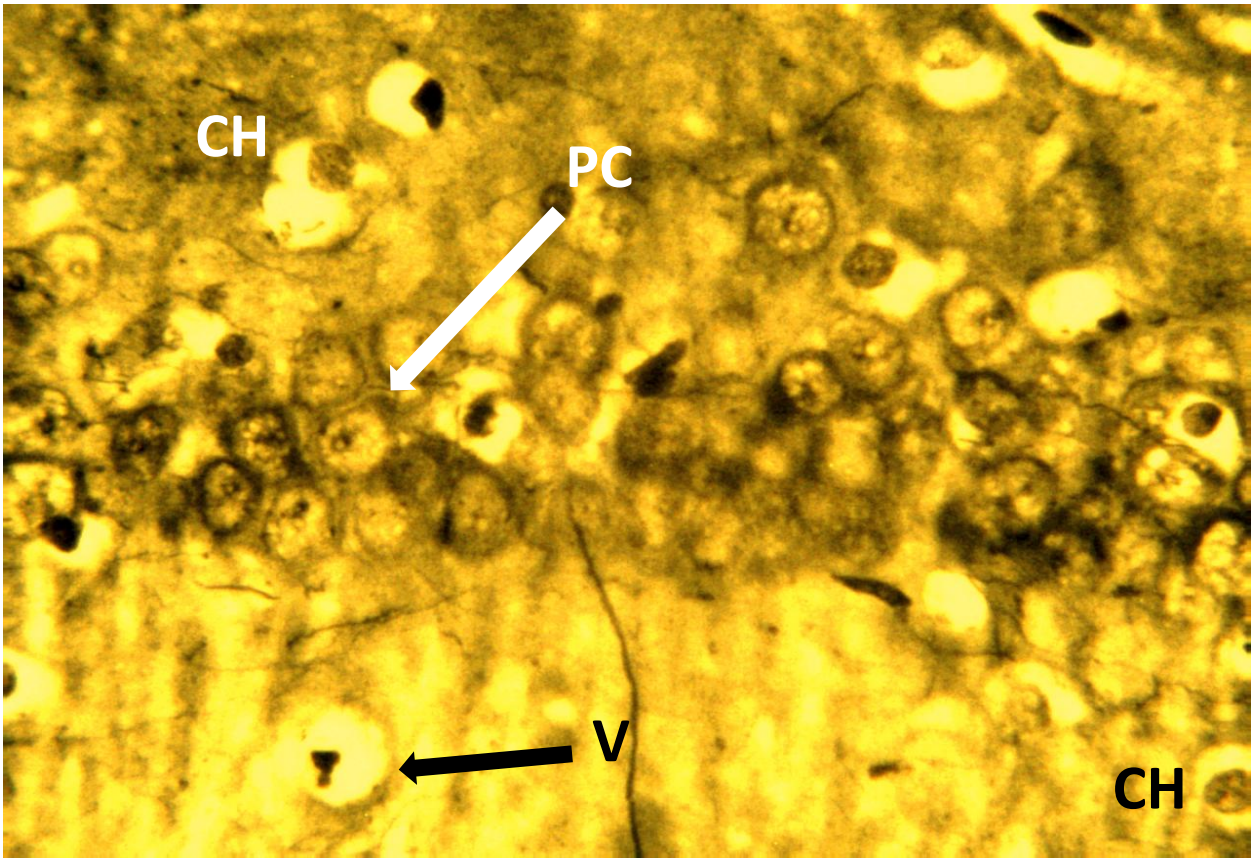


Plate L: A longitudinal section of the CA3 hippocampal region of rats treated with 2.86/8.75 mg/kg AS/AQ + 500 mg/kg AEPD, showing a near normal compaction of the pyramidal cells (PC) but with evident chromatolysis (CH) and vacuolations (V). Bielschowsky (×400)

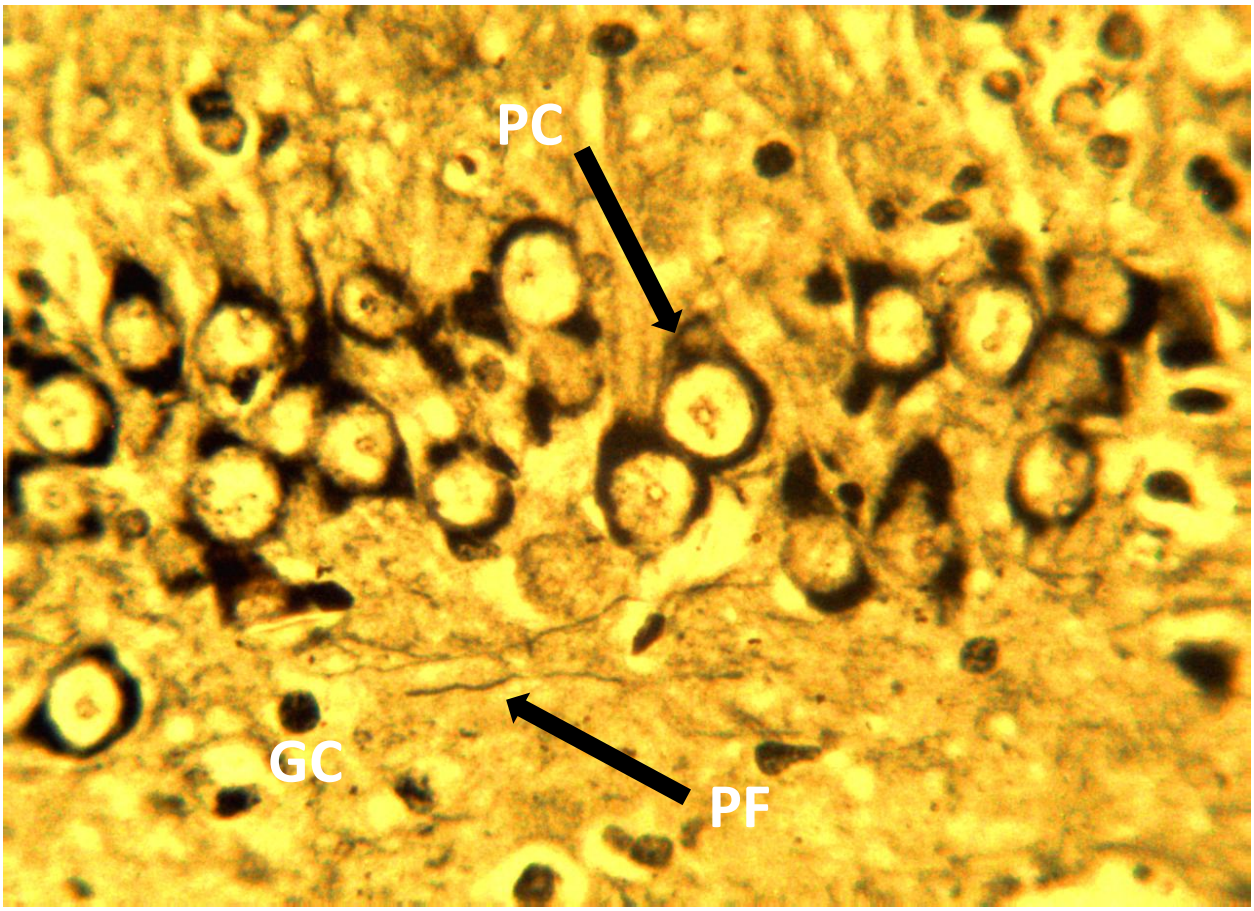


Plate LI: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg AEPD showing a normal organization of the pyramidal cells (PC), parallel fibres, and sparsely distributed granule cells (GC). Bielschowsky (×400)

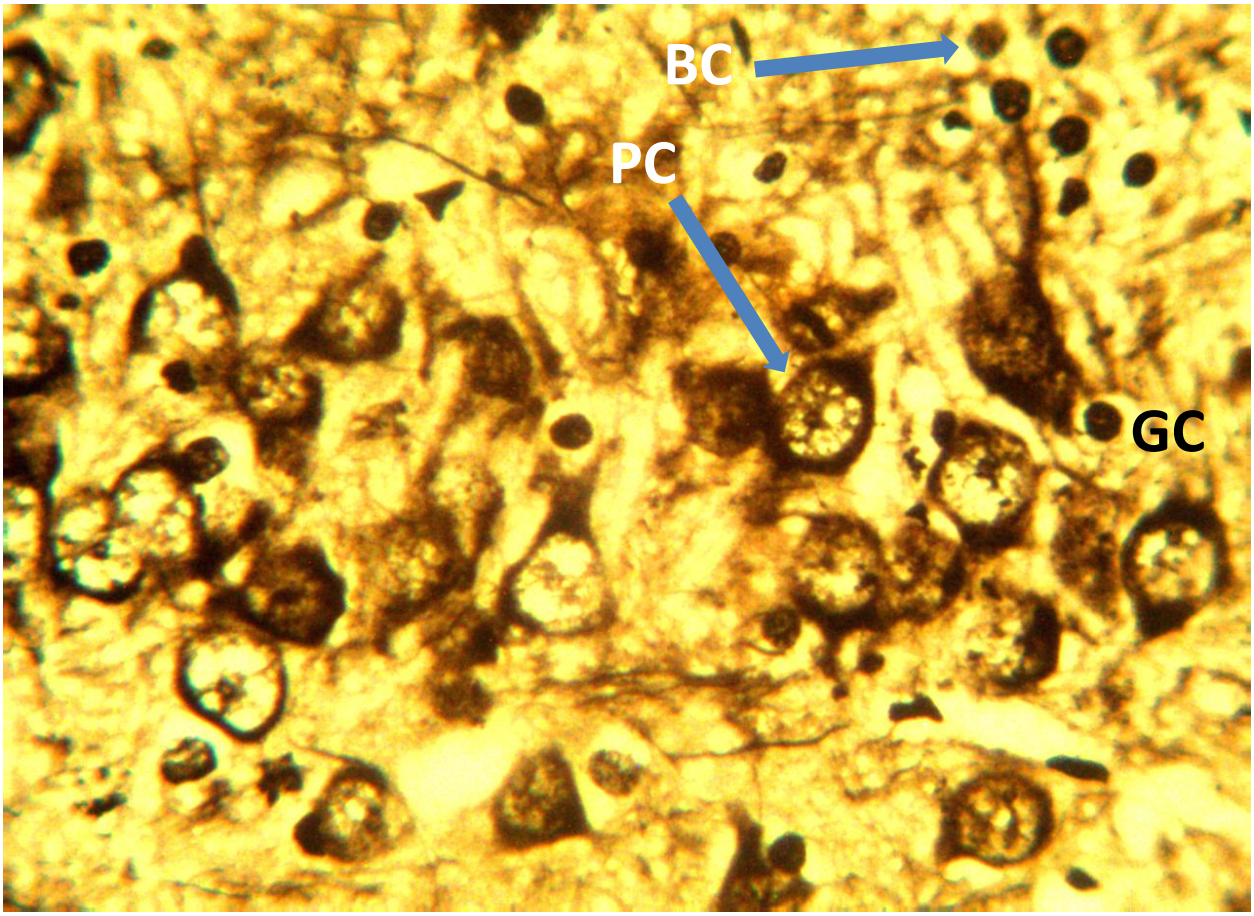


Plate L1I: A longitudinal section of the CA3 hippocampal regions of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg AEPD showing a normal histoarchitecture of the pyramidal cells (PC) interspersed with granule cells (GC) and basket cells (BC) Bielschowsky (×400)

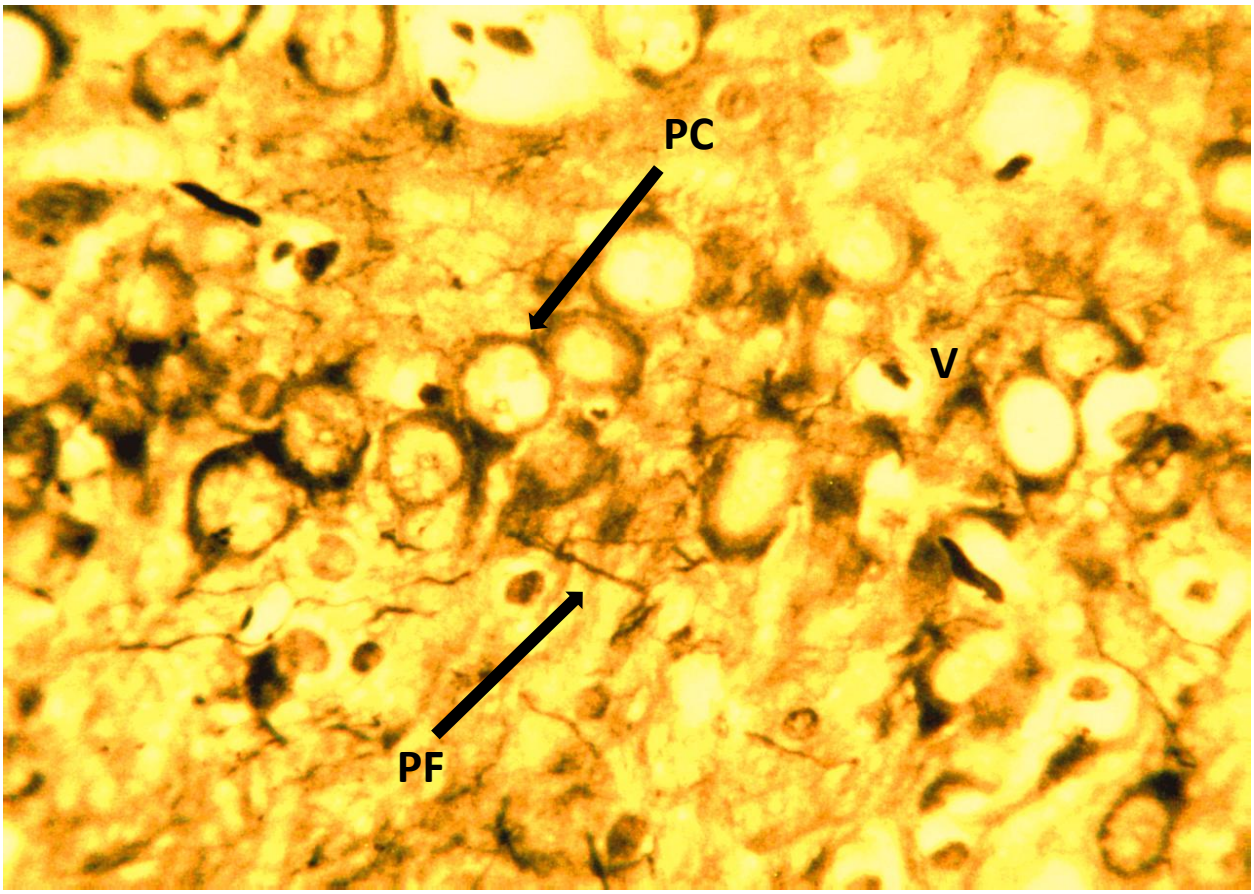


Plate LIII: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 500 mg/kg EEPD showing the pyramidal cells (PC), granule cells with little vacuolations (V) and parallel fibres (PF). Bielschowsky (×400)

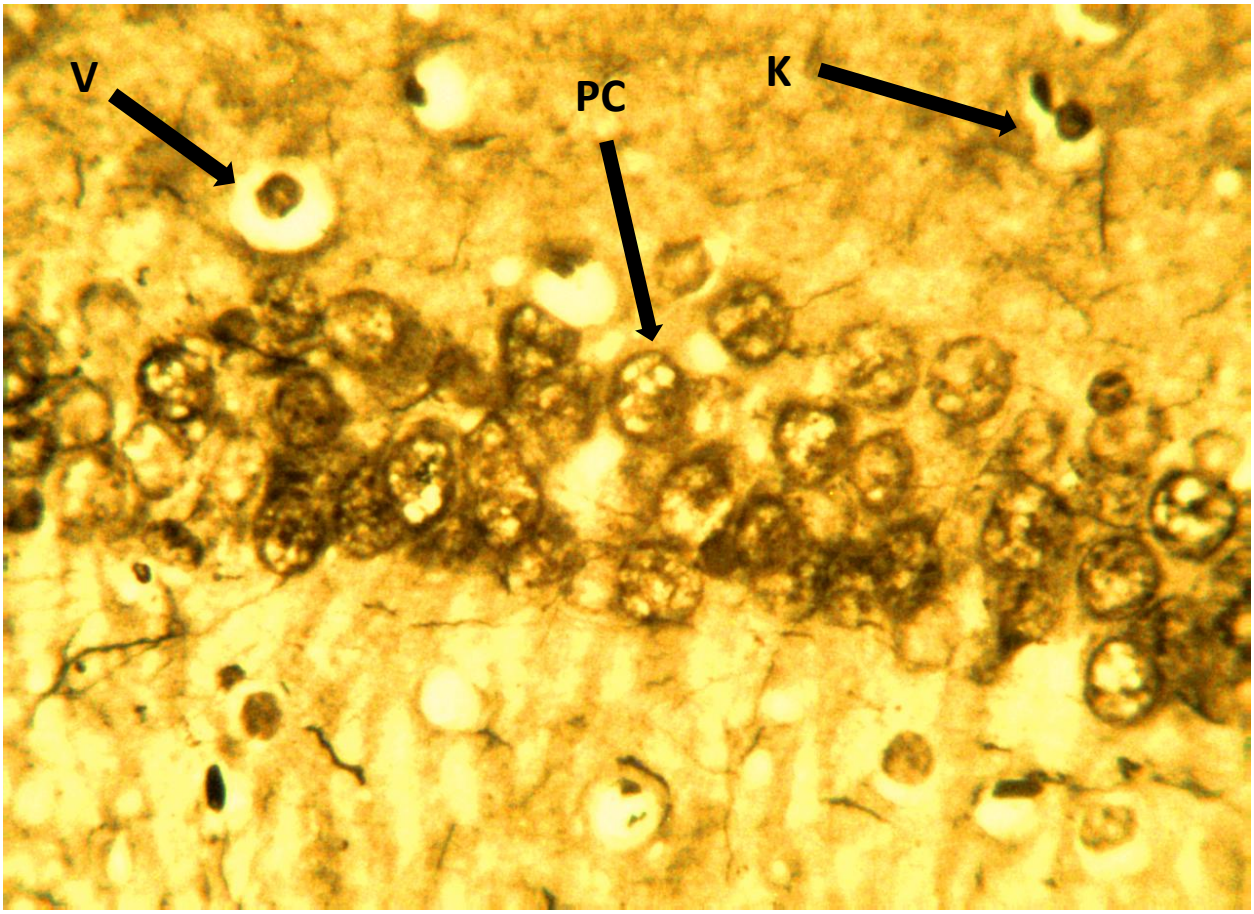


Plate LIV: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1000 mg/kg EEPD showing the pyramidal cells (PC), vacuolation (V) of granule cells and karyorrhexis (K) of basket cells. Bielschowsky (×400)

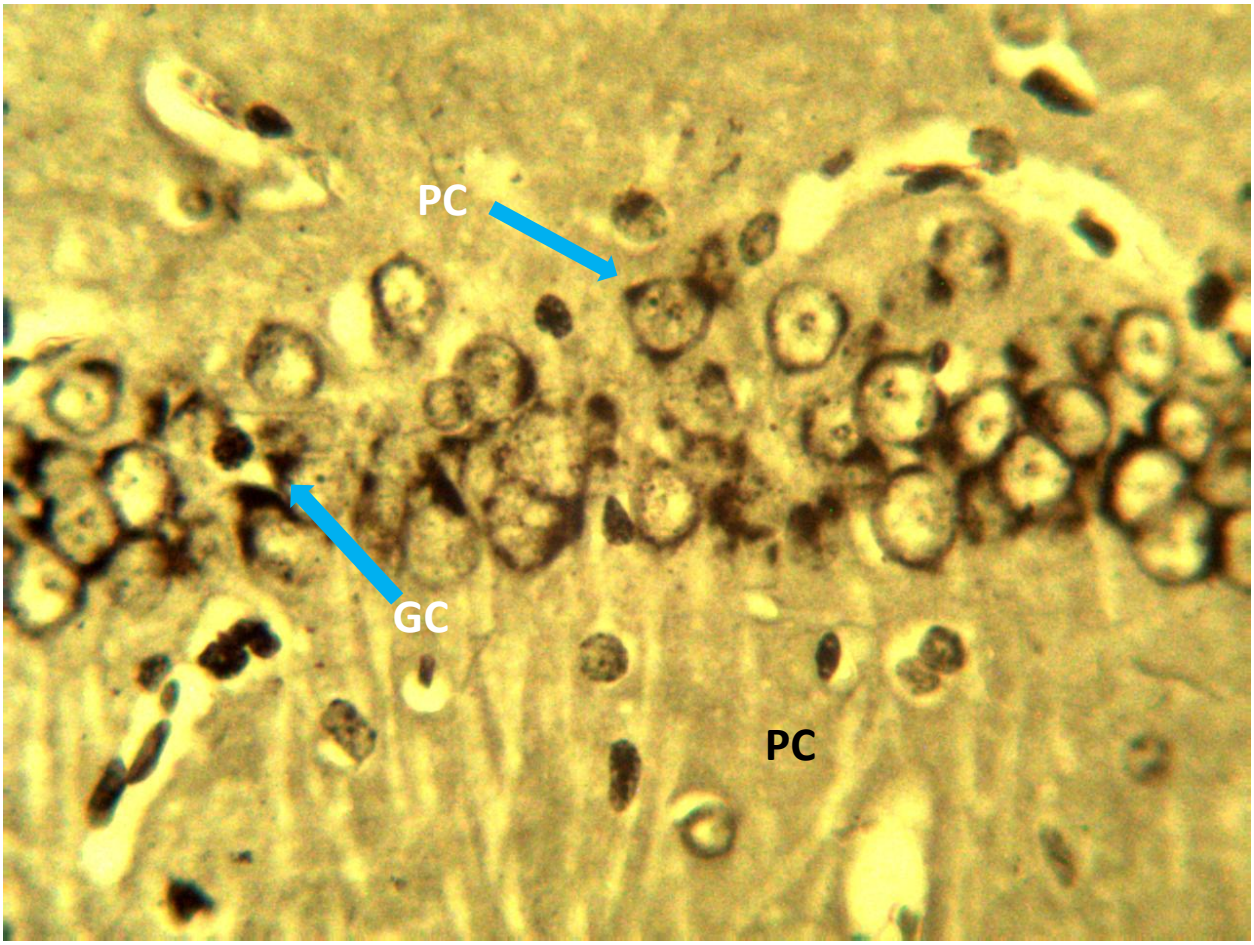


Plate LV: A longitudinal section of the CA3 hippocampal region of the rats treated with 2.86/8.75 mg/kg AS/AQ + 1500 mg/kg EEPD showing a normal compaction of the pyramidal cells (PC) interspersed with granule cells (GC). Bielschowsky (×400)

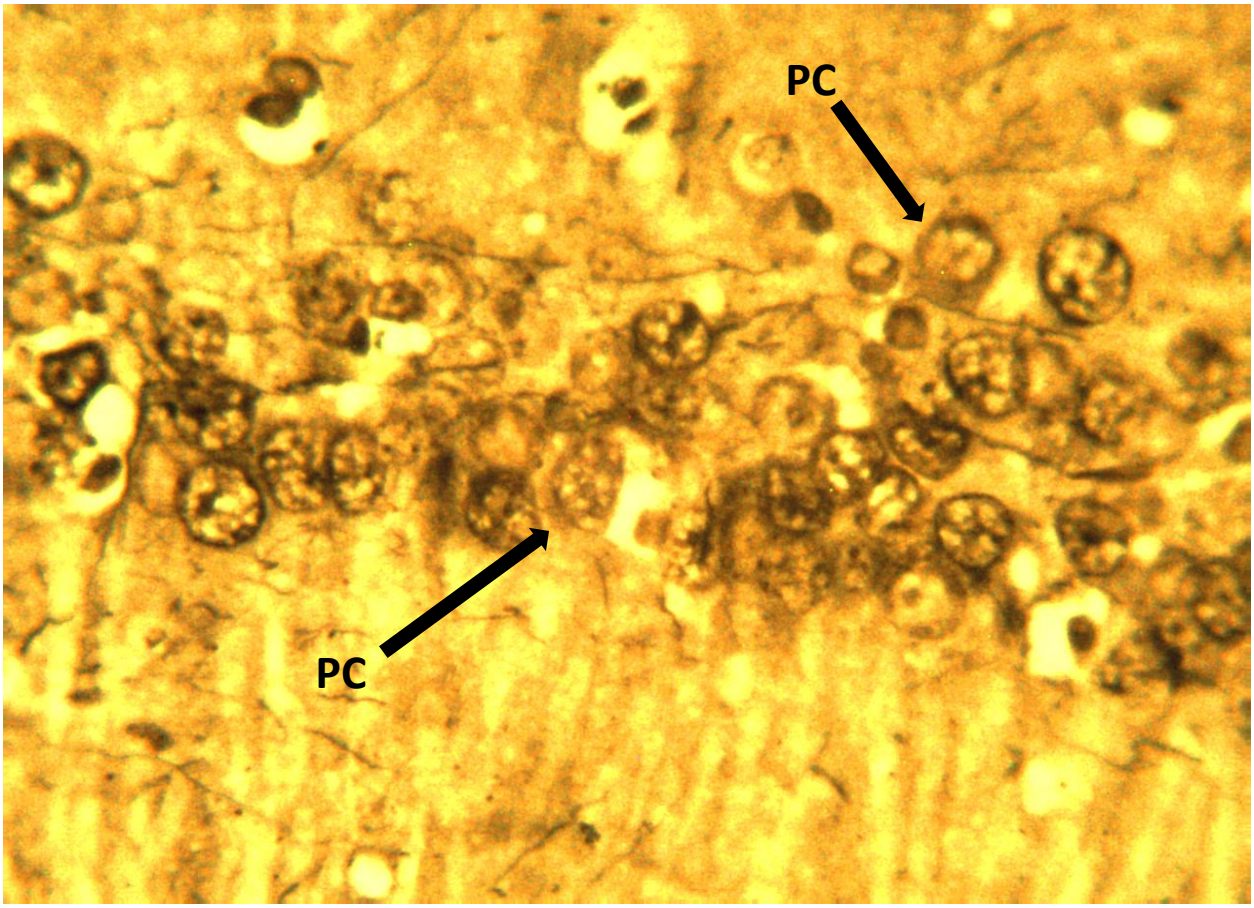


Plate LVI: A longitudinal section of the CA3 hippocampal region of rats treated with 2.86/8.75 mg/kg AS/AQ + 100 mg/kg ascorbic acid (standard drug) showing a normal compact organization of the pyramidal cells (PC). Bielschowsky (×400)

CHAPTER FIVE

5.0 DISCUSSIONS

The Beam walking test result showed that treatment groups (AS-AQ, AEPD, EEPD and AA) did not differ significantly from the control with respect to the latency time observed. This is consistent with previous reports by Genovese *et al.,* (1995; 1998) that showed artemisinin to have no overt behavioural signs of toxicity such as tremor of gait disturbances reflected on latency time. Furthermore, neither AS-AQ, both extracts, nor ascorbic acid altered general behaviour. This suggests that dopaminergic neurotransmitter levels are unaltered by either AS-AQ, *P. dactylifera* or ascorbic acid, as reported by Doan *et al.,* (2008) that animals with dopaminergic depletions displayed pronounced sensory and motor deficits, especially those in walking. For memory, the cholinergic neurotransmitters (such as gamma aminobutyric acid (GAMA), glutamate, ACH and their receptors) in the hippocampus were possibly unaffected, since their loss in the hippocampal area is principally involved in loss of memory and other cognitive functions, as typified in Alzheimer's disease (Nordberg and Svensson, 1998; Siddiqui *et al*., 1999)

In the Morris water maze test, the observed swim latency of the treatment groups (aqueous and ethanol extracts of *P. dactylifera*, and ascorbic acid) did not differ significantly from the Control group. This outcome differs from the findings of Meera *et al.,* (2014) that EEPD decreases swim latency. It however partly lends belief to the report of Onaolapo *et al.,* (2013) on the one hand that artesunate and amodiaquine, when combined, have less impairment than when used singly. On the other hand, it disagrees with their findings that AS-AQ altered neurobehaviour in mice by affecting their cognition and anxiety. It also gives credence to studies that report AS-AQ to cause cerebellar alterations, though not agreeing with the reported cerebellar dysfunction (Ekong *et al*.,

2009). The phytochemical alkaloid may have affected the central nervous system, including nerve cells of the brain which control many direct body functions and behavior, as well as interfere or compete with the action of serotonin in the brain (Pearson, 2001), causing a decreased firing in the brain, and thus, non-significant improvement in cognitive functions.

The result of the CAT quantification revealed a non-significant increase in the concentration in all the treatment groups. This is at variance with previous reports that associated ACT treatment with oxidative stress (Adegbesan *et al*., 2014; Olayinka and Ore, 2013) and *P. dactylifera* treatment with increase in the antioxidant CAT (Hassan *et al*., 2012).

The result of the SOD and GPx did not show any significant difference in both the Control and treatment groups (AS-AQ, AEPD, EEPD and AA). This does not support the reports that SOD levels are decreased by ACT (Olayinka and Ore, 2013) or increased by treatment with *P. dactylifera* (Ragab *et al*., 2013; Dania and Ezerioha, 2016)

The MDA level was observed to have increased significantly in the AS-AQ treated group, with a corresponding decrease in the AEPD and EEPD groups in a dose-dependent manner (significantly decreased in the 1500 mg/kg AEPD, $p<0.035$), as well as in the ascorbic acid treated group. This is consistent with previous reports that ACT treatment increases MDA levels significantly (Olayinka and Ore, 2013; Chiagoziem *et al*., 2012) and decreased by treatment with *P. dactylifera* extracts (Dania and Ezerioha, 2016; Ragab *et al.*, 2013; Pujari *et al*., 2011). Increase in MDA levels is an indication of lipid peroxidation in the cerebellum and hippocampus, and this suggested participation of free radical-induced oxidative cell injury in mediating neurotoxicity of ACT. Lipid peroxidation of biological membranes results in the loss of membrane fluidity, changes in membrane potentials, an increase in membrane permeability

and alteration in receptor functions (Nehru and Anand, 2005; Albendia *et al*., 2007). ROS can induce lipid peroxidation and disrupt the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability (Girotti, 1985). Products of lipid peroxidation, such as MDA, are capable of inactivating many cellular proteins by forming protein cross-linkages (Siu and Draper, 1982; Hagihara *et al*., 1984). ROS can cause fragmentation of the peptide chain, alteration of electrical charge of proteins, cross linking of proteins, and oxidation of specific amino acids and therefore lead to increased susceptibility to proteolysis by degradation by specific proteases (Kelly and Mudway, 2003). This oxidation cause conformational changes, protein unfolding, and degradation which is a measure of neurotoxicity (Lyras *et al*., 1997). The AEPD, EEPD and AA are assumed to have inhibited the ROS generation and thus, prevent the membranes of cells from lipid peroxidation following a dose-dependent fashion.

The concentrations of Zinc (Zn) and copper (Cu) were observed to have a non-statistically significant decrease across all the treatment groups when compared to the control. This is consistent with the report that Zn is vital and fairly very stable as it functions in modulation of synaptic transmission, synaptic plasticity and for operation of certain neurotransmitter receptors that are found in high density in the hippocampus (Law *et al*., 2003; Yanik *et al*., 2004). It plays a neuromodulator role in regulating diverse populations of neurons and myelinating oligodendrocytes, as well as essential for maintaining the integrity of the myelin sheath (Law *et al.*, 2003). it is stored in specific synaptic vesicles by glutamatergic neurons and can modulate brain excitability (Bitanihirwe and Cunningham, 2009). It plays a key role in synaptic plasticity and so in learning (Nakashiman and Dyck, 2009). It can also be a neurotoxin, suggesting that Zn

homeostasis plays a critical role in normal functioning of the brain and CNS. Therefore, its non-significant decrease could be a possible reason for the unaltered neurobehaviour of the animals.

Similarly, copper concentration was observed to also decrease, though not significantly, across all treatment groups when compared to the control. The outcome of this study lends belief to the report that Cu, if deficient, will result in poor myelination, necrosis of nerve tissue (Osredkar and Sustar, 2011) and hence, decreased motor activity. As reported by Araya *et al.*, (2006), Cu can act as both an antioxidant and a pro-oxidant. As an antioxidant, it scavenges or neutralizes free radicals and may reduce or help prevent some of the damage they cause (Bonham *et al*., 2002; Rakel, 2007). When it acts as a pro-oxidant, at times, it promotes free radical damage and may contribute to the development of Alzheimer's disease (Christen, 2000).

The concentration of Fe increased significantly in the ascorbic acid treated group but relatively unchanged in both AEPD and EEPD treated groups when compared to the Control. This is consistent with the work of Shiau and Jan (1992) that associated ascorbic acid deficiency with reduction in the absorption and redistribution of Fe. At the cellular level in the CNS, Fe levels are the highest in myelin, and in the astrocyte and oligodendrocyte glial cells that support neurons (Connor and Menzies, 1996). In these cells, Fe is required for the increased metabolic demands of myelination (Schulz *et al*., 2011).

The concentration of Mn was observed to be significantly high in the 2.86/8.75 mg/kg AS-AQ + 1500 mg/kg AAE and ascorbic acid treated group. It did not, however, increase in the other AEPD and EEPD treated groups. This elevated concentration of Mn, however, as seen, does not support the claims that associate its accumulation with neurobehavioural deficits such as hyperactivity, and neuron damage and lesion formation within the brain structures (Canavan *et*

al., 1934; Sani *et al*., 2007). Mn functions as a cofactor for multiple enzymes, including glutamate in the CNS (Sidoryk-Wegrzynomicz and Aschner, 2013a). at the cellular level, it functions in free radical defense and sequestration (Aschner *et al*., 2005)

The histology of the control group showed a normal histoarchitecture of the cerebellum with its three distinct layers – the molecular layer, Purkinje cell layer and granule layer. The Nissl substance as well as the dendritic network of the Purkinje neuronal fibres, were observed to be normally and evenly distributed. Similarly, the hippocampus of the Control group showed a normal arrangement of Pyramidal cells in the CA3 region, with deeply stained Nissl substance.

The ACT-treated group showed neurodegenerative insult to the cerebellum evidenced by a complete loss of Purkinje cell dendritic network of neuronal fibres, neuronal cell loss, vacuolations, nuclear pyknosis, as well as eosinophilic cytoplasm. Staining of Nissl substance of the Purkinje neurons and granular necrosis was also revealed. This finding is at variance with the report of Izunya *et al.,* (2011) and Kasim *et al.,* (2012) that neuropathological damage such as lesions, chromatolysis and necrosis were not observed following ACT treatment in Wistar rats. The result obtained here, however, is consistent with other reports that ACT treatment cause histological alterations in the cerebellum (Eweka and Adjene, 2008; Ekong *et al*., 2009), testes (Akinsomisoye and Raji, 2011; Timbuak *et al*., 2014), heart (Akinranide *et al*., 2014) and kidney (Amasa *et a*l., 2013). Contrary to the report of Ekong *et al.,* (2009) however, the observed neurodegeneration did not manifest in behavioural disturbance or deficits.

The hippocampus of the ACT treated group also revealed a distortion of the pyramidal cell layer organization, karyorrhexis, basophilic nuclei, pyknosis and a complete absence of the Nissl substance. In support of this observation, Nissl substances have been reported to be altered by

chemicals, toxins and certain drugs (Davis and Robertson, 1991). This occurs, during necrosis as ribosomes are dispersed from the rough endoplasmic reticulum and polyribosomes disassociate resulting in a number of monomeric ribosomes that are found free in the cytoplasm (Martin *et al*., 1978)

The histological sections of the aqueous extract treated groups showed a progressively improved amelioration of the AS-AQ-induced histologic insults in the cerebellum and hippocampus in a dose-dependent manner of 500 mg/kg (Group 3), 1000 mg/kg (Group 4) and 1,500 mg/kg (Group 5), respectively. At 500 mg/kg, there was neuronal pyknosis, vacuolations, diminished network of Purkinje neuronal fibres, indistinct granules and eosinophilic basket cells in the cerebellum, and distorted pyramidal cell layer of the hippocampus evidenced by necrosis, vacuolations and karyorrhexis, with a corresponding failure to stain the Nissl substance.

However, at doses of 1,000 mg/kg and 1,500 mg/kg in Groups 4 and 5, there was remarkable restoration of the cerebellar and hippocampal histoarchitecture to normal as revealed by distinct and intact layers of both brain regions: distinct Purkinje neuronal cells with numerous rich dendritic neuronal fibres, granule cells and restored organization of pyramidal cells arrangement The cerebellar cortex, and hippocampus (at 1,500 mg/kg only) clearly showed the Nissl substance. This result is in accord with the findings of the ameliorative effects of aqueous extracts of *P. dactylifera* on histopathological indices such as necrosis, ulceration (Al-Qawari *et al*., 2005), nephroprotection (Al-Qawari *et al.,* 2008), hepatoprotection (El-Gazzar *et al*., 2009; Abdulrahman *et al*., 2012; Agbon *et al*., 2013)

The histological sections of the ethanol extract-treated groups showed a similar ameliorative trend in the cerebellum and hippocampus as the doses increased from 500 mg/kg (Group 6) to

1,000 mg/kg (Group 7) to 1,500 mg/kg (Group 8). At a dose of 500 mg/kg, the cerebellar sections revealed the presence of neuronal pyknosis, Purkinje cell swelling and vacuolations, eroded dendritic network of the Purkinje cells, and several indistinct granule cells. A poor staining of the Nissl substance of the Purkinje cells was also revealed. The hippocampal section showed scattered and distorted arrangement of the pyramidal cell layer having necrosis, chromatolysis, vacuolations, and poor staining of the Nissl substance. There was a noticeable improvement in the cerebellar and hippocampal histology of the groups administered higher doses of 1,000 mg/kg and 1,500 mg/kg, respectively. However, nuclear pyknosis, chromatolysis, karyorrhexis, necrosis and vacuolations in the cerebellum, as well as pyramidal cells distortion in the hippocampus of the 1,000 mg/kg dose administered group, was observed, together with poor staining of the Nissl substance while at the 1,500 mg/kg dose, there was no obvious histological insults in both the cerebellum and hippocampus. There was, however, a consistently poor Nissl substance staining across all the EEPD-treated hippocampal sections. This finding is also consistent with the report that ethanol extract of *P. dactylifera* markedly ameliorate histopathological indices in stomach sections of gastric ulcerated rats (Al-Qawari *et al.*, 2005), and in lead-induced hematotoxicity (Wahab *et al*., 2010). The observed amelioration of histopathological degeneration in the cerebellum and hippocampus could equally be attributed to the free radical scavenging actions of the phytochemicals such as flavonoids, tannins and phenols as suggested by Ateeq *et al.,* (2013).

Comparatively, the aqueous extracts revealed a more potent ameliorative activity than the ethanol extracts, evidenced by fewer neurodegenerative parameters at all the compared doses. At all doses, the AEPD ameliorated the neurohistopathologic effects of AS-AQ much better than

the ethanol extract. What accounts for, and the mechanism underlining this difference, however, is uncertain.

The ascorbic acid treated group, when compared to the AS-AQ treated group, was observed to have ameliorated the histological insults incurred by the latter. Also, the histology sections, when compared with the control, revealed a near-normal cerebellar and hippocampal sections, with few vacuolations and degenerated Purkinje cells, but having a distinct dendritic circuitry, moderate staining of Nissl substance (in the cerebellum) normal organization of the pyramidal cells (though with poorly stained Nissl substance). Comparatively, the higher doses of both the AEPD and EEPD ameliorated the neurohistopathologic effects of AS-AQ much better than that observed with the ascorbic acid treated group. This lends belief to the reports of Bastway *et al*., (2008) that *P. dactylifera* extracts compare favourably with ascorbic acid in protecting the liver against thioacetamide-induced hepatotoxicity in rats.

The result of the ascorbic acid treated group supports the findings of Ibegbu *et al.,* (2013) that ascorbic acid ameliorated induced degenerative changes in the brain caused by mercuric chloride toxicity in rats. Ascorbic acid is considered an important neuroprotective agent (Naseer *et al*., 2011) since it is potent, scavenging ROS production and sustaining superoxide dismutase and catalase activities (Santos *et al*., 2009). It also protects neurons against glutamate excitotoxicity, which is associated with neurodegenerative processes (Qiu *et al*., 2009). It affects synaptic neurotransmission by preventing neurotransmitter binding to receptors (Majewska and Bell, 1990) by modulating their release and reuptake and also acting as a cofactor in neurotransmitter synthesis, thus it is considered a neuromodulator of neural transmission (Harrison and May, 2009)

Ascorbic acid is not only a simple antioxidant but also a neuromodulator in the central nervous system (Dai *et al.,* 2006). In addition, it can interfere with glutamatergic, dopaminergic, cholinergic and GABAergic transmission and related behaviours (Fiona *et al*., 2009). These neurotransmitter systems have a basic and crucial role in learning and memory processes (Myhrer, 2003).

Neuronal injury may result in reversible or irreversible cell damage or cell death (Seilhean *et al.,* 2004). Cell death has been reported to result from neuronal degeneration (Waters *et al*., 1994) and could result from necrosis, a pathological 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)

Histoarchitectural distortions such as chromatolysis, necrosis and indistinct neurons due to reduced staining intensity, when compared to the control as observed is indicative of AS-AQ induced neurotoxicity. Central chromatolysis is a histopathologic change seen in the cell body of a neuron, 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). It involves the cytoplasmic organelles and the cell membrane which ruptures, leading to cell death. Features include cytoplasmic shrinkage, disappearance of Nissl substances and intense eosinophilia ("red neurons") (Mena *et al*., 2004; Seilhean *et al*., 2004), small, shrunken darkly stained (pyknotic) nucleus, or the eventual fragmentation of such shrunken nucleus (karyorrhexis). Degenerating

neurons have a very poorly stained Nissl substance (rather than darkly) as a result of disassociation of ribosomes from the rough endoplasmic reticulum which occurs in the early stages of cell degeneration (Garman, 2011). These degenerative changes could have been induced by damage to mechanisms of DNA repairs (Hartwig *et al*., 1994)

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study, the following conclusions were arrived at:

1. Aqueous and ethanol extracts of *P. dactylifera* have both neuroprotective and ameliorative potentials against artesunate-amodiaquine toxicity as reflected by histological, histochemical and biochemical parameters in the cerebellar cortex and hippocampus.
2. The observed amelioration could be attributed to the free radical scavenging actions of its phytochemicals tannins and flavonoids.

6.2 Recommendations

1. Molecular biology analysis via gel electrophoresis should be employed to check the extent of amelioration of *P. dactylifera* extracts.
2. Further studies using Stereological techniques should be carried out to investigate the quantitative potency of the aqueous and ethanol, as well as other solvent extracts of *P. dactylifera* extracts
3. More research should be be carried out to establish pharmacognistic profile of the whole plant for inclusion into the Nigerian/African Pharmacopoeia which will assist in standardization of the plant as a tool for quality control.
4. Further work needs to be done to determine, identify, purify and quantify the ameliorative compound within this plant and also to determine its full spectrum of efficacy.

6.3 Contribution to Knowledge

The aqueous and ethanol fruit extracts of *P. dactylifera*:

1. Aqueous and ethanol extracts of *P. dactylifera* ameliorate artesunate - amodiaquine - induced histo-architectural insults at 1000 and 1500 mg/kg, and 1500 mg/kg body weight, respectively.
2. Aqueous extract of *P. dactylifera* ameliorates artesunate-amodiaquine-induced lipid peroxidation at 5.17 ($p<0.035$)

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