EFFECT OF ETHANOL EXTRACT OF *Vernonia amygdalina* LEAF IN CEREBRAL AND CEREBELLAR CORTICES OF YOUNG MICE INOCULATED WITH *Plasmodium berghei*

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BY

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FACULTY OF BASIC MEDICAL SCIENCES,
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NIGERIA

MAY, 2018.

DECLARATION

I declare that the research work in this dissertation entitled "**Effect of Ethanol Extract of *Vernonia amygdalina* Leaf in Cerebral and Cerebellar Cortices of Young Mice Inoculated with *Plasmodium berghei*** has been carried out by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree at this or any other Institution.

_____ _____

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CERTIFICATION

This dissertation entitled "**EFFECT OF ETHANOL EXTRACT OF *Vernonia amygdalina* LEAF IN CEREBRAL AND CEREBELLAR CORTICES OF YOUNG MICE INOCULATED WITH *Plasmodium berghei*"** by **Abdulazeez MOHAMMAD AWWAL** meets the regulations governing the award of the degree of Master of Science (M.Sc.) of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Almighty **ALLAH**, His Noble Prophet **Mohammad** (S.A.W.) his households, companion and those who follows his footsteps.

To my Murishid (Sheikh Mohammad Amin Abdullah) and lastly to my late Mother (Hajiya .S. Abdulazeez). May ALLAH have mercy on her and grant her the companion of the noble ones.

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ABSTRACT

Malaria is the world's most important parasitic disease especially when *Plasmodium falciparum* is the cause of most of the mortality and morbidity. Malaria has been challenging human health and losing the lives of many people since long period of time. Malaria has been one of the most extensively studied parasitic infectious diseases for millennia and the recent failure of chemotherapy through parasite resistance to drugs and alternative therapy is paramount. The present study was aimed at evaluating the effect of ethanol extracts of *vernonia amygdalina* leaf in cerebral and cerebellar cortices of young mice inoculated with *plasmodium berghei*. Sixty (60) young mice aged 5-7 weeks, weighing between 10-15g were divided into three categories in which category A, B, C contain twenty mice each which represent the Suppressive, Curative, and the Prophylactic design. Each category contains five groups with four mice in each group. In all the categories each Group 1 was administered distilled water, Group 2 was inoculated with *P. berghei* parasite, Group 3 was treated with *P. berghei* + ELVA 2500mg/kg, Group 4 was treated with *P. berghei* + ELVA 1250mg/kg and Group 5 was treated with *P. berghei* + 10mg/kg of Chloroquine respectively where ELVA is Ethanol Leaf extract of *Vernonia amygdalina.* In the Suppressive design the mice were inoculated with *P. berghei* parasite three hours after then parasite load is quantified follow by treatment with ELVA and on the 4^{th} day parasite quantification and sacrifice is done. In the Curative design the mice were inoculated with *P. berghei* parasite 48 hours after then parasitemia level was taken follow by treatment with ELVA, on the 7^{th} day parasite quantification and sacrifice was carried out. In the prophylactic design the mice were treated with ELVA for 72 hours thereafter inoculation with *P. berghei* parasite was carried out with parasite quantification noted up till the 8^{th} day before sacrifice. The mice were sacrificed and brain tissues were removed and fixed in Bouin's fluid, processed for histopathological studies using Haematoxyline

and Eosin (H and E), and Cresyl Echt Violet stains. The result for the suppressive design indicates that the mean percentage parasitemia in group 3 mice treated *P. berghei* + ELVA 2500mg/kg (2.84±0.10) was significantly lower (P<0.001) compare to Group 2 (4.44±0.16), group 4 (3.28±0.12) and group 5 (3.34±0.13). The result from the curative design shows that the parasitemia level of the mice in group 3 (2.48±0.22) was significantly lower compare to Group 2 (4.34±0.57) and group 4 (3.76±0.35) group 5 (2.64±0.10) with a (P value) of 0.015. The ethanol leaf extract of *V. amgydalina* exhibited different degrees of suppression at the doses (1250 and 2500) mg/kg and Pyrimethamine 1.2mg/kg which were (72.7, p<0.057; 61, p<0.057 and 76.2%, p<0.057) respectively as compared to normal control group. For the mean calculation of the level of parasitemia the result indicates that there is no significant difference observed in the group. In the suppressive and curative design, the histopathological changes in cerebral and cerebellar cortices of the young mice inoculated with *P. berghei* parasites and the effects were ameliorated when treated with ELVA when compared with the control. However, in the prophylactic design ELVA doesn't not ameliorate the effects of the inoculated parasite when compare to the suppressive and curative. Thus, the present study has established that ethanol extract of *Vernonia amygdalina* leaf at doses of 2500 mg/kg and 1250 mg/kg was able to ameliorate the effects of Malaria parasite inoculated and may justify the local usage by the traditionalist and the mass population in endemic areas that are exposed to the malaria parasite.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Malaria is the world's most important parasitic disease especially when *Plasmodium falciparum* is the cause of most of the mortality and morbidity (Cropper *et al*., 2004). Malaria has been challenging human health and losing the lives of many people since long period of time. It is one of the most prevalent; devastating parasitic infectious diseases in the world (Moss *et al*., 2008). Malaria has been one of the most extensively studied parasitic infectious diseases for millennia. In 2012, there were around 627,000 malaria deaths worldwide, 90% of which were in the African region, followed by Southeast Asia (7%) and the Eastern Mediterranean (3%) (WHO, 2013). Most of these deaths were due to *Plasmodium falciparum*. However, *Plasmodium vivax* is now increasingly recognized as a cause of severe malaria and death (WHO, 2013b).

Malaria infections are characterized by defective immune responses with poor efficacy against infection and in some cases with immunopathology (Mackintosh and Beesonk 2004). Severe malaria is a complex multisystem disorder with complications such as cerebral malaria, anaemia, acidosis, jaundice, respiratory distress, renal insufficiency, coagulation anomalies and hyperparasitemia (Penet *et al.,* 2005).

Most cases of endemic P. falciparum malaria remain uncomplicated; however, some develop a number of severe complications including cerebral malaria, severe anaemia and placental malaria. Severe malaria occurs when the parasite reaches the erythrocytic phase of the infection and starts to proliferate inside the erythrocytes. A characteristic feature of P. falciparum malaria is the ability of the parasite infected red blood cells (pRBCs) to

adhere to host endothelium (cytoadherence) and to non-infected erythrocytes (rosetting). These binding events eventually lead to the occlusion of the microvasculature in various tissues and organs, such as the brain in cerebral malaria (Jerrard *et al.,* 2002) hence contributing directly to the pathogenesis of severe malaria disease. A number of host molecules have been implicated as receptors for parasite adhesion and P. falciparum erythrocyte membrane protein 1 (PfEMP1) has been suggested as the key adhesive ligand of pRBCs.

Approximately 300,000 foetal and infant's deaths and 2,500 deaths of pregnant women are attributable to malaria in the endemic areas (Brabin *et al.*, 1990). Both mother and foetus are at risk of poor outcome with pregnancy associated malaria and complications like premature delivery, anaemia, abortion, intrauterine growth retardation, low birth weight and perinatal and maternal mortality are well (Brabin *et al.,* 1991 and Menendez 1995) known. The frequency and severity of malaria is increased (Brabin *et al.,* 1990) during pregnancy. Pregnancy complicates the clinical course, diagnosis, and treatment of malaria. Pregnancy is associated with downregulation of maternal immune responses, to protect the foetus from rejection. This altered immunity explains, in part, the association of pregnancy with more severe malaria (Abrams *et al*., 2009). Anaemia is more pronounced in pregnant patients due to haemolysis from rupture of infected erythrocytes exacerbated by erythrocyte sequestration in the spleen and liver, as well as iron deficiency (Steketee *et al*., 2001; Jerrard *et al*., 2002; Desai and Steketee, 2003; Danquah *et al.,* 2008). Thrombocytopenia, another key hematologic feature of malaria, is more common in the pregnant patient (Jerrrd *et al.*, 2002; Trampuz *et al.,* 2003; Greenbaum *et al.,* 2009). Hypoglycemia is more commonly seen in the pregnant patient with malaria, due to increased consumption by host and parasite, as well as glycogen depletion and impaired gluconeogenesis (Nayak *et al.,* 2009). This may be exacerbated by drug treatments for malaria (Whitty *et al.,* 2005). Plasmodium

parasites uniquely sequester in the placenta in the case of falciparum malaria, contributing to miscarriage, premature labour, and low-birth-weight infants (Jerrard *et al.*, 2002; Whitty *et al.*, 2005; Mali *et al*., 2007; Abrams *et al.*, 2009).

1.2 Medicinal Plants

Medicinal plants have formed the basis of healthcare worldwide. Hence, the use of plant derived natural products as part of herbal preparations for alternative sources of medicine continues to play a major role in chemotherapy especially in third world countries (Joy *et al.*, 1998). Plants by means of secondary metabolism contain a variety of herbal and non-herbal ingredients that can ameliorate a disease condition by acting on a variety of targets in host organism. *Vernonia amygdalina* have been known and identified by many researchers to possess herbal ingredients for the treatment of malaria parasite infection and have also been studied for their effect on hematology (Akinnuga *et al.*, 2011; Oyedeji *et al.*, 2013).

Vernonia amygdalina is commonly known as bitter leaf which is a shrub or small tree of 2-5m belonging to the family; "Asteraceae". The leaves are green with characteristic odour and a bitter taste (Singha *et al.*, 1996). The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides (Ologunde *et al.*, 1999). *Vernonia amygdalina* produces no seeds. Hence, they are distributed through cutting (Tende *et al.*, 2013). It is known as "Ewuro" in Yoruba, "Etidot" in Ibibio, "Onugbu" in Igbo and "Chusa"in Hausa tribes of Nigeria (Egedigwe *et al.*, 2010). The leaves are mostly used as vegetables for preparation of soup to stimulate the digestive system and also used for treatment of fever in South Southern and South Eastern Nigeria. It contains a wide variety of phytochemicals such as oxalates, phytates and tannins (Eleyimi *et al.*, 2008), as well as high amount of flavonoid (Igile *et al.*, 1994). It also contains major minerals like calcium,

potassium, magnesium (Mensah *et al.*, 2008), and vitamins A, C and E, as well as niacin, riboflavin, thiamine (Atangwho *et al*., 2009). It grows under a range of ecological zones in Africa and produces large mass of foliage and is tolerant to drought (Bonsi *et al.*, 1995).

1.3 Statement of Research Problem

Malaria is the world most important parasitic infection (Hay *et al*., 2004) and it remains a major impediment to health in Africa, south of the Sahara (Snow, 1999). It is reported to be a major cause of morbidity and mortality amongst Nigeria's children population (Ayoola *et al*., 2005). *Plasmodium falciparum* is responsible for 13–28% of deaths in children under 5 years of age (Tulu *et al*., 1993). It is estimated that 1-2 million people die yearly as a result of malaria (Sudhanshu *et al*., 2003). Efforts to reduce the high malaria morbidity and mortality rates have been hampered by the development to resistance, particularly by falciparum to long-standing conventional drugs such as chloroquine (Okoyeh *et al*., 1993; Ejov *et al*., 1999; Wongsrichanalai *et al*., 2002).

Nigeria has the highest prevalence of malarial cases in Africa (WHO, 2008a) as transmission of the disease occur all year round in the southern part of the country while in the northern part, the disease is more seasonal mostly occurring during the rainy season. As reported by (Davidson *et al.*, 2000), pregnant women, their unborn foetus and children below the age of 5 years are more vulnerable to malaria which serves as the major cause of maternal and infant anaemia.

1.4. Significance of the Study

Of all populations at risk of acquiring malarial disease, children 5 years or younger in Sub-Saharan Africa are most susceptible to developing malaria and the severe form (Cerebral Malaria CM) (Dorovini-Zis *et al.*, 2011) as a manifestation of clinical malaria. Due to

immunological factors children under five years and pregnant women have a higher risk for both uncomplicated and severe malaria. In high transmission areas the risk of severe *Falciparum* malaria developing is greatest among young children. (WHO, 2011). Hence this study will provide more information on the established potency or therapeutic value of ethanol extract of *Vernonia amygdalina* on young children malaria.

1.5 Justification of the Study

Despite the intense efforts made by the research community and the Global Eradication program (Khadjavi *et al.,* 2010), no effective vaccines or adjuvant therapies are available for malaria. There are evidences that *Vernonia amgydalina* has benefitting effects on induced malaria in mice but little is known of its effects on the nervous system of under aged mice i.e. young mice. The study seeks to provide information on administered effects of *Vernonia amgydalina* on the cerebral and cerebellar cortices in young ones. The study outcome could serve as the basis on which further studies could be carried out to broaden the understanding of paediatric malaria management.

1.6 Scope of the Study

The study was focused on the parasitic load indices and histology of the cerebrum and cerebellum as a result of young mice exposure to *Plasmodium berghei* malaria parasite. Haematoxylin and Eosin (H and E) and Cresyl Echt Violet was employed in the study of the tissues.

1.7 Aim and Objectives of the Study

1.7.1 Aim of the study

To study the effect of Ethanol leaf extract of *Vernonia amgydalina* on cerebral and cerebellar cortices in young mice inoculated with *Plasmodium berghei* parasite.

1.7.2 Objectives of the study

The objectives of this study were to:

i. Determine the suppressive effects of the ethanol leaf extract of *Vernonia amygdalina* against *Plasmodium berghei* inoculated in young mice.
ii. Determine the curative effects of the ethanol leaf extract of *Vernonia amygdalina* against *Plasmodium berghei* inoculated in young mice.
iii. Determine the prophylactic effects of the ethanol leaf extract of *Vernonia amygdalina* against *Plasmodium berghei* inoculated in young mice.
iv. Investigate the histological changes in cerebral and cerebellar cortices of the *Plasmodium berghei* inoculated young mice.

1.8 **Research Hypothesis**

Ethanol leaf extract of *Vernonia amygdalina* have an in vivo modulatory effect on induced experimental malaria in young mice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Related Literature

The crude water and ethanol extract of *Vernonia amygdalina* was found to show a significant inhibitory activity in-vitro were tested on trophozoites, mainly ring forms, and there was a significant reduction in the number of parasitized cells relative to control (P > 0.05) and showed the highest antimalarial activity of 78.1 %, with IC50of 11.2 µg/mL. The water extract had the parasite growth inhibition of 74.0 % and IC50of 13.6µg/mL (Sha'a *et al*., 2011).

Melariri *et al*. (2011) has also described that the extracts of in vitro and in vivo antiplasmodial activities of extracts of Cymbopogon citratus Staph and *Vernonia amygdalina* Del. by petroleum ether, ethyl acetate, and methanol extracts of C. citrates showed IC50 of 9.1µg/mL, 12.1µg/mL, 15.9µg/mL respectively while the petroleum ether, ethyl acetate, and methanol extracts *Vernonia amygdalina*a recorded IC50s of 14.1µg/mL, 10.7µg/mL and >50µg/mL respectively. Water extracts of both plants showed IC50 > 50µg/mL. The dichloromethane extracts of the two plants recorded the greatest antiplasmodial activity invitro. Simultaneously, the in vivo antimalarial activities showed a marked growth inhibition of parasites with values of 87.2% and 95.8% by the dichloromethane extracts of C. citratus and *Vernonia amygdalina*, respectively at a dose of 800 mg/kg. It was found that the Ethanolic, aqueous, and hydroethanolic extracts both In vitro and in vivo antiplasmodial activity from *Vernonia amygdalina* Leaves showed that the ethanolic extract of the plant leaves had the highest ($p<0.05$) antiplasmodial activity (IC50 = 9.83 ug/mL) and cytotoxicity (IC50 = 60.33) with moderate selectivity index of 6.14 when compared with the other extracts and the ethanolic extract was also significantly

active in vivo against P. berghei in a dose-dependent manner with maximum activity observed at 1000 mg/kg (% inhibition of 82.3 %) (Omoregie *et al*., 2010).

Recent in vitro study by Odeh and Usman, (2014) of Antimalarial activity and phytochemical analysis of aqueous leaf extract of *Vernonia amygdalina* showed at different concentration ranging from 20 mg/mL, 40mg/mL, 60mg/mL, 80mg/mL and 100mg/mL. It was observed that the extract showed a higher zone of inhibition (15.0±0.58) at 100mg/mL concentration on P. malariae and (14.00±1.00) on *P. falciparum. P. ovale* showed a moderate zone of inhibition of (14.00±0.58) while P. vivax showed the least zone of inhibition (13.00±0.57). The analysis was controlled with Coartem (14.3±0.58). The phytochemical screening of the aqueous leaf extract of *Vernonia amygdalina* was investigated which showed the presence of Saponins, Tannins, Alkaloids, Cardiac glycosides, Glycosides, Steroids and Anthraquinones which have an antimalarial activity on Plasmodium parasites.

Najan *et al*. (2008) found that the analgesia and toxicity of the aqueous extract of *Vernonia amygdalina* extract caused a significant ($p<0.05$) and dose-dependent reduction in mean parasitemia in mice infected with P. berghei in comparison to Chloroquine(CQ) (5 mg/kg). The extract caused a parasitemia reduction of 52% with 50 mg/kg, 64% with 100 mg/kg, and 73% with 200 mg/kg (i.p.). All the mice in the saline group died within 15 days throughout the 30-day observation period of the experiment, while the remaining mice recovered fully. Ethanolic leaf and root-bark extracts of *Vernonia amygdalina* were found to possess significant antimalarial activities against drug-sensitive P. berghei in mice in-vivo (Abosi and Roseroka, 2003). Moreover, Iwalokun, (2008) reported that prophylactically, chloroquine (5mg/kg) in combination with *Vernonia amygdalina* extracts

achieved a dose-dependent (57.2 – 72.7%) suppression of parasitaemia due to CQ sensitive and resistant *P. berghei* strains in the experimental animals.

2.2 Traditional Medicine and Anti-Malarial Drugs from Medicinal Plants

In developing countries traditional medicine still plays an important role in local health care systems (Xu *et al*., 2011). The WHO estimates that up to 80% of some Asian and African countries depend on traditional medicine for their health care needs (Xu, 2011; WHO, 2008). It is difficult to assign one definition to the broad range of characteristics and elements of traditional medicine. The WHO thus defines traditional medicine as the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being (WHO, 2008).

Chan, (2005) concludes that traditional healing is "holistic" in nature. It does not focus on symptoms or diseases. Instead, it deals with the total individual. Healing focuses on the person, not just the illness and healing does not follow written guidelines, that is different people may receive the same medication or treatment even if they suffer from different diseases, in contrast to modern medicine where different people may receive different treatments even if they suffer from the same disease. Plants have been a source of medicine throughout the history of medicine. For thousands of years' natural compounds, mostly from plants, have been the manifestation of traditional medicine (Ginsburg and Deharo, 2011). Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary health care system of resource limited communities and the local therapy is the only means of medical treatment for such communities (Yineger and Yewhalaw, 2007).

WHO has stated that 74% of 119 plant-derived pharmaceutical medicines applied in modern ways resembles the method used by native cultures. Utilization of plants as primary source of medicinal agents by the majority of the population is not only due to the high cost of Western pharmaceuticals, but also because the traditional medicine is generally more acceptable from a cultural and spiritual perspective. Even in the Western world, the use of herbal medicines is steadily growing with approximately 40% of the population reporting use of herbs to treat medical illness (Kirkpatrick, 2002).

In malarial regions, affordable treatments against malaria are mainly based on the use of traditional herbal remedies. Indeed, indigenous plants play an important role in the management of the disease, and they seem to be the most convenient solution because of their accessibility and diversity in tropical and sub-tropical regions (Phillipson and Wright, 1991). According to several reports, up to 80% of world's populations still rely on traditional medicine mainly on herbal remedies as primary source of medicinal agents for the treatment of diseases including malaria (Kirkpatrick, 2002). Malaria ranks as the most important disease treated with herbal remedies in ethno medical practices (Phillipson and Wright, 1991).

Plant based drugs provide an outstanding contribution to the modern therapeutics (Ginsburg and Deharo, 2011). It is estimated that at least 25% of all modern medicines are derived either directly or indirectly from medicinal plants primarily through the application of modern technology to traditional knowledge. In the case of certain classes of pharmaceuticals such as antitumoral and antimicrobial medicines, this percentage may be as high as 60% (Sucher, 2008). The development of useful and widely used drugs like digoxin and digitoxin from digitalis leaves, quinine from the cinchona bark, reserpine from Rauwolfia serpentine, morphine from Papaver somniferum, cocaine from Erythroxzion

coca and the anticancer vincristiner and vinblastine from Cartharathus troseus of Madagascar and again the anticancer compound bruceatin from the Ethiopian plant Brucea antidysentrica are just to name a few examples of the contributions of traditional medicinal plants to the modern therapeutics agents (Bekele,2007). Many plant species continue to be used in traditional medicine for the treatment of malaria and many people depend upon such remedies as they cannot afford or do not have access to standard antimalarial drugs. Recently, the WHO estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care (WHO, 2010c), especially developing countries where malaria is endemic which depends strongly on traditional medicine as a source for inexpensive treatment of this disease. However scientific data to validate the antimalarial properties of these herbal remedies are scarce (Ramalhete *et al*., 2008).

2.3 Description of the Study Plant

The largest family of flowering plants that contains about 900 genera and some 13,000 species. Members are herbs, shrubs or rarely small trees or climbers. Leaves are alternate or opposite, simple or variously divided (Hutchinson, 1963). Members are known to store carbohydrate as polyfructose, notably inulin and commonly producing polyacetylenes which are borne in the resin-canals. Polyacetylenes are characterized by the presence of cyclic, aromatic or heterocyclic end-group; bitter sesquiterpenes (especially sesquiterpene lactones), terpenoid volatile essential oils and often one or another sort of alkaloids, notably the pyrrolizidine alkaloids, but without tiridoid compounds (Cronquist, 1981). Asteraceae are known to contain pyridine, quinoline alkaloids and occasionally diterpenes. Glycosides, saponins, flavonoids and tannins are their chief constituents and flavonoids are used as taxonomic markers (Evans, 1999).

The Genus: Vernonia Shrub. The genus has about 60 members that are trees, shrubs or woody climbers. They are characterized by involucre bracts with coloured or whitish appendages; capitula 1.3-3cm or more diameters; achenes often black and ribbed; pappus-setae more or less uniform. They are usually deciduous and buff in colour and leaves are often toothed (Hutchinson, 1963).

They are classified in according to Sanogo (2005) as follows: Kingdom: Plantae Division: Magnoliophyta Class: Magnoliopsida Order: Asterales Family: Asteraceae Genus: Vernonia Species: *Vernonia amygdalina* Del.

The Species: *Vernonia amygdalina* Del. *Vernonia amygdalina* commonly known as bitter leaf (English), grawa (Amharic), Eebicha (Afaan Oromo) is a tropical shrub that grows up to 3 meters high in the African tropics and other parts of Africa particularly Nigeria, Cameroon, Zimbabwe Ethiopia and it is also known as "Ewuro" in Yoruba, "Etidot" in Ibibio, "Onugbu" in Igbo and "Shuwaka" in Hausa tribes of Nigeria (Egedigwe *et al.,* 2010). The leaves are dark green coloured with a characteristics odor and a bitter taste. It is reputed to have several health benefits. It is effective against amoebic dysentery, gastrointestinal disorder and has antimicrobial and anti-parasitic activity (Erasto *et al*., 2006). *Vernonia amygdalina* is a perennial herb belonging to the Asteraceae family. The species is indigenous to tropical Africa and is found wild or cultivated all over sub Saharan African. The leaves are eaten after crushing and washing thoroughly to remove the bittern. However, almost all parts of the plant are pharmacologically useful, both the root and the leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort among others (Nwanjo, 2006; Erasto *et al*., 2006).

Vernonia amygdalina has been ascertained to provide various culinary and medicinal properties, these medicinal properties exert bacteriostatic and bacteriocidal effect on some

bacteria. Antihelmitic and Antimalarial properties (Abort and Raserika, 2003) as well as antitumourigenic properties (Izevbigie *et al*., 2004) have also been reported for extracts from the plant. Furthermore, other studies have demonstrated hypoglycemic and hypolipidaemic effect of the leaf extract in experimental animals (Nwanjo, 2006). Many herbalist and native doctors in Africa recommend its aqueous extract for their patients for the treatment of varieties of ailment ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexual transmitted diseases and diabetes mellitus among others. *Vernonia amygdalinaa* and its relatives are good sources of sesquiterpenes lactones. Compounds have been isolated and identified from the plant leaves including vernolide, vernodalin and hydroxyvernolide (Kraft *et al*., 2003). The occurrences of steroidal saponins, terpenes, coumarins, tannins, alkaloids and flavonoids have also been reported (Erasto *et al*., 2006). The phytochemicals present in the plant have been suggested to play a role in its biological activity (Erasto *et al*., 2006).

2.4 Historical Background

Malaria, the name is derived from the Italian word mal'aria, for ''bad air". This is to describe the swampy areas in Europe in which the disease was prevalent (Amorosa *et al*, 2005). But later, the etiological agent for the disease was discovered and is known to be the protozoan parasite Plasmodium (Ridder *et al*., 2008). Malaria is one of the world's most serious infectious diseases caused by Plasmodium parasites (Cropper *et al*., 2004). There are more than 100 species of Plasmodium parasite that can infect many animal species such as reptiles, birds, rodents, monkeys and humans (NIAID, 2007). Human malaria caused by genus Plasmodium is mainly transmitted through the bite of infected female Anopheles mosquitoes during blood meal (Cox, 2010; Echhoff, 2011) and other rare mechanisms

including congenitally acquired, blood transfusion, sharing of contaminated needles and organ transplantation (Guerin *et al*., 2002).

Four species of genus Plasmodium; P. falciparum, P. vivax, P. ovale and P. malarae have long been recognized to infect humans in nature all of which are transmitted by the female anopheles mosquito. In addition, there is one species; P. knowelesi that naturally infects macaque monkeys has recently been recognized as a cause of zoonotic malaria in humans (Eede *et al*., 2009).

About 300-500 million clinical cases and 1.2–2.8 million deaths due to malaria occur each year (Linares *et al.,* 2007; Sahu *et al.,* 2008). In 2015, there were 214 million cases of malaria worldwide resulting in an estimated 438,000 deaths with 90% cases of death in Africa (WHO, 2016). Malaria is commonly associated with poverty and has a major negative effect on economic development (Gollin and Zimmermann *et al.,* 2007). Africa loses an estimated 12 billion dollars yearly to malaria through health care costs, lost ability to work and negative effects on tourism (Greenwood *et al.,* 2005). The physiologic changes associated with pregnancy make the patient more prone to congestive heart failure which is exacerbated by anaemia (Greenbaum *et al.,* 2009). Anti-malarial drug treatment requires consideration for the potential adverse effects on the foetus, balanced against the need for rapid and effective therapy to attack the parasites within the mother, to reduce complications for both. Previous studies of the pathology of malaria have identified several specific features of the disease (Penet *et al.,* 2005). As the *Plasmodium* parasite matures within host erythrocytes, it disappears from the peripheral circulation and localizes preferentially within the vascular beds of the vital organs such as the liver, brain, lung, and kidney. This process is termed sequestration. There has been some debate as to the role of sequestration in causing disease within a particular organ such as the brain in cerebral

malaria (CM). Some investigators believe that malaria infection causes generalized vital organ dysfunction as a result of the release of systemic cytokines such as tumour necrosis factor alpha, or local release of mediators such as nitric oxide (Penet *et al.,* 2005 and Chandel *et al.,* 2010). It has thus been important to try and relate the presence of sequestration within a particular organ to clinical disease within that organ before death. The pathophysiology of severe malaria is complex and poorly understood (Mackintosh and Beesonk *et al.,* 2004, Penet *et al.,* 2005 and Linares *et al.,* 2007). Nearly all severe and fatal malaria in humans is caused by *Plasmodium falciparum*, and manifests clinically as a spectrum of disease ranging from asymptomatic infection through mild, severe, and fatal disease. Death can result from a variety of syndromes including renal failure, severe anaemia, cardiovascular shock, multi-system organ failure, and cerebral malaria (CM). Cerebral malaria is the most common clinical presentation and accounts for the majority of deaths in severe malaria (Sahu *et al.,* 2008). The pattern of vital organ dysfunction is different in adults and children, although CM is common at all age groups (Basco *et al.,* 1994, Ibezim *et al.,* 2008 and Chandel *et al.,* 2010).

2.5 The Global Burden of Malaria

Malaria is one of the major diseases of poor people in developing countries and one of the leading causes of avoidable death, especially in children and pregnant women. The highest risk is found in the sub-Sahara Africa in which the populations living in this area have the highest risk of acquiring malaria of all geographical locations. On contrary, in other regions of the world, particularly Latin America and most of Asia, levels of transmission are much lower and malaria tends to affect people of all ages causing severe morbidity, but less commonly resulting in death (WHO, 2011(a); UNICEF, 2011).

The global burden of malaria is terrible. Bio medically, the burden of disease is a function of many things and various public health disciplines define it differently. Epidemiologists, on the other hand, refer to burden in terms of morbidity and mortality, while economists refer to a quantification of the costs (direct and indirect), and effects on levels of productivity, national growth, and development. Common to both of these approaches is a focus on disease and risk factors, with the aim of establishing causative linkages with broad quantitative outcomes (Jones and Williams, 2004).

In spite of promising progress in controlling the diseases, malaria remains one of the major public health problems on the Africa in that it has a heavy burden on individual families and national health systems. In many African countries 30 % or more of outpatient's visit and hospital admissions due to malaria (PMI, 2014b). Malaria affects the economy in which it follows different channels. The direct effect is that adults are unable to work during episodes of the disease, and may be significantly weakened for a period afterward. Repeated infection with malaria is associated with anaemia in children and adults (Weil, 2010).

Malaria also retards economic and social development through effects such as reduced working hours due to sickness or attending to the sick, income spent on financing health care, which in turn, lead to impacts at national level due to massive health care budgets, reduced productivity of the work force, and the like (Pierre-Louis *et al.*, 2005). The disease subsists in more than 100 countries in different regions of the world, including India, Southeast Asia, and Central and South America, although sub-Saharan Africa is the most strongly affected (Figure 1.1). Efforts to reduce poverty and childhood mortality in those vulnerable societies will fail if this devastating disease is not adequately controlled (Alonso, 2010).

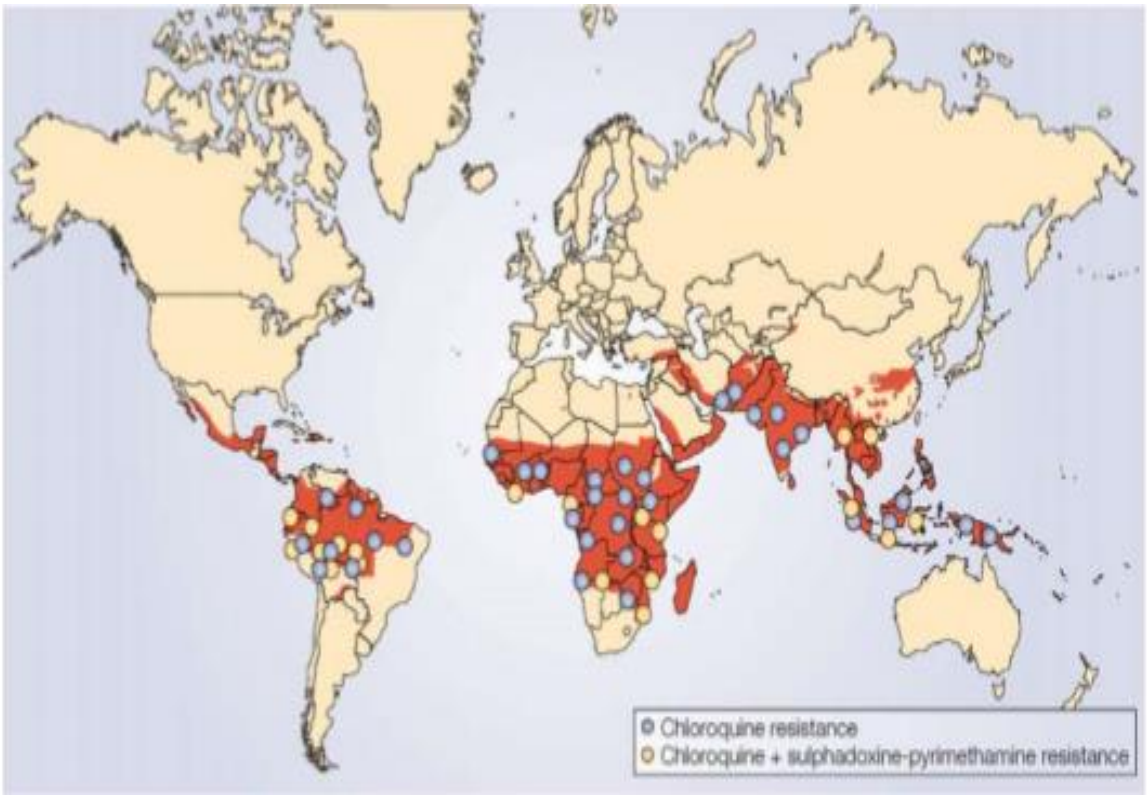


Figure 2.1: Estimates of World Malaria Burden and Resistance (Fidock *et al*., 2004).

2.6 Life Cycle of Malaria

Malaria, an infectious disease associated with fever, anaemia and other pathologies, is caused by species of Plasmodium. This genus infects mammals, birds and lizards, and is transmitted by the bite of female mosquitoes (Anopheles species in mammals, or Culex species in birds and lizards) in which part of its life cycle is spent. Mammalian malarias are confined to antelopes, lemurs, bats, rodents and primates (including humans), and absent in felids, canids, equids or bovids, for unknown reasons (Cox-Singh and Singh, 2008).

Human and other mammalian Plasmodium species are transmitted by anopheles' mosquitoes. The parasite is injected with the saliva during mosquito feeding and first undergoes a round of merogony in the liver followed by multiple rounds of merogony in the erythrocytes (Figure 1.2). Gametogony begins within the erythrocytes of the vertebrate host and is completed within the mosquito where sporogony takes place. This life cycle exhibits the general features of other apicomplexan parasites characterized by asexual replication and the formation of invasive stages with typical apical organelles (Clark *et al*., 2003).

The infection is initiated when sporozoites are injected with the saliva during mosquito feeding. The sporozoites enter the circulatory system and within 30-60 minutes will invade a liver cell. The sporozoites gain access to the hepatocytes by first invading and traversing a Kupffer cell. Host cell entry, as in all apicomplexa, is facilitated by the apical organelles. After exiting the Kupffer cell, the sporozoite can traverse several hepatocytes before developing into an exoerythrocytic (or pre-erythrocytic) schizont. Schizogony refers to an asexual replicative process in which the parasite undergoes multiple rounds of nuclear division without cytoplasmic division followed by a budding, or segmentation, to form progeny called merozoites. The merozoites are released into the circulatory system

following rupture of the host hepatocytes. Recent observations suggest that the merozoites are released as a membrane bound aggregate, called a merosome, from the dying hepatocytes and the merosomes are delivered to the blood stream. This presumably provides protection from phagocytosis of the free merozoites by the Kupffer cells of the liver (Hyde, 2007).

In *P. vivax* and *P. ovale* some of the sporozoites do not immediately undergo asexual replication, but enter a dormant phase known as the hypnozoite. This hypnozoite can reactivate and undergo schizogony at a later time resulting in a relapse. Relapse has a specific meaning in regards to malaria and refers to the reactivation of the infection via hypnozoites. Recrudescence is used to describe the situation in which parasitaemia falls below detectable levels and then later increases to a patent parasitaemia (Hyde, 2007).

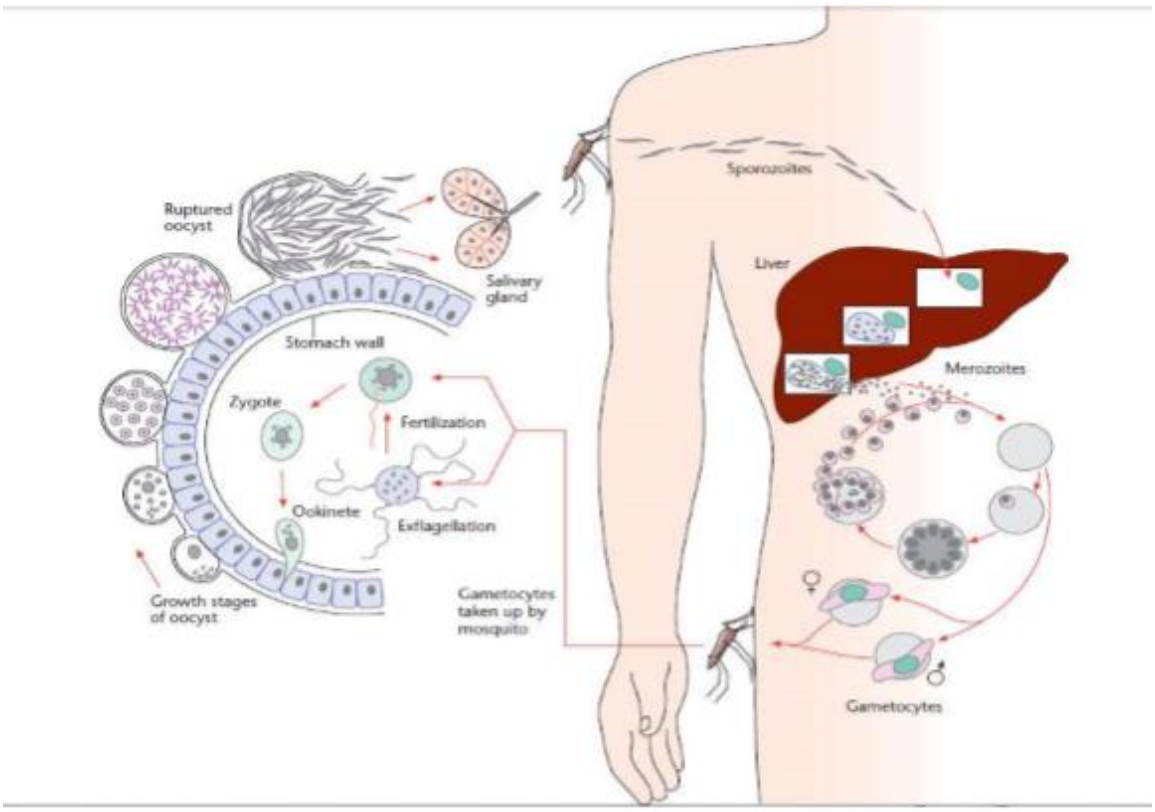


Figure 2.2: Life cycle of malaria parasite (Hyde, 2007).

In Blood Stage, Merozoites released from the infected liver cells invade erythrocytes. The merozoites recognize specific proteins on the surface of the erythrocyte and actively invade the cell in a manner similar to other apicomplexan parasites. After entering the erythrocyte, the parasite undergoes a trophic period followed by an asexual replication. The young trophozoite is often called a ring form due to its morphology. As the parasite increases in size this 'ring' morphology disappears and it is called a trophozoite. During the trophic period the parasite ingests the host cell cytoplasm and breaks down the haemoglobin into amino acids. A by-product of the haemoglobin digestion is the malaria pigment, or hemozoin. These golden-brown to black granules have been long recognized as a distinctive feature of blood-stage malaria parasites. Nuclear division marks the end of the trophozoite stage and the beginning of the schizont stage. Erythrocytic schizogony consists of 3-5 rounds (depending on species) of nuclear replication followed by a budding process. Late stage schizonts in which the individual merozoites become discernible are called segments. The host erythrocyte ruptures and releases the merozoites. These merozoites invade new erythrocytes and initiate another round of schizogony. The blood-stage parasites within a host usually undergo a synchronous schizogony. The simultaneous rupture of the infected erythrocytes and the concomitant release of antigens and waste products accounts for the intermittent fever paroxysms associated with malaria. Blood stage schizogony in *P.falciparum* differs from the other human malarial parasites in that trophozoite- and schizont-infected erythrocytes adhere to capillary endothelial cells and are not found in the peripheral circulation. This sequestration is associated with cerebral malaria and other complications (Idro, 2005).

As an alternative to schizogony some of the parasites will undergo a sexual cycle and differentiate into either micro- or macrogametocytes. The factors involved in the induction of gametocytogenesis are not known. However, commitment to the sexual stage occurs

during the asexual erythrocytic cycle that immediately precedes gametocyte formations since the daughter merozoites from a particular schizont will develop into either all asexual forms or all sexual forms. Gametocytes do not cause pathology in the human host and will disappear from the circulation if not taken up by a mosquito. Gametogenesis, or the formation of micro- and macrogametes, is induced when the gametocytes are ingested by a mosquito. After ingestion by the mosquito, the microgametocyte undergoes three rounds of nuclear replication. These eight nuclei then become associated with flagella that emerge from the body of the microgametocyte. The macrogametocytes mature into macrogametes. The highly mobile microgametes will seek out and fuse with macrogametes. Within 12-24 hours the resulting zygote develops into an ookinete. The ookinete is a motile and invasive stage which can transverse both the peritrophic membrane and the midgut epithelium of the mosquito. Traversing the peritrophic membrane probably involves secretion of chattiness. Penetration of the midgut epithelium involves invading and exiting several epithelial cells before emerging on the basal side of the epithelium. The invasion process is similar to other apicomplexan except that the ookinete does not have rhoptries and does not form a parasitophorous vacuole after invading the host cell (Greenwood, 2008).

After reaching the extracellular space between the epithelial cells and the basal lamina, the ookinete develops into an oocyst. Oocysts undergo an asexual replication, called sporogony, which culminates in the production of several thousand sporozoites. This generally takes 10-28 days depending on species and temperature. Upon maturation the oocyst ruptures and releases the sporozoites which cross the basal lamina into the hemocoel (body cavity) of the mosquito. These sporozoites are motile and have an ability to specifically recognize the salivary glands. After finding the salivary glands the sporozoites will invade and transverse the salivary gland epithelial cells and come to lie within its lumen. These sporozoites will be expelled into the vertebrate host as the mosquito takes a

blood meal, and thus reinitiate the infection in the vertebrate host. Although the hemocoel and salivary gland sporozoites are morphologically similar, they are functionally distinct. Salivary gland sporozoites efficiently invade liver cells, but cannot re-invade the salivary glands, whereas the hemocoel sporozoites are inefficient at invading liver cells (Greenwood, 2008)

2.7 Cerebellum

The cerebellum, is the largest part of the hindbrain as is dorsal to the pons and medulla, and its median region is separated from them by the fourth ventricle. It is joined to the brain stem by three pairs of cerebellar peduncles, which contain afferent and efferent fibres. The cerebellum occupies the posterior cranial fossa, where it is covered by the tentorium cerebelli. It is roughly spherical, but somewhat constricted in its median region, and flattened - its greatest diameter is transverse. In adults the weight ratio of cerebellum to cerebrum is c.1:10, in infants c.1:20.

2.7.1 Functions of the cerebellum

The cerebellum performs the following function:

i. Coordination of Movement; the cerebellum controls the timing and pattern of muscle activation during movement.
ii. Maintenance of Equilibrium in conjunction with the vestibular system.
iii. Regulation of Muscle Tone and modulation of spinal cord and brain stem mechanisms involved in postural control (Linas *et al*., 2004).

2.7.2 Morphology of the cerebellum

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

2.7.3 Cellular layers of the cerebellum

The cerebellar cortex of adults is divided into three layers. The layers from the lower to the upper include the followings as shown in Figure 2.3.

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

ii. The Purkinje layer lies in the middle, forming a narrow zone that contains only the cell bodies of Purkinje cells (Linas *et al*.,2004). They are distinguished by the shape of the dendritic tree. The dendrites branch very profusely, but are severely flattened

in a plane perpendicular to the cerebellar folds. Thus, the dendrites of a Purkinje cell form a dense planar net, through which parallel fibres pass at right angles (Bell, 2008; Linas *et al*.,2004). The dendrites are covered with dendritic spines, each of which receives synaptic input from a parallel fibre. Purkinje cells receive more synaptic inputs than any other type of cell in the brain. The large, spherical cell bodies of Purkinje cells are packed into a narrow layer, one cell thick of the cerebellar cortex, called the Purkinje layer (Linas, *et al.*, 2004).

iii. The molecular layer is thick. It contains a sparse population of neurones,dendritic arborizations, non-myelinated axons and radial fibres of the neuroglial cells. (Gray, 2000; Clerke, 1996). Purkinje cell dendritic trees extend towards the surface and spread out in a plane perpendicular to the long axis of the cerebellar folia. Purkinje cell dendrites are flattened. This outermost layer of the cerebellar cortex also contains two types of inhibitory interneurons, stellate cells, and basket cells (Linas *et al*., 2004).

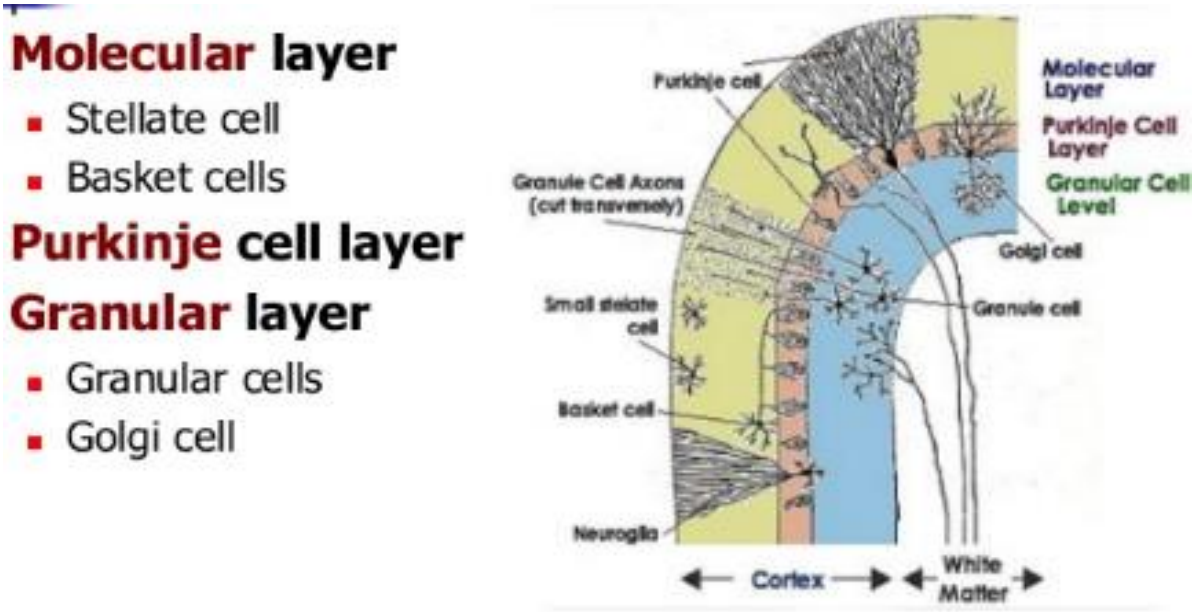


Figure 2.3: Layers of the Cerebellar Cortex (Kandel *et al.*, 2000)

The cerebellum, is the largest part of the hindbrain as is dorsal to the pons and medulla, and its median region is separated from them by the fourth ventricle. It is joined to the brain stem by three pairs of cerebellar peduncles, which contain afferent and efferent fibres. The cerebellum occupies the posterior cranial fossa, where it is covered by the tentorium cerebelli. It is roughly spherical, but somewhat constricted in its median region, and flattened - its greatest diameter is transverse. In adults the weight ratio of cerebellum to cerebrum is c.1:10, in infants c.1:20.

The basic internal organization of the cerebellum is of a superficial, highly convoluted cortex (a laminated sheet of neurones and supporting cells) overlying a dense core of white matter. The latter contains deep cerebellar nuclei, which give rise to the efferent cerebellar projections. Although the human cerebellum makes up approximately one-tenth of the entire brain by weight, the surface area of the cerebellar cortex, if unfolded, would be about half that of the cerebral cortex. The great majority of cerebellar neurones are small granule cells, which are so densely packed that the cerebellar cortex contains many more neurones than the cerebral cortex. Unlike the cerebral cortex, where a large number of diverse cell types are arranged differently in different regions, the cerebellar cortex contains a relatively small number of different cell types, which are interconnected in a highly stereotyped way. Consequently, one region of the cerebellar cortex looks very much like another (Gray's anatomy).

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 cell perikaryons, is also easily recognizable. The molecular layer is rich in axons and dendrites as well as capillaries

that penetrate deep into this layer. Four pairs of nuclei are located within the substance of the cerebellar white matter. These are the fastigial, dentate, emboliform, and globose nuclei. The connections between the cortical regions and the deep nuclei of the cerebellum permit the subdivision of the cerebellum into three zones— the vermal, paravermal, and hemispheric where each zone is composed of deep cerebellar nuclei, white matter, and cortex. (Gross anatomy of the brain)

Pre and postnatal Stage

This stage is characterised by extensive growth of the brain. In rats, neuronal cell populations are produced up to postnatal day 21; in the rat cerebellum, around half of all cell population is produced is produced in the postnatal period (Krinke and Eisenbrandt, 1994). According to Bayer and Altman (1995), central nervous system proliferation and differentiation continues after birth in many species.

2.8 Cerebrum

2.8.1 The morphology of the cerebrum

The cerebrum comprises a large portion of the brain. It lies in front or on top of the brainstem and in humans is the largest and best-developed of the five major divisions of the brain. The cerebral cortex is the largest part of the brain; it is formed by grey matter and cerebral hemisphere on the surface as shown in Figure 2.4. The cortex is convoluted with projections called sulci and gyri. The cerebral cortex is about 1.5 - 4mm thick, and contains nerve cells, fibres, neuroglia and blood vessels. The cerebral hemisphere is supplied by cerebral branches of polygon of Willis i.e. the anterior, middle and posterior cerebral arteries (Kandel *et al.,* 2000).

2.8.2 Anatomy frontal lobe

The frontal lobe extends from the frontal pole to the central sulcus**.** On its lateral aspect, the frontal lobe extends from the frontal pole to the central sulcus, constituting the anterior one-third of the cerebral cortex. Its posteriormost gyrus, the precentral gyrus, consists of the primary motor area and is bordered anteriorly by the precentral sulcus and posteriorly by the central sulcus. The region of the frontal lobe located anterior to the precentral sulcus is subdivided into the superior, middle, and inferior frontal gyri. This subdivision is due to the presence, though inconsistent, of two longitudinally disposed sulci, the superior and inferior frontal sulci. The inferior frontal gyrus is demarcated by extensions of the lateral fissure into three subregions: the pars triangularis, pars opercularis, and pars orbitalis. In the dominant hemisphere, a region of the inferior frontal gyrus is known as Broca's area, which functions in the production of speech.

On its inferior aspect, the frontal lobe presents the longitudinally disposed olfactory sulcus. Medial to this sulcus is the gyrus rectus (also known as the straight gyrus), and lateral to it are the orbital gyri. The olfactory sulcus is partly occupying by the olfactory bulb and olfactory tract. At its posterior extent, the olfactory tract bifurcates to form the lateral and medial olfactory striae. The intervening area between the two striae is triangular and is known as the olfactory trigone and it abuts the anterior perforated substance. On its medial aspect, the frontal lobe is bordered by the arched cingulate sulcus, which forms the boundary of the superior aspect of the cingulate gyrus. The quadrangular-shaped cortical tissue anterior to the central sulcus is a continuation of the precentral gyrus and is known as the anterior paracentral lobule (Maria and leslie, 2006).

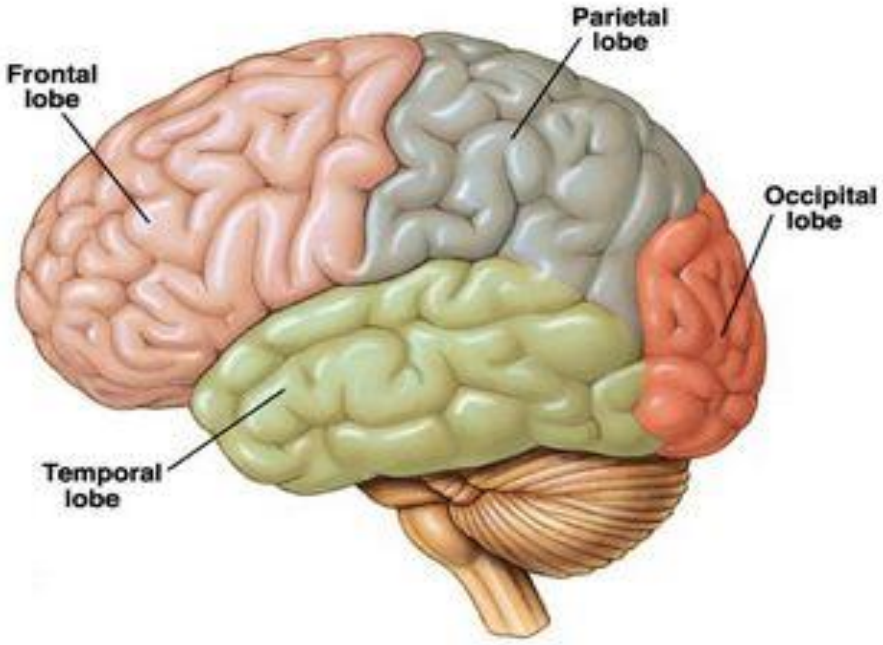


Figure 2.4: The lobes of the cerebral cortex (Anusa, 2012).

2.8.3 Cellular layers of the cerebral cortex

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

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

The stellate cells: They are small and multipolar and form 1/3 (one third) of the total population of neurons of the cortex under low magnification. They look like granular cells. They are therefore being termed granular neurons (cells) by earlier workers.

Stellates cell are of various types depending on their bodies and on the pattern of their ramification processes. Their axons are short and end within the cortex and their processes extend chiefly in radical direction within the cortex in some case horizontally.

Some Stellates cells may be uniform rather than Stellates, with one process arising at either end. Stellates neurons are classified as spiny and non-spiny neuron. The spiny Stellates

cells use glutamate as neurotransmitter, while in most non-spiny Stellates cells it is Gamma amino Butyric Acid (GABA). The neurons of the cerebral cortex are grouped into six main layers, from outside to inside namely as shown in Figure 2.5, according to Saladin, (2010); Shipp, (2007); Kandel *et al*. (2000).

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

Layer II, the External granular layer, contains small pyramidal neurons and numerous stellate neurons (Kandel *et al*., 2000).

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

Layer IV, the Internal Granular layer, contains different types of stellate and pyramidal neurons, and is the main target of thalamocortical afferents from thalamus type C neurons as well as intra-hemispheric corticocortical afferents (Kandel *et al.*, 2000).

Layer V, the Internal Pyramidal layer, contains large pyramidal neurons such as the Betz cells in the primary motor cortex. It is the principal source of subcortical efferent, as such

there are large pyramidal cells which give rise to axons leaving the cortex and running down through the basal ganglia, the brain stem and the spinal cord (Kandel *et al*., 2000).

Layer VI, the Polymorphic or Multiform layer contains few large pyramidal neurons and many small spindle-like pyramidal and multiform neurons. Layer VI sends efferent fibres to the thalamus, establishing a very precise reciprocal interconnection between the cortex and the thalamus. These connections are both excitatory and inhibitory. Neurons send excitatory fibres to neurons in the thalamus and also from collateral to other ones via the thalamic reticular nucleus that inhibit these thalamus neurons or ones adjacent to them (Kandel *et al*., 2000). Since the inhibitory output is reduced by cholinergic input to the cerebral cortex, this provides the brainstem with adjustable "gain control for the relay of lemniscus inputs (Kandel *et al.*, 2000).

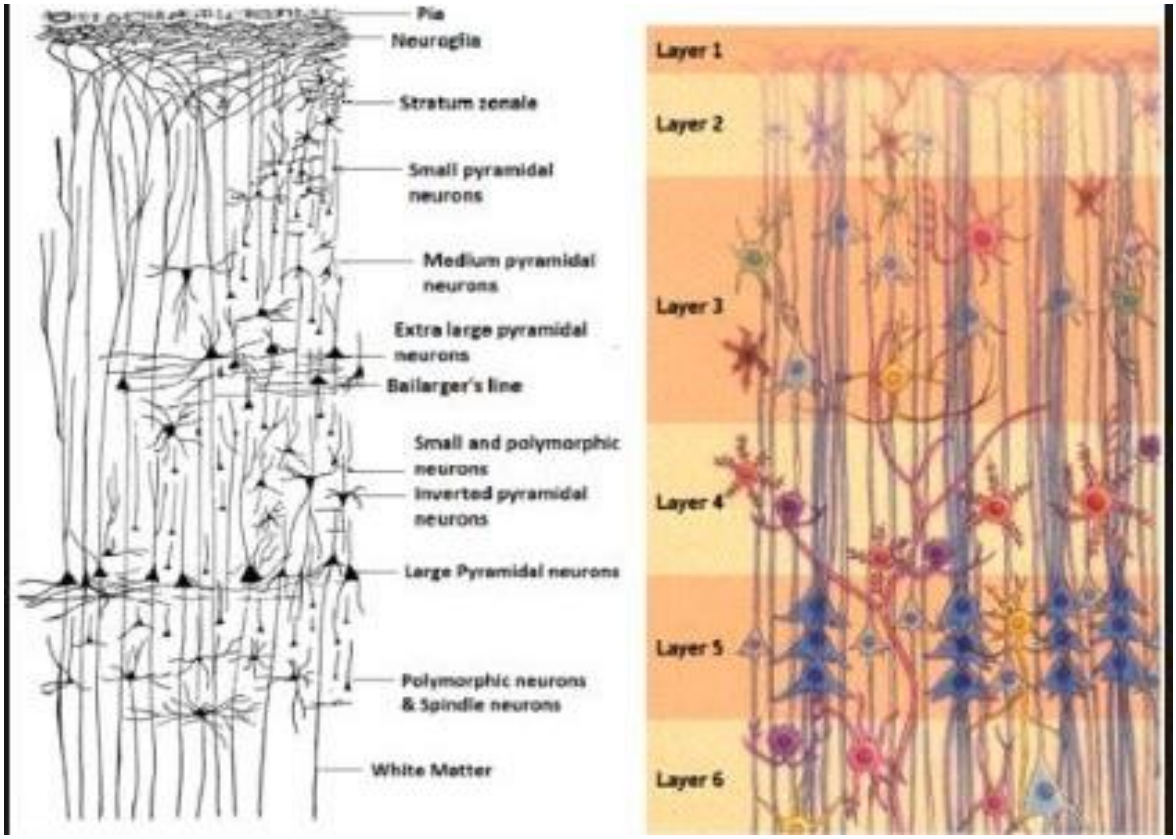


Figure 2.5: Layers of the cerebral cortex (Kandel *et al.*, 2000)

2.8.4 Variations of cortical structure

The structure of the cerebral cortex shows considerable variations in the region, both in terms of thickness and in prominence of the various laminae. These variations are as follows: In the agranular cortex, the external and internal granular Laminae are inconspicuous. This type of cortex is referred to as precentral gyrus (area 4) and is therefore to be of typical area. In the granular cortex, the granular cortex is highly developed while the pyramidal and ganglion layer are poorly developed or absent. This type of cortex is mostly present in sensory area including the post-central gyrus (Kandel *et al*., 2000).

2.8.5 Functions associated with cerebrum

The cerebrum is the most highly developed part of the human brain and is responsible for determining intelligence, motor function, planning and organization, touch sensation, thinking, perceiving, producing and understanding language. Most information processing occurs in the cerebral cortex. The cerebral cortex is divided into lobes of which each has a specific function (Kandel *et al*., 2000).

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

possibly part of area 6, which lies in the precentral gyrus, the specific region in the area responsible for movement of specific part of the body (Kandel *et al.*, 2000).

Sensory processing: The primary sensory areas of the cerebral cortex receive and process visual, auditory, somato-sensory, gustatory, and olfactory information. Together with association cortical areas, these brain regions synthesize sensory information into our perceptions of the world around us (Kandel *et al.*, 2000).

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

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Digital Microscope (Celestron and Olympus), Distilled water, Computers, Bouins fluid, Beakers, Specimen bottles, Digital weighing balance, 1ml and 5ml syringes and injecting needles, Dissecting tray, Chloroform and Dissecting kit.

3.2 Plant Collection and Extraction

The fresh leaves of *Vernonia amgdalina* plant were collected based on Ethnobotanical description and with the help of local traditional healers around Mada Area Development Council in Gusau local government area of Zamfara state, Nigeria, in May 2017. The plants were identified and authenticated at Botany Department, Faculty of Life Science, Ahmadu Bello University Zaria, with voucher number (12063). The Fresh leaves of *Vernonia amgdalina* were cleaned from extraneous materials, air-dried under shade at room temperature then pounded into powder in Biochemistry Laboratory, of Umaru Musa Yardua University Katsina state, Nigeria. The powdered plant material was weighed (500g) using SCIENTECH Mode No SL 3100D Rev-c accuracy class (II) (Debella, 2002).

3.3 Ethanol Extract Preparation

Powdered *Vernonia amgdalina* (500g) was macerated with 80 % ethanol for 72 hours with intermittent agitation by Orbital shaker at 120 revolutions per minute. The supernatant part of agitated material filtered with 15 cm Whatman grade1 filter paper two times. The filtrate of *Vernonia amgdalina* was then concentrated using Rotary evaporator (BUCHI R250,Switzerland) at 40^{o}c to remove methanol and further dried using in a lyophilizer

(CHRIST, 3660 Osterode/harz/, France) to remove water and the extract were kept at −20°C until used (Debella, 2002).

Table 3.1: Percentage Yield of Ethanolic Extract of *Vernonia amygdalina*

Plant Species	Solvent	Weight of powder	Vol. of solvent	Weight of Yield (g)	% of yield
Vernonia amygdalina	Ethanol	500g	5000ml	235	53

3.4 In Vivo Acute Toxicity Tests

Acute oral toxicity of ethanolic leaf extract of *Vernonia amygdalinaa* was evaluated. Healthy adult Swiss albino mice maintained under standard laboratory conditions were used for acute toxicity test according to (LORKE, 1983). The varying dose was orally administered in accordance to the guideline of lorke's and no mortality observed in all mice. According to the up and down guideline of the lorke's method the mice were observed for 24 hrs for any toxicity signs like changes in physical appearance, behavioural change, motor and feeding activities, hair erection, rigidity, lacrimation, reduction in motor.

3.5 Experimental Animals Preparation

The animals employed for this study were premature young male and female 5-7 weeks of Albino Swiss mice (10- 15g). The mice were obtained from the Animal Facility, Faculty of Pharmaceutical Sciences of Ahmadu Bello University Zaria, Nigeria. The mice were put to full experimental condition because the experiment took place in the same venue of purchase by first screening the mice for any possible parasitic infection. Blood was collected from the tail of each mice to make smear which thereafter parasite level was screen for. Also, the PCV of the mice was taken into account. Finally, all the mice had a

relative parasite free blood and PCV count which justify the mice for the experiment. They were then housed in cages and had unrestricted access to a standard pellet diet and water *ad libitum*. The animals were maintained under the natural light-dark cycle throughout the duration of the study. For in-vivo anti-malarial assays of plant extracts, the mouse-infective chloroquine sensitive strain of *P. berghei* (ANKA) obtained from National Institute of Medical agency Lagos State was kept alive by continuous intraperitoneal (i.p.) serial blood passage from mice to mice on research demand by the laboratory staff of the animal facility of Faculty of Pharmaceutical science, Ahmadu Bello University Zaria.

3.6 In Vivo Evaluation of the Anti-Malarial Activity of Plant Extracts

3.6.1 Four-day suppressive test

The standard four-day suppressive method as described by Peter (1967) was employed. Twenty (20) Swiss albino young mice weighing (10-15) g were injected with infected RBC (infected with *P. berghei*) and randomly divided into respective groups of four mice per cage.

Table 3.2: Grouping and Treatment (Suppressive Design)

GROUPS	TREATMENT	DURATION
GROUP 1	0.2ml distilled water orally	4 days
GROUP 2	*P. berghei* parasite	4 days
GROUP 3	*P. berghei* + ELVA 2500mg/kg	4 days
GROUP 4	*P. berghei* + ELVA 1250mg/kg	4 days
GROUP 5	*P. berghei* + 10mg/kg of Chloroquine	4 days

After 3 hours, Post inoculation treatment is ELVA. ELVA: Ethanol Leaf Extract of *Vernonia amygdalina*

All the extracts and the drug were given through oral route using an intragastric tube to ensure safe ingestion of the extracts and the drug. Blood sample was collected from the tail of each mouse to investigate for parasite load and PCV before the commencement of treatment. Treatment were started after 3 hours of inoculation on day 0 and continued daily for four days (i.e. from day 0 to day 3). On the fifth day (Day-4), 24 h after the last dose (i.e. 96 h post-infection), blood sample were collected from tail snip of each mouse according to methods of Kalra *et al*. (2006). The smears were applied on microscope slides fixed with absolute methanol for 15 min and stained with 15% Geimsa stain at pH 7.2 for 15 min. The stained slides were then washed gently using distilled water and air dried at room temperature. Then, each stained slide was examined under Olympus microscope (CHK2-F-GS, Taiwan) with an oil immersion objective of 100x magnification power to evaluate the percent suppression of each extract with respect to the control groups. The microscope eye piece shows about 100 red blood cells per field (Dikasso *et al*., 2006). The parasitaemia level was determined by counting minimum of five fields per slide with about 100 RBC in random field of the microscope.

Percent parasitaemia and percentage of suppression was calculated using formula described in all model (Adhroey *et al*., 2011).

$$\% \text{ Parasitaemia} = \frac{\textbf{Number of Parasitized RBC}}{\textbf{Total RBC counted}} \text{ X } 100\ \%$$

$$\% \text{ Suppression} = \frac{(\textbf{Parasitaemia in normal control}) - (\textbf{parasitaemia in treated group})}{\textbf{Parasitaemia in normal control}} \text{X } 100\ \%$$

3.6.1.1 Determination of Body Weight and PCV

The body weight of each mice in all the groups was measured before infection (day 0) and on (day 4) in case of treatment, it was measured before and after the different doses were given by a digital weighing balance. Blood collected from the tail of the mice were used in estimating the PCV before and after the treatment. Rectal temperature (Electronic thermometer model ZC) was also measured by a digital thermometer before infection and four hours after infection. (Satayavivad *et al*., 1998).

3.6.2 Seven-day curative test (Rane's test)

Evaluation of curative anti-malarial potential of the extract was done using a method described by Ryley and Peters, (1970) and modified by Carvalho *et al*., (1990). To assess the curative potential of the ethanol extracts on established *P. berghei* infection, 20 Swiss albino young mice were divided into five groups with each group containing four mouse, they were then inoculated with *P. berghei*. The mice were injected by intra peritoneal on the first day and infection was allowed to be established for 72 hrs.

Table 3.3: Grouping and Treatment (curative design)

GROUPS	TREATMENT	DURATION
GROUP 1	0.2ml distilled water orally	7 days
GROUP 2	*P. berghei* parasite	7 days
GROUP 3	*P. berghei* + ELVA 2500mg/kg	7 days
GROUP 4	*P. berghei* + ELVA 1250mg/kg	7 days
GROUP 5	*P. berghei* + 10mg/kg of Chloroquine	7 days

After 72 hours, Post inoculation treatment is ELVA. ELVA: Ethanol Leaf Extract of *Vernonia amygdalina*

Thin blood films stained with Giemsa were prepared from tail blood of each mouse on day 3 and day 7 to monitor the parasitaemia level. The animals were observed till the 7th days. Rectal temperature was measured day 3 and day 7 using digital rectal thermometer, while PCV and body weight were also measured at day 0(day 3) and day 4(day 7).

3.6.3 Prophylactic test (Repository Test)

Evaluation of prophylactic potential of the extract was done using Peters methods with slight modification (Peters, 1967). 20 Swiss albino young mice were divided into five groups with four mice per group (cage).

Table 3.4: Grouping and treatment (prophylactic design)

GROUPS	TREATMENT	DURATION
GROUP 1	0.2ml distilled water orally	8 days
GROUP 2	*P. berghei* parasite	8 days
GROUP 3	*P. berghei* + ELVA 2500mg/kg	8 days
GROUP 4	*P. berghei* + ELVA 1250mg/kg	8 days
GROUP 5	*P. berghei* + 1.2mg/kg of Pyrimethamine (Newton and Krishna, 1998)	8 days

Pre-inoculation treatment is ELVA. ELVA: Ethanol Leaf Extract of *Vernonia amygdalina*

After 72 Hrs, thin blood films stained with Giemsa were prepared from tail blood of each mouse on day 8 to monitor the parasitaemia level. Rectal temperature was also measured by a digital thermometer before infection and four hours after infection. The Packed Cell Volume (PCV) was measured to predict the effectiveness of the test extracts. This test was done on each mouse just before infection and on the 8th day of post infection and body weight were also measured at day 5 and day 8 and all parameter calculated based on the formula described (Adhroey *et al*., 2011).

3.7 Animal Sacrifice

Animals were humanely euthanized through cervical dislocation at completion of each test according to the test battery. Blood sample was collected for parasitaemia quantification and the brain tissue was then dissected out and fixed immediately in Bouin's fluid (Bancroft and Gamble, 2008).



Plate I: (A), (B), (C) and (D) Showing the Photograph of the Different Stages of the Research.

3.8 Tissue Processing

The Brain tissue were collected and fixed in Bouin's fluid for proper fixation. The tissue was processed routinely, sectioned and proceeded for staining using haematoxylin and eosin (H and E) technique and also a special stain using Cresyl Echt Violet method for the identification of neurons in tissue sections or the clear demonstration of both Nissl substance and nuclei or the loss of Nissl substance (Freida, 2007)

3.8.1 Haematoxylin and eosin (H and E) staining method

The methods of H and E staining were carried out by de-waxing the tissue in two changes of xylene for three minutes each, hydrated by passing them through descending grades of alcohol (100%, 95%, 90%, 70%) for three minutes each, then stain in Harris haematoxylin for ten minutes and then washed in tap water to remove excess stain. The slides were then flooded with acid alcohol for few seconds for differentiation and then washed in tap again. The slides were then blued in Scott's tap water for five minutes and then counter stain in Eosin for three minutes. The sections were rinsed in tap water and then dehydrated in ascending grades of alcohol and cleared in xylene. The sections were then cover slipped using a media (Frieda, 2007). Sections of the slides were made using digital Am scope (MD 900) microscope.

3.8.2 Cresyl Echt Violet method

Frozen sections were deparaffinized and hydrated to distilled water and then stain for 3 to 5 minutes in cresyl echt violet solution. The stained sections were rinsed in two changes of distilled water and placed in 95% alcohol for 30 seconds. Sections were transferred to absolute alcohol for 30 seconds and then placed in xylene and balsam-xylene mixture for 1minute and 2 minutes respectively. Differentiations were made in absolute alcohol, two

changes for 10 to 30 seconds each. The sections were taken through several changes of xylene and mounted with synthetic resin. Digital photomicrographs at the magnification of 250 were taken from all the experimental groups with the aids of MD 900 Am scope microscope digital camera (Freida, 2007).

3.9 Statistical Analyses

Data collected is presented as Mean ± SEM which was statistically analyses using Statistical Package for Social Science (SPSS), Version 20 (IBM, Incorp, NY). A *p*-value < 0.05 was considered significant. ANOVA was use to compare the levels of parasitaemia of the *P. berghei* infected mice between the control and extract treated groups at a fixed time. In addition, recommended charts were used to determine percent change of PCV and body weight of *P. berghei* infected mice between the comparison days. Also LSD Posthoc test was carried out. The results were presented as the Mean ± SEM (Standard Error of the Mean) and statistical significance was considered at a 95% confidence interval ($P<0.05$).

3.10 Ethical Consideration

Ethical clearance form was submitted to Ahmadu Bello University, research and ethics committee on the use of animals for this research project.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute Toxicity Test for the Plant Material (*Vernonia amygdalina*)

In vivo studies on the effect of ethanol extract from *Vernonia amygdalina* in the tested animals were carried out in the present study. The value of the ethanol extract of *Vernonia amygdalina* for acute toxicity given were 2900 and 5000mg/kg body weight. No death and other toxicological changes were observed. Brief representation of the observation is in table 4.1.

Table 4.1 Observed Parameters of the Toxicity Study of Ethanolic Extract of *Vernonia amygdalina*

Sign of Toxicity	2900mg/kg of *V. amygdalina*	5000mg/kg of *V. amygdalina*
Hyperactivity	**	**
Rigidity	**	**
Irritability	**	**
Jumping	**	**
Sedation	**	**
Abnormal Secretion	**	**
Coma	**	**
Death	**	**

** No sign of Toxicity.

4.2 Physical Observations

During the course of the research from the induction of malaria to the treatment process we observed that there are features of feverish condition in the beginning which includes reduction in appetite resulting in decreased intake of food resulting in weight loss until the end of the administration in all the groups.

There was gross difference in the fur of the animals which were exposed to the malaria and parasite than the animals which were exposed and where been treated with ethanol extract of *Vernonia amygdalina*.

Rectal Temperatures were taken to serve as an outcome to positive inoculation of the malaria parasite *P. berghei.* Some of the physical observed traits are express in a descriptive form in the respective designs of the in vivo activity of *Vernonia amygdalina* on *P. berghei.*

4.3 Four Day Suppressive Test

4.3.1 Percentage parasitaemia and suppression on a four day suppressive test

Antimalarial suppressive test results of ethanolic extracts of *Vernonia amygdalina* against *P. berghei* in Swiss albino mice are summarized in (Tables 4.2). The result indicates that the mean percentage parasitaemia in group 3 (2.84±0.05) was significantly lower (P value of 0.001) compare to Group 2 (4.44±0.16), group 4 (3.28±0.12) and group 5 (3.34±0.13).The Group 3 treated with (ELVA 2500mg/kg) shows highest percentage suppression of 74.4 follow by (ELVA 1250mg/kg) of 67.6 and (CQ 10mg/kg) of 64.5 respectively. Ethanol extract of the plant showed dose dependent suppressive effect at all doses used and the percentage suppression is a measure of parasite clearance. However, it did not clear the parasite completely on day four. Whereas, the high dose group (treated with 2500mg/kg ethanolic extract) had very less detectable parasitaemia at day four.

Table 4.2 Mean and SEM of Percentage Parasitaemia and Suppression at Day 4

Groups (n=4)	Treatment	Dose	% Parasitaemia	% Suppression	P-Value
1	Distilled water	P/bw	--------	----------	------
2	P.b	0.3ml	4.44 ± 0.16^{b}	----------	
3	V.A	2500mg/kg	2.84 ± 0.05^{abc}	74.4	<0.001
4	V.A	1250mg/kg	3.28 ± 0.12^{c}	67.6	
5	CQ	10mg/kg	3.34 ± 0.13^{a}	64.5	

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, CQ- Chloroquine Where N = Numbers of animal, P/bw- Per body weight

4.3.2 Body weight expression on the fourth day of suppressive test

The Mean ad SEM of the suppressive design at Day 0 indicates that in group 2 (10.76± 0.56) was significantly lower compare to Group 4 (13.42±0.47), group 3 (11.78±0.62), group 5 (11.28±0.27) and group 1 (11.08±0.49), with (P value) of 0.005 while at Day 4 group 4 (13.32±0.47) was significantly higher compared to group 2 (10.80±0.55), group 3 (12.04±0.50) and group 1 (11.18±0.49) with (P value) of 0.010.

Table 4.3 Body Weight of the Animals at Day 0 and Day 4 in a Four-Day Suppressive Test

Groups(n=4)	Treatment	Dose	BW (g)		P-value	
			Pre D0	Post D4	D0	D4
1	Dist. H_2O	P/bw	11.08 ± 0.49^{d}	11.18 ± 0.49^{c}		
2	P.b	0.2ml	10.76 ± 0.56^{a}	10.8 ± 0.55^{a}	0.005	0.010
3	V.A	2500mg/kg	11.78 ± 0.62^{b}	12.04 ± 0.50^{babcd}		
4	V.A	1250mg/kg	13.42 ± 0.47^{abcd}	13.32 ± 0.47^{abc}		
5	CQ	10mg/kg	11.28 ± 0.27^{c}	11.48 ± 0.27		

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, CQ-Chloroquine Where n: Numbers of animal, P/bw: Per body weight, Pre D0: before (Day 0), Post D4: After (Day 4), BW- Body Weight (g), Dist: Distilled water

4.3.3 Packed cell volume determination on 4 day suppressive test

The result indicates that the Packed cell volume at Day 0 in group 5 (53.20±1.59) was significantly higher compare to Group 1 (39.60±1.29), group 2 (40.40±1.03), Group 3 (40.20± 0.37) and group 4 (41.00±0.32) with P value of <0.001 while there is no significant difference observed in Day 4.

Table 4.4. PCV of the Animals at Day 0 and Day 4 in a Four-Day Suppressive Test

Groups	Treatment	Dose	PCV		P-Value	
			D 0	D 4	D 0	D 4
1	Distilled	P/bw	39.60±1.29[a]	39.00±0.95		
2	P. b	0.2ml	40.40±1.03[b]	41.80±1.63	<0.001	0.069
3	V.A.	2500mg/kg	40.20±0.37[c]	41.20±0.58		
4	V.A	1250mg/kg	41.00±0.32[d]	43.80±1.24		
5	CQ	10mg/kg	53.20±1.59[abcd]	41.40±0.51		

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, CQ- Chloroquine Where N = Numbers of animal, P/bw- Per body weight, Pre D0- before (Day 0), Post D4- After (Day 4).

4.3.4 Temperature of the mice before, during and after the treatment with *Vernonia amygdalina*

The mean temperature of the animals in the suppressive design of the activity of *Vernonia amygdalina* was taken and represented in figure 4.1 below. Group 1 administered with distilled water shows no temperature difference throughout the duration of the research compare to Group 2 which was inoculated with *P. berghei* parasite which present high temperature and a corresponding feverish condition which is characterised with reduction in feeding appetite.

Group 3 and Group 4 shows a similar pattern of representation in figure 4.4 where there is no difference between the period before inoculation and the period after inoculation but at day four which is the final day shows much graphical difference in temperature. The group which was treated with chloroquine shows no statistical difference in the temperature three hours after inoculation but with increment on the final day of the experiment which is at day four.

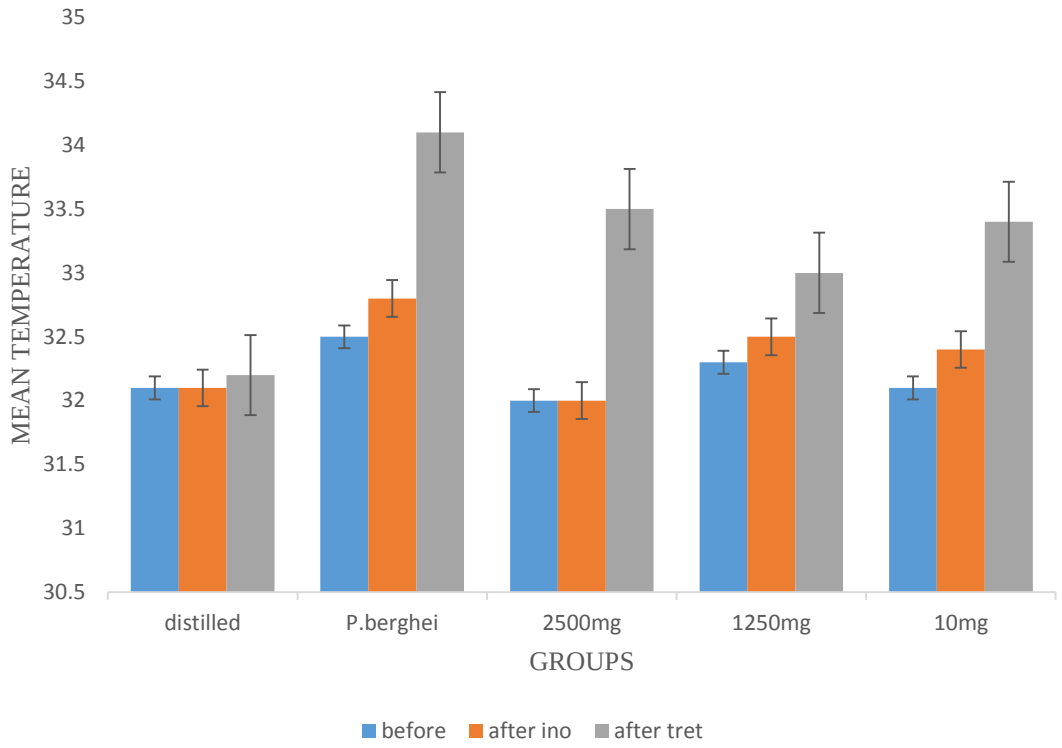


Figure 4.1: Representation of the Varying Mean Temperature

4.4 Seven-Day Curative Test (Rane's test)

On established infection, both dose (high, 2500mg/kg and low, 1250mg/kg) of ethanol extract caused a significant reduction in comparison to normal control which showed a daily increment of parasitaemia (table 4.5). The result indicates that the parasitaemia level of the mice in group 3 (2.64±0.10) was significantly lower compare to Group 2 (4.34±0.57) and group 4 (3.76±0.35) with a (P value) of 0.015 while there is no significant difference noted in the parasitaemia level at day 3 which is 72 hrs after inoculation and onset of treatment with (P value) of 0.619.

The ethanol extract of *Vernonia amygdalina* at doses (1250 and 2500) mg/kg and CQ 10mg/kg had shown statistically significant ($p<0.015$) parasitaemia suppression of 50.6%, 59.5%and 45.6% as compared to normal control group, respectively. From the investigation, it was observed that the extract exhibited a dose dependent curative effect with a slight difference between dose.

Table 4.5: Percentage of Parasitaemia and Suppression of the Curative Test of Ethanolic Extract of *V. amygdalina*

Group	Treatment	Dose	% Parasitaemia		% Suppression	P-value	
			PRE (D3)	POS D7)		D3	D7
1	Dist. H_2O	p/bw	-------	---------	-------	-----	------
2	P.b	0.3ml	3.58±0.24	4.34±0.57[b]	----------		
3	V.A	2500mg/kg	4.00±0.71	2.48±0.22	59.5	0.619	0.015
4	V.A	1250mg/kg	4.32±0.39	3.76±0.35[a]	45.6		
5	CQ	10mg/kg	3.60±0.24	2.64±0.10[ab]	50.6		

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, CQ- Chloroquine Where N = Numbers of animal, P/bw- Per body weight, Pre D3- before (Day 3), Pos D7- After (Day 7), D3- Day 3, D7- Day 7

4.4.1 Body weight determination of the mice in the curative test

In Curative test, the ethanolic extract treated mice exhibited statistically significant ($p<0.002$) increments of body weight across all groups between day 7 (Table 4.5). The result indicates that the body weight of the mice on day 3 in group 3 (10.76±0.56) was significantly lower compare to Group 2 (12.98±0.35), group 4 (14.06±0.54), group 5 (13.04±0.33), while at day 7 which is the final day of the design just before sacrifice Group 3 (10.94±0.59) was significantly lower compared to group 2 (12.62±0.49), group 4 (14.08±0.52) and group 5 (13.18±0.32) with (P value) of <0.001.

Table 4.6 Body Weight at Day 3 and Day 7 on a Seven-Day Curative Test

Groups	Treatment	Dose	Body Weight (g)		P-value	
			Pre D3	Post D7	D0	D4
1	Distilled H_20	P/bw	11.62±0.31	11.96±0.39		
2	P.b	0.3ml	12.98±0.35^{c}	12.62±0.49^{c}	<0.001	0.002
3	V.A	2500mg/kg	10.76±0.56^{abc}	10.94±0.59^{abc}		
4	V.A	1250mg/kg	14.06±0.54^{a}	14.08±0.52^{a}		
5	CQ	10mg/kg	13.04±0.33^{b}	13.18±0.32^{b}		

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, CQ- Chloroquine Where N = Numbers of animal, P/bw- Per body weight, Pre D0- before (Day 0), Post D7- After (Day 7), D3- Day 3, D4- Day 4

4.4.2 Packed cell volume determination on a seven day curative test

The result in table 4.7 indicate that the packed cell volume on the mice on day 7 in group 5 (32.80±0.74) was significantly lower ($p<0.001$) compare to Group 1 (39.60±1.29), group 2 (39.00±1.23) and group 3 (39.80±0.37) while there is no significant difference in the mice across the groups at day 3.

Table 4.7 PCV at Day 3 and Day 7 on a seven-day curative test

Groups	Treatment	Dose	PCV		P-Value	
			D 3	D 7	D 3	D 7
1	Distilled	P/bw	39.60±1.29	39.60±1.29[a]		
2	P. b	0.2ml	40.80±1.72	39.00±1.23[b]	0.570	< 0.001
3	V.A.	2500mg/kg	41.00±0.71	39.80±0.37[c]		
4	V.A	1250mg/kg	41.40±1.03	40.60±0.93[d]		
5	CQ	10mg/kg	39.00±0.84	32.80±0.74[abcd]		

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, CQ- Chloroquine Where N = Numbers of animal (N= 4), P/bw- Per body weight, D3- (Day 3), D7- (Day 7).

4.4.3 Temperature of the pups before, during and after the treatment with *Vernonia amygdalina*

The mean temperature of the animals in the suppressive design of the in vivo activity of *Vernonia amygdalina* was taken and represented in figure 4.2 below. Group 1 administered with distilled water shows no temperature variation compare to Group 2 which was inoculated with *P. berghei* parasite and was characterised with increased temperature.

Group 3 and Group 4 shows a similar pattern of representation in figure 4.2 where there is no difference between the period of inoculation and after inoculation but at day four which is the final day shows graphical increase in temperature. The group which was treated with chloroquine shows no distinguishing difference in the temperature before and after inoculation but with increment on the final day of the experiment which is at day four. In all there is no significant difference noted.

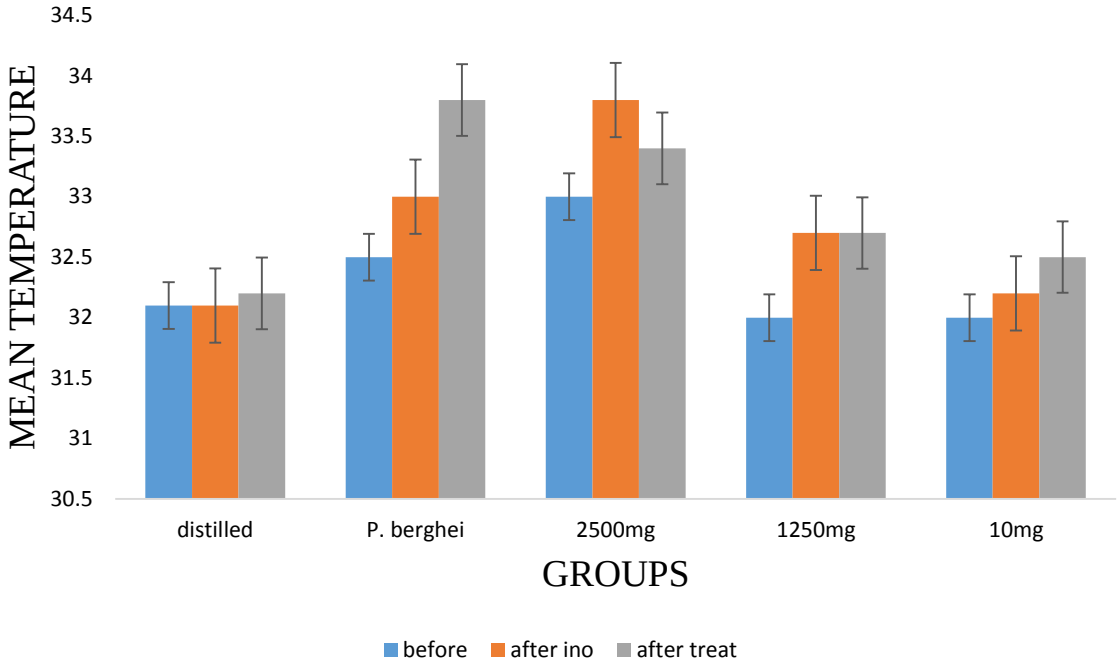


Figure 4.2: Representation of the Varying Temperature in a Seven-Day Curative Test

4.5 Prophylactic Test (Repository Test)

The prophylactic test which is basically (anti-plasmodial effect on residual infection) was also conducted on the ethanol extract of *Vernonia amygdalina* and exerted a dose dependent reduction of parasitaemia but with higher values of percentage parasitaemia when compared with the suppressive and curative test.

The ethanol extract of *V. amgydalina* exhibited different degrees of suppression at the doses (1250 and 2500) mg/kg and Pyrimethamine 1.2mg/kg which were (72.7, 61.7 and 76.2%, with $p<0.057$) respectively as compared to normal control group.

For the mean calculation of the level of parasitaemia the result indicates that there is no significant difference observed in the group.

Table 4.8. Percentage Parasitaemia and Suppression in a Prophylactic Test

Groups	Treatment	Dose	% Parasitaemia	% Suppression	P-Value
1	Distilled water	P/bw	---------	----------	
2	P.b	0.3ml	17.82±0.53	----------	
3	V.A	2500mg/kg	11.38±2.62	76.2	0.057
4	V.A	1250mg/kg	12.28±0.85	72.7	
5	Py	1.2mg/kg	16.26±0.38	61.7	

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, Py- Pyrimitamine Where N = Numbers of animal (4), P/bw- Per body weight

4.5.1 Body Weight Determination on Prophylactic Test

In Prophylactic test the ethanol extract treated mice shows statistically significant ($p<0.019$) increments of body weight across all groups on day 5 i.e. before induction of malaria parasite. (Table 4.9). The result indicates that at Day 5 the mice in group 3 (12.54 ±0.33) was significantly higher compare to Group 2 (10.76±0.56), group 4 (11.50± 0.16), group 5 (11.02±0.28) and group 1 (11.36±0.29), while there is no significant difference at Day 8 across the groups.

Table 4.9: Body Weight at Day 5 and Day 8 on a Prophylactic Test

Groups	Treatment	Dose	Body Weight (g)		P-value	
			Pre D4	Post D8	D4	D8
1	Distilled H_20	P/bw	11.36 ± 0.29^{d}	11.06±0.28		
2	P.b	0.3ml	10.76 ± 0.56^{a}	10.94±0.59	0.019	0.203
3	V.A	2500mg/kg	12.54 ± 0.33^{abcd}	12.56±0.72		
4	V.A	1250mg/kg	11.50 ± 0.16^{b}	11.52±0.36		
5	Py	1.2mg/kg	11.02 ± 0.28^{c}	11.74±0.45		

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, CQ- Chloroquine Where N = Numbers of animal (N= 4), P/bw- Per body weight, Pre D5- before (Day 5), Post D8- After (Day 8).

4.5.2 PCV Determination on Day 5 and Day 8 of the Prophylactic Test

The Packed cell volume result indicates that at Day 8 the mice in group 5 (29.60±0.51) was significantly lower ($p< 0.001$) compare to Group 1 (39.80±0.49), group 2 (38.40±0.68), Group 3 (38.80±0.37) and group 4 (39.40±0.40) while at Day 5 which is the onset of the test there is no significant different observed across the groups.

Table 4.10: PCV at Day 5 and Day 8 on a Prophylactic Test

Groups	Treatment	Dose	PCV		P-Value	
			D 5	D 8	D 5	D 8
1	Distilled	P/bw	39.60±1.29	39.80±0.49[a]		
2	P. b	0.2ml	38.80±0.66	38.40±0.68[b]	0.029	< 0.001
3	V.A.	2500mg/kg	40.80±0.66[b]	38.80±0.37[c]		
4	V.A	1250mg/kg	34.40±1.08[a]	39.40±0.40[d]		
5	Py	1.2mg/kg	37.00±2.03	29.60±0.51[abcd]		

P.b- induced with *Plasmodium berghei*, V.A- *Vernonia amygdalina* treated, Py- Pyrimithamine Where N = Numbers of animal (N= 4), P/bw- Per body weight, D5- (Day 5), D8- (Day 8).

4.5.3 Temperature of the mice before, during and after the treatment with *Vernonia amygdalina*

Figure 4.3 below shows the effect of the activity of *V. amygdalina* where Group 1 administered with distilled water shows no temperature difference throughout the duration of the research compare to Group 2 which was inoculated with *P. berghei* parasite.

In the graphical presentation below Group 3 and Group 4 shows a similar no significant difference between the period before inoculation and the period after inoculation but at day four which is the final day shows much graphical difference in temperature. The group which was treated with chloroquine shows no distinguishing difference in the temperature before and three hours after inoculation but with increment on the final day of the experiment which is at day four.

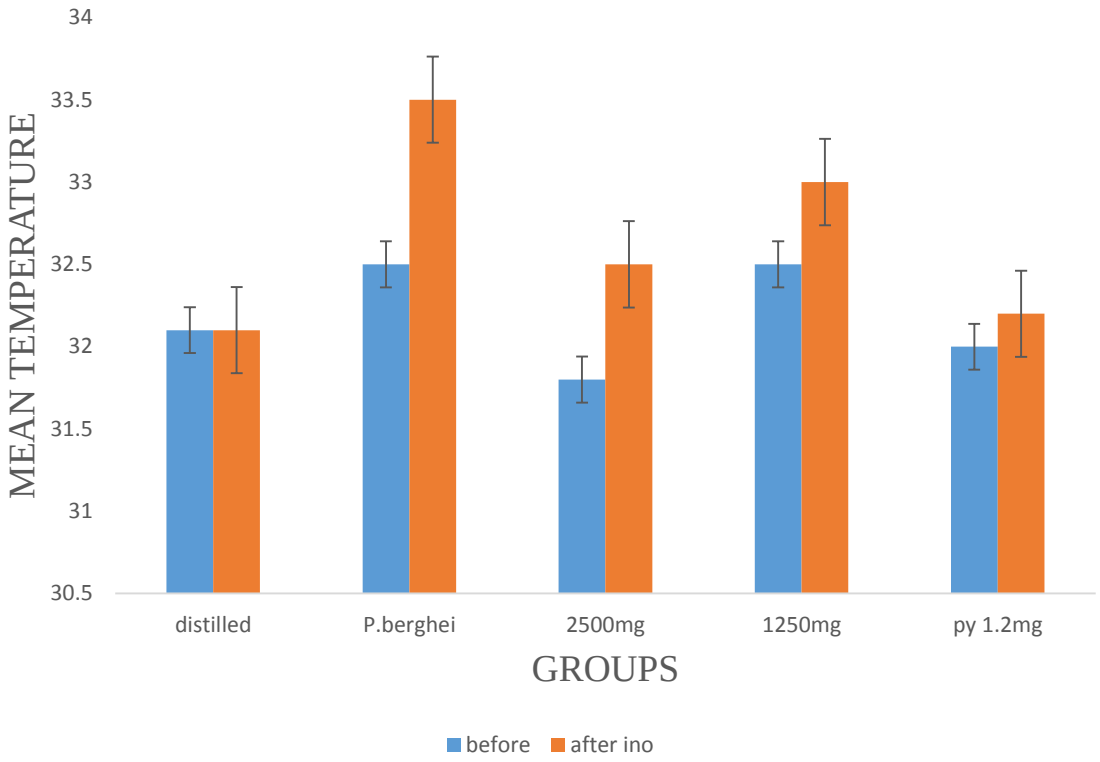


Figure 4.3: Representation of the Mean of the Varying Temperature in a Prophylactic Test

4.6 Histological Studies

4.6.1 Histopathological studies of cerebral cortex on a suppressive test, curative test and prophylactic test

The cerebral cortex of the mice in Group 1 shows normal architecture with normal pyramidal and few Stallete cells which are contained in layer 1 and layer II (Plate I). The layer 1 and II of the cerebral cortex of the mice in Group 2 shows distorted architecture with loss of majority cells content and degenerated characteristics. (Plate II). Cerebral cortex of layer I and layer II of the Group 3 and Group 4 mice shows degeneration in the cell structure and orientation of the cells are also affected (Plates III and IV), thus the Group 5 mice present similar cell structure degeneration which is in (Plate V).

Group 1 shows normal architecture with normal pyramidal and few Stellate cells which are contained in layer 1 and layer II of the cerebral cortex (Plate I). The layer 1 and II of the cerebral cortex of the mice in Group 2 shows distorted architecture with loss of majority cells content and degenerated characteristics. (Plate II). Group 3 and Group 4 mice treated with varying doses of the extract shows similar degeneration in the cell structure and orientation of the cells are also affected (Plates III and IV), thus the Group 5 mice present similar cell structure degeneration which is in (Plate V).

The cerebral cortex of the mice in Group 1 shows normal architecture with normal pyramidal and Stellate cells (Plate XI). The layer 1 and II of the cerebral cortex of the mice in Group 2 shows distorted architecture with loss of majority cells content and degenerated characteristics. (Plate XII). Cerebral cortex of layer I and layer II of the Group 3 and Group 4 mice shows degeneration in the cell structure and orientation of the cells are also affected (Plates XIII and XIV), thus the Group 5 mice shows degenerative features like nuclei displacement and disintegrated nuclei which is in (Plate XV).

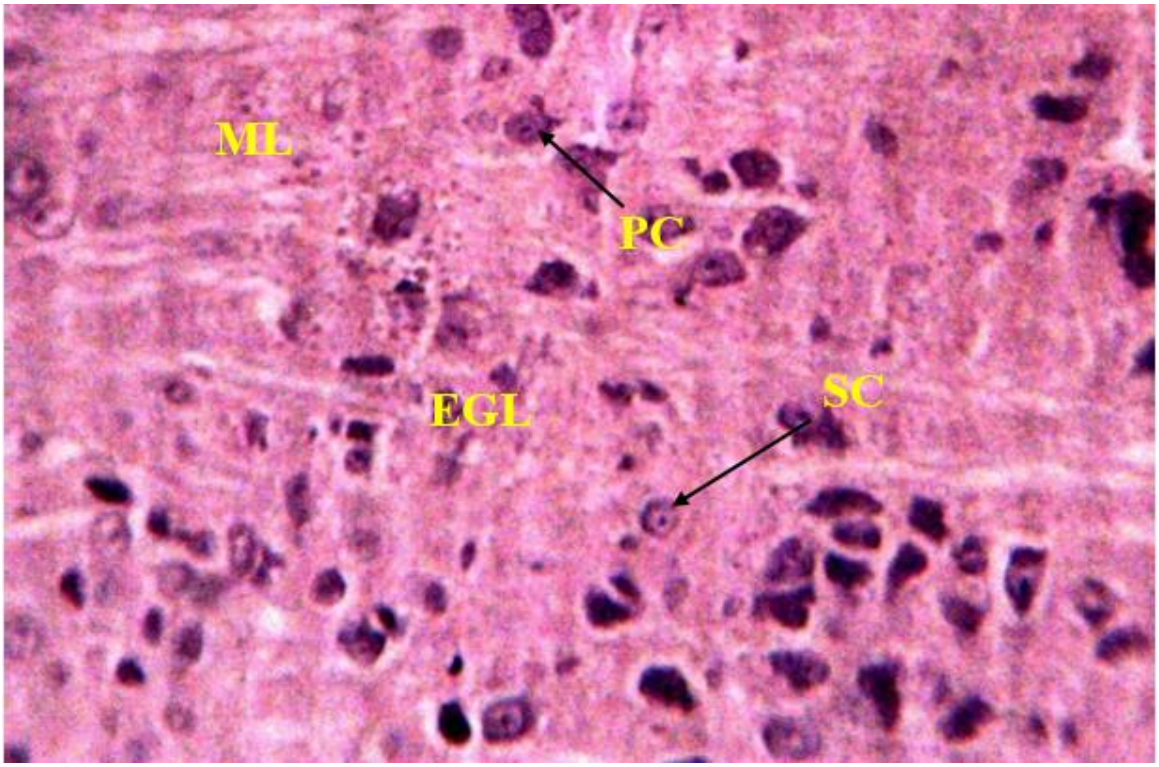


Plate II: Section of cerebral cortex of Group 1 (Control Group) showing normal architecture. EGL- External Granular layer, PC- Pyramidal cell, ML- Molecular layer, SC- Stellate cell (H & E X 250).

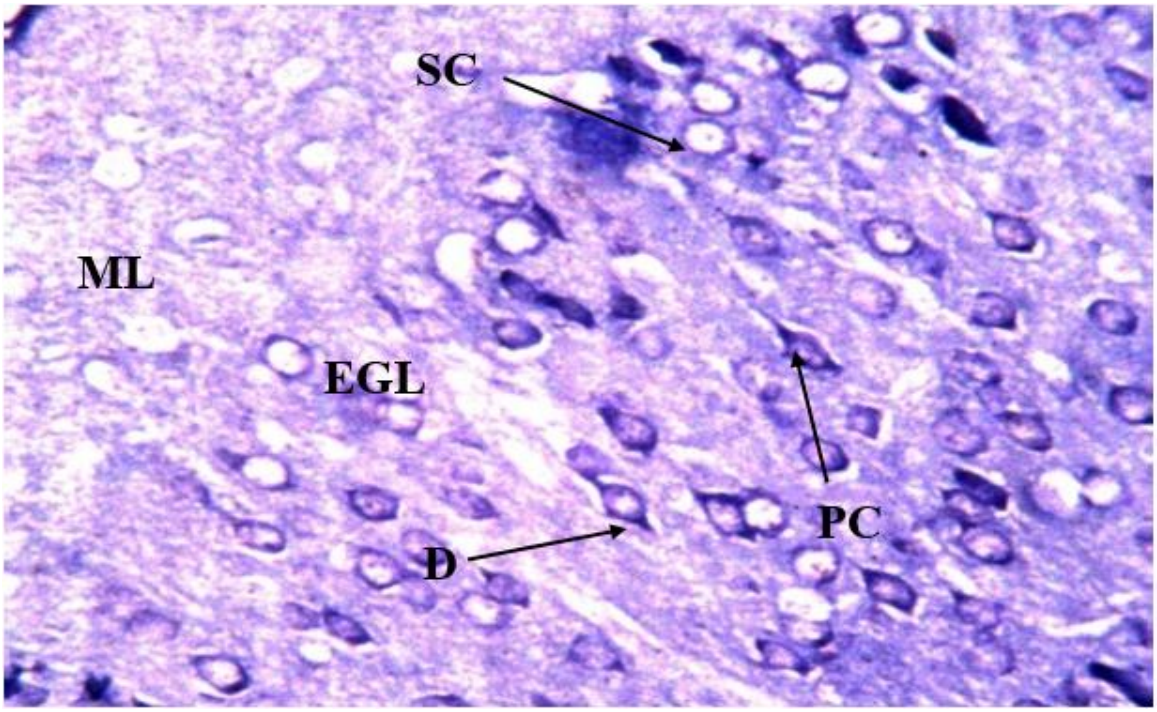


Plate III: Section of cerebral cortex of Group 1 (Control Group) showing normal architecture. SC- Stellate cell, PC- Pyramidal cell, EGL- External Granular layer, ML- Molecular layer, D- Dendrite (Cresyl Echt Violet X 250).

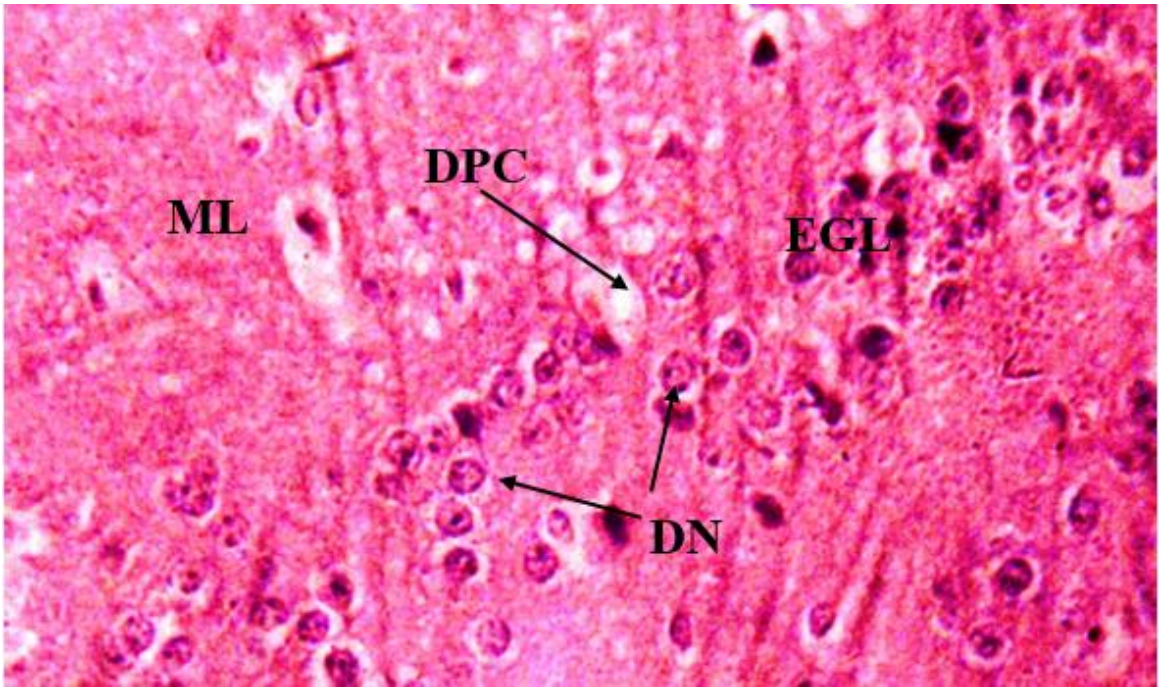


Plate IV: Section of cerebral cortex of Group 2 (Untreated Group) showing an overview of distorted architecture. DPC- Degenerated Pyramidal cells, EGL- External Granular layer, ML- Molecular layer, DN- Disintegrated nuclei (H & E X 250).

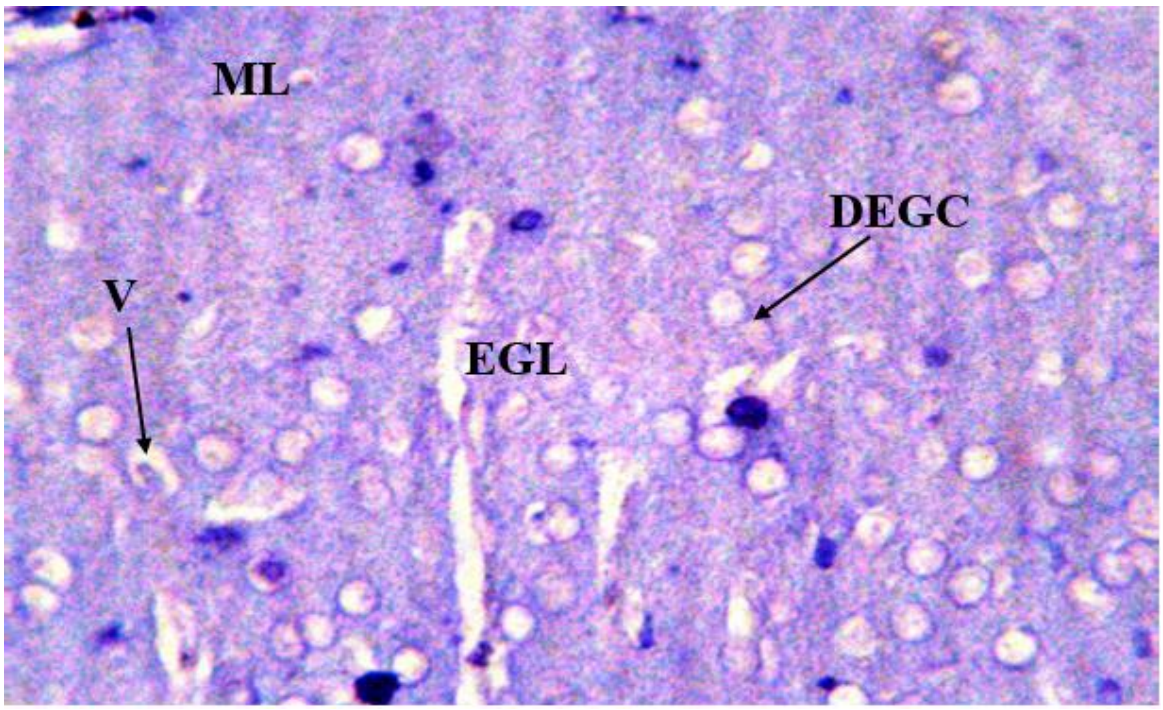


Plate V: Section of cerebral cortex of Group 2 (Untreated Group) showing an overview of distorted architecture. DEGC- Degenerating external granular cells (Stellate cells), EGL- External Granular layer, ML- Molecular layer, V- Vacuole (Cresyl Echt Violet X 250).

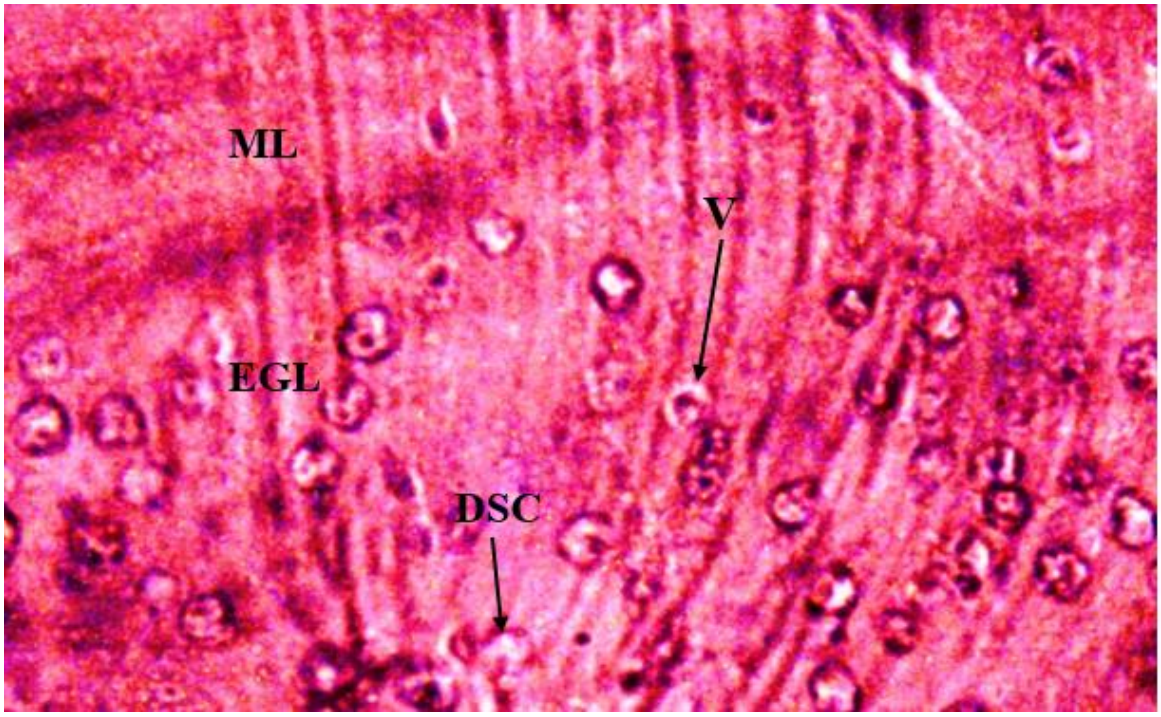


Plate VI: Section of cerebral cortex of Group 3 (Treated Group 2500mg/kg) showing sparse cellular degeneration. DSC- Degenerated Stellate cells, EGL- External Granular layer, ML- Molecular layer (H & E X 250).

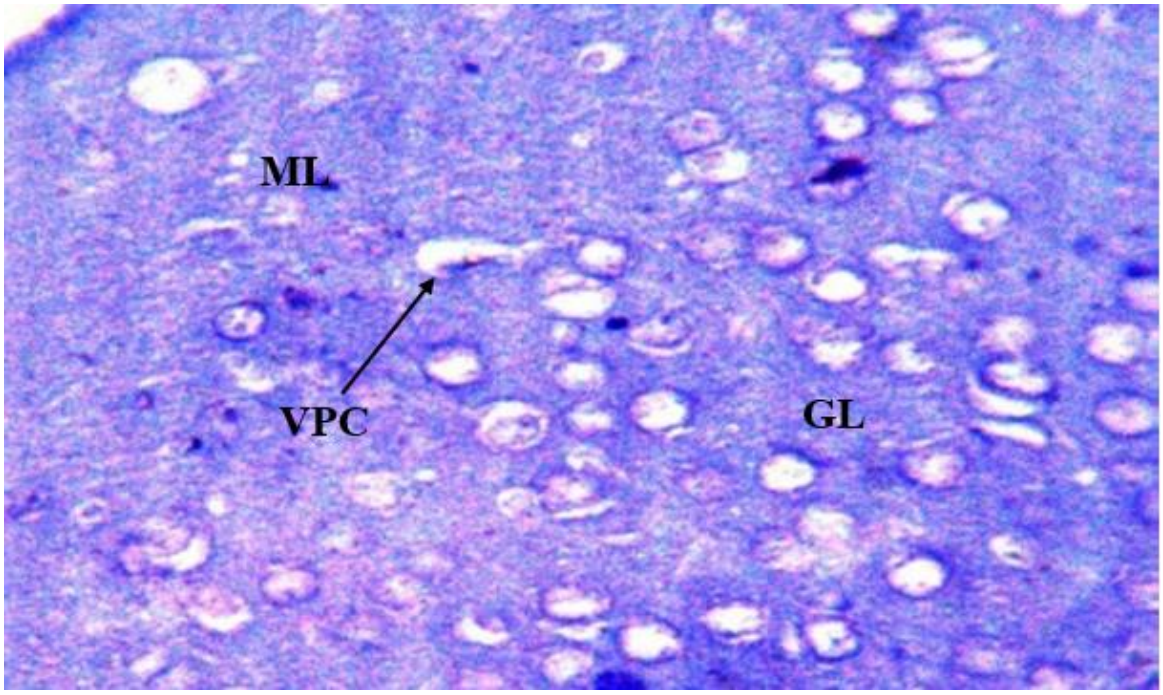


Plate VII: Section of cerebral cortex of Group 3 (Treated Group 2500mg/kg) showing sparse cellular degeneration. VPC- Vacuolated pyramidal cells, GL- Granular layer, ML- Molecular layer which is normal (Cresyl Echt Violet X 250).

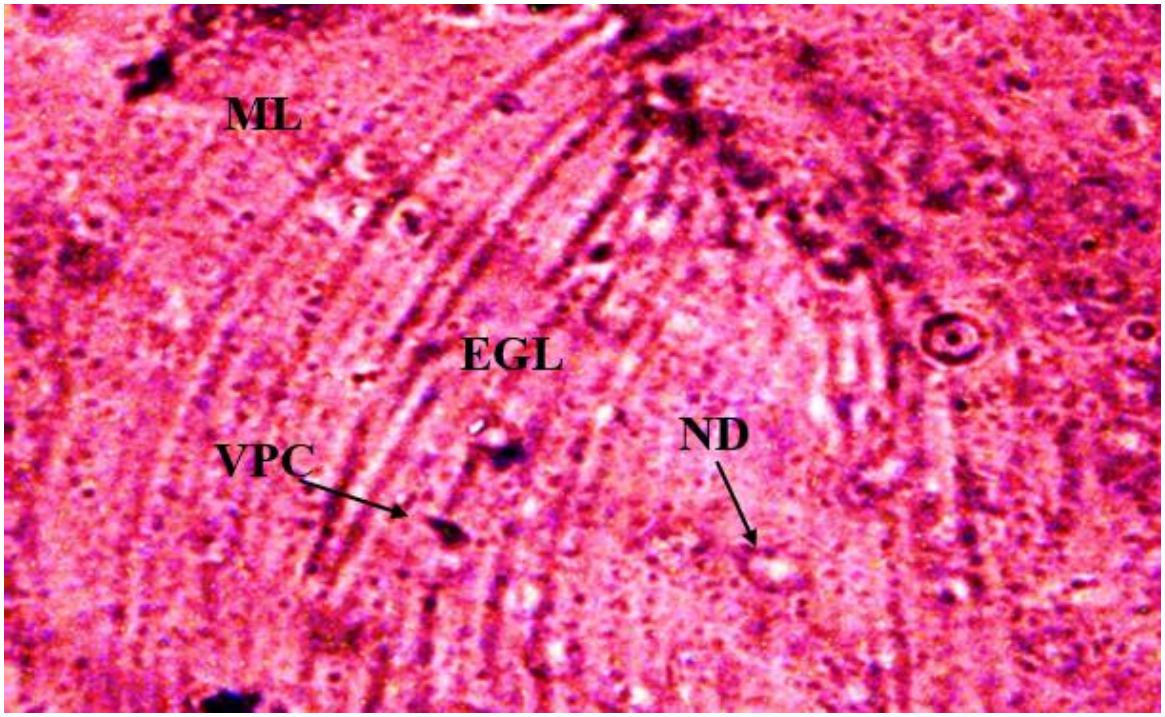


Plate VIII: Section of cerebral cortex of Group 4 (Treated Group 1250mg/kg). VPC- Vacuolated pyramidal cell, EGL- External Granular layer, ML- Molecular layer, ND – Nuclei displacement (H & E X 250).

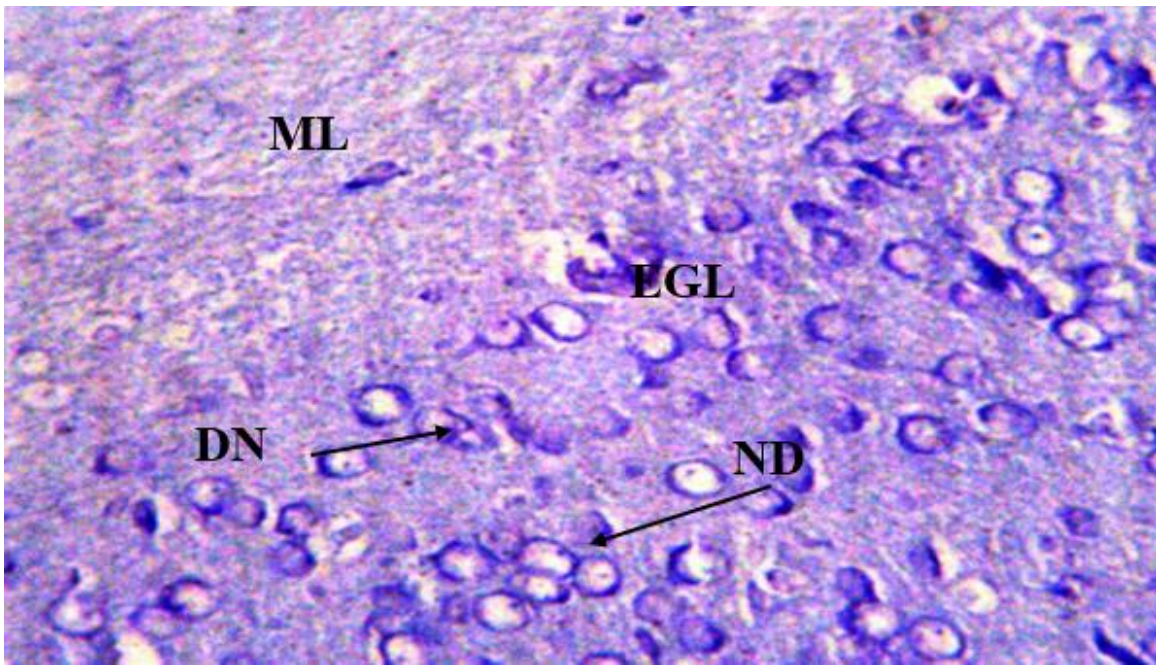


Plate IX: Section of cerebral cortex showing distorted cytoarchitecture (Treated Group 1250mg/kg). EGL- External Granular Layer, DN- Disintegrated nuclei of stellate cell, ML- Molecular layer (Cresyl Echt Violet X 250).

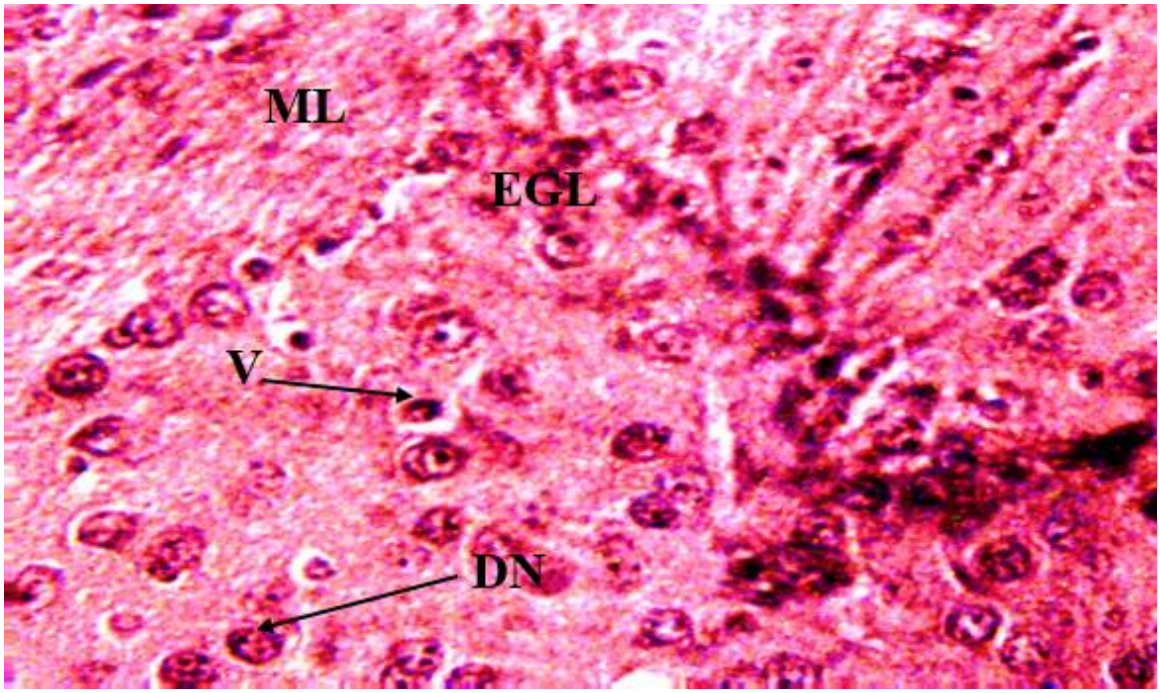


Plate X: A is Section of cerebral cortex of Group 5 (Treated Group 10mg/kg) shows features of cellular degeneration. DN- Disintegrated nuclei, EGL- External Granular layer, ML- Molecular layer, V- Vacuole (H & E X 250).

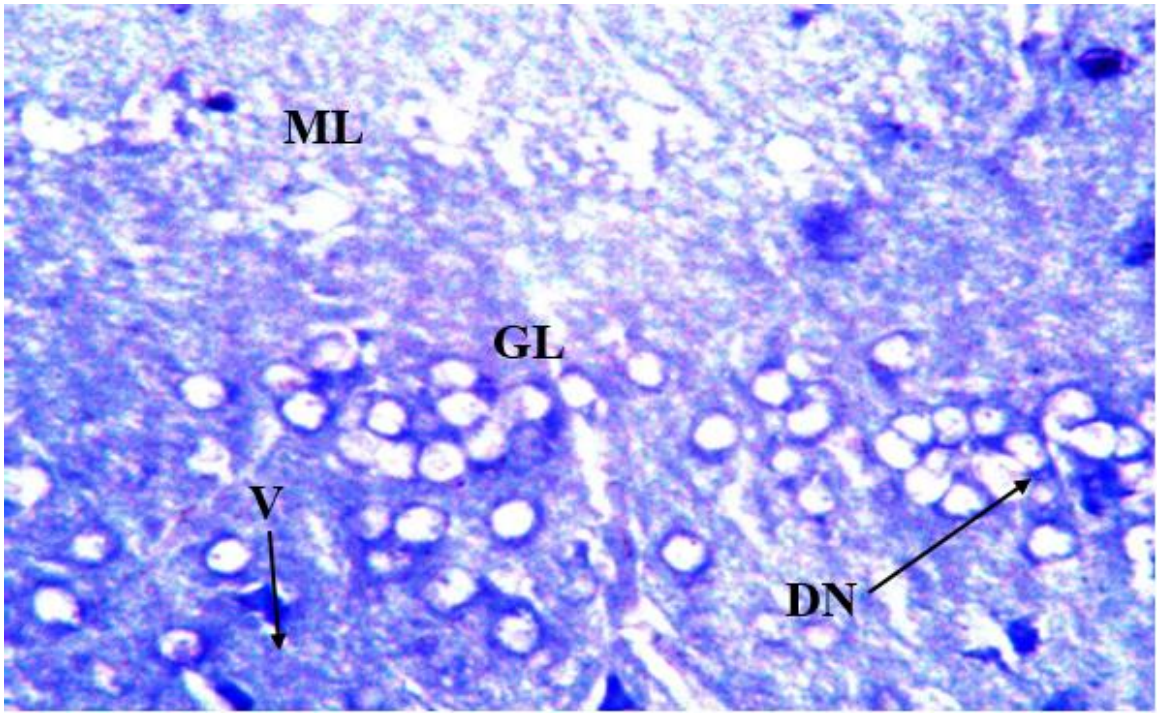


Plate XI: Section of cerebral cortex of Group 5 (Treated Group 10mg/kg) showing sparse cellular degeneration. GL- Granular layer, ML- Molecular layer, DN- Disintegrated nuclei, V- Vacuolation (Cresyl Echt Violet X 250).

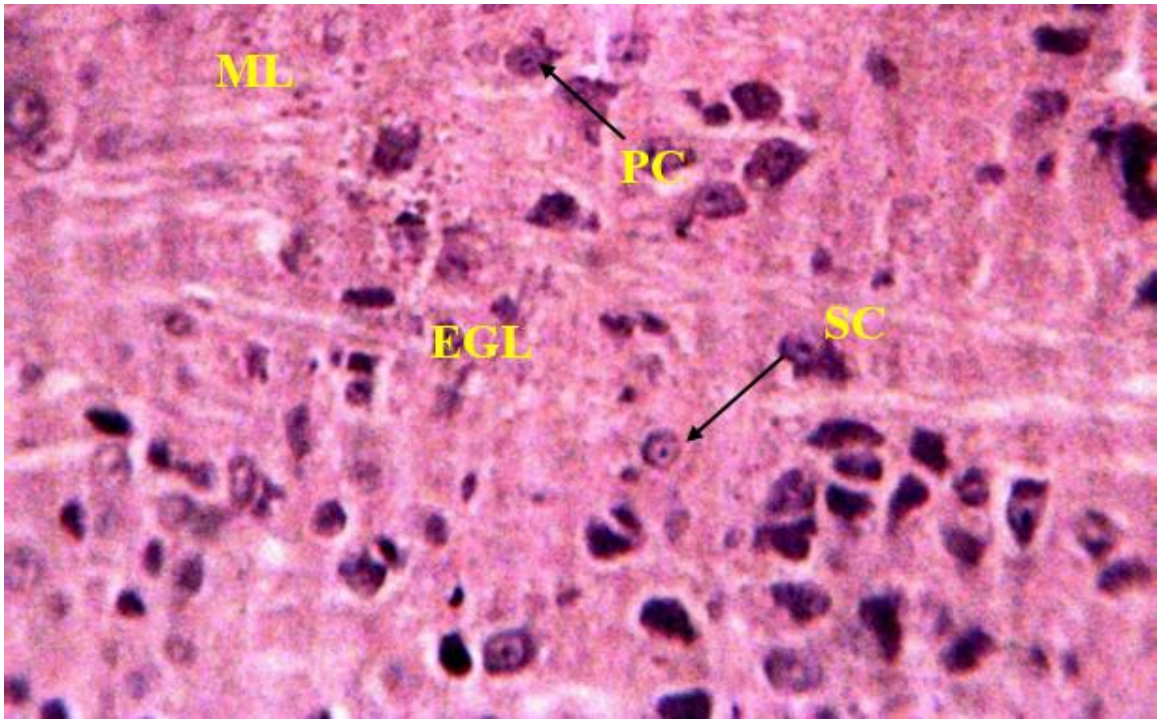


Plate XII: Section of cerebral cortex of Group 1 (Control Group) showing normal architecture. EGL- External Granular layer, PC- Pyramidal cell, ML- Molecular layer, SC- Stellate cell (H & E X 250).

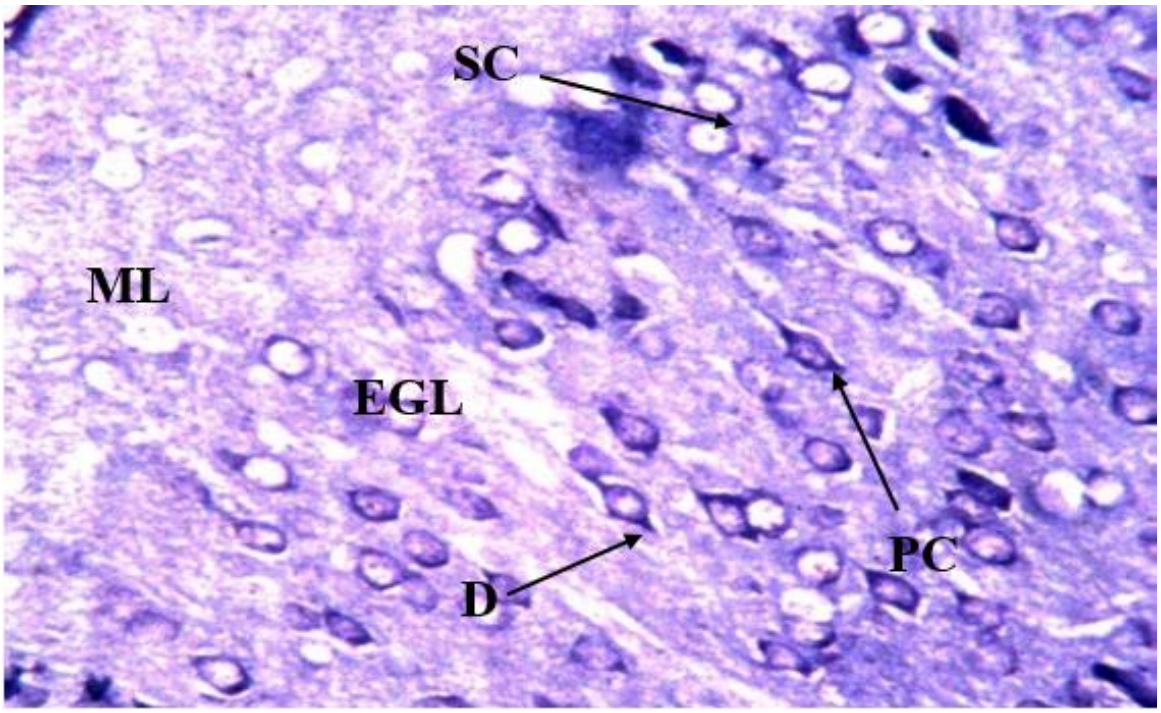


Plate XIII: A is Section of cerebral cortex of Group 1 (Control Group) showing normal architecture. SC- Stellate cell, PC- Pyramidal cell, EGL- External Granular layer, ML- Molecular layer (Cresyl Echt Violet X 250).

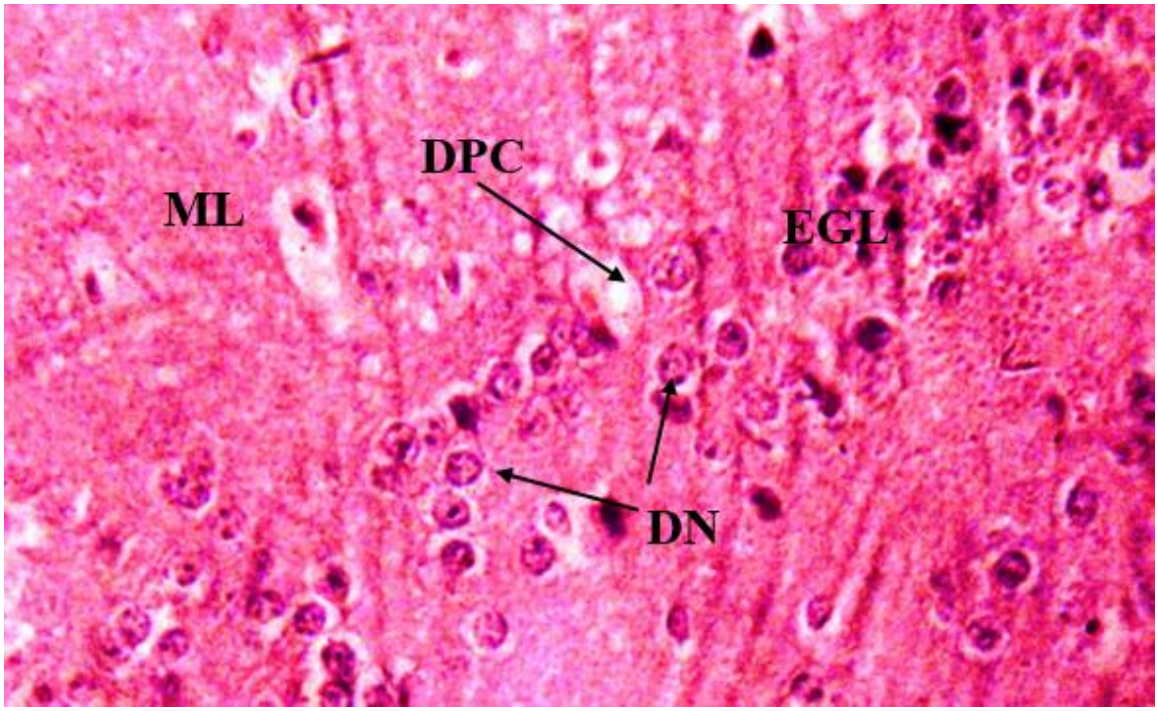


Plate XIV: Section of cerebral cortex of Group 2 (Untreated Group) showing an overview of distorted architecture. DPC- Degenerating Pyramidal cells, EGL- External Granular layer, ML- Molecular layer, DN- Disintegrated nuclei (H & E X 250).

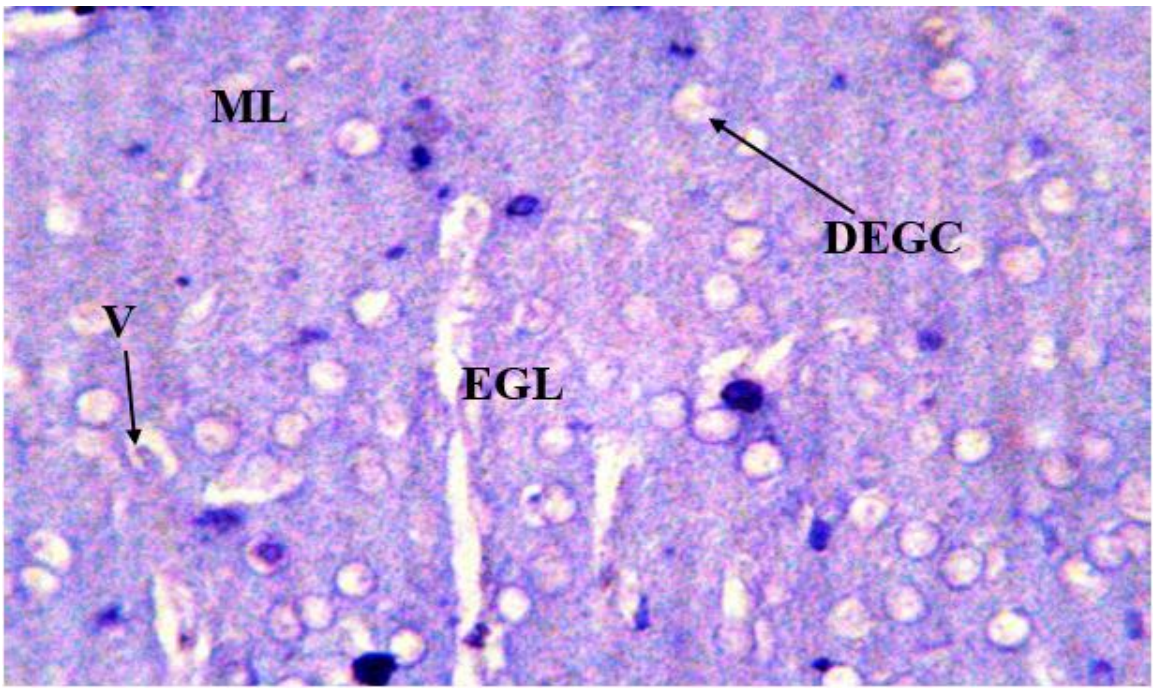


Plate XV: Section of cerebral cortex of Group 2 (Untreated Group) showing an overview. DEGC- Degenerating external granular cells (Stellate cells), EGL- External Granular layer, ML- Molecular layer, V- Vacuole (Cresyl Echt Violet X 250).

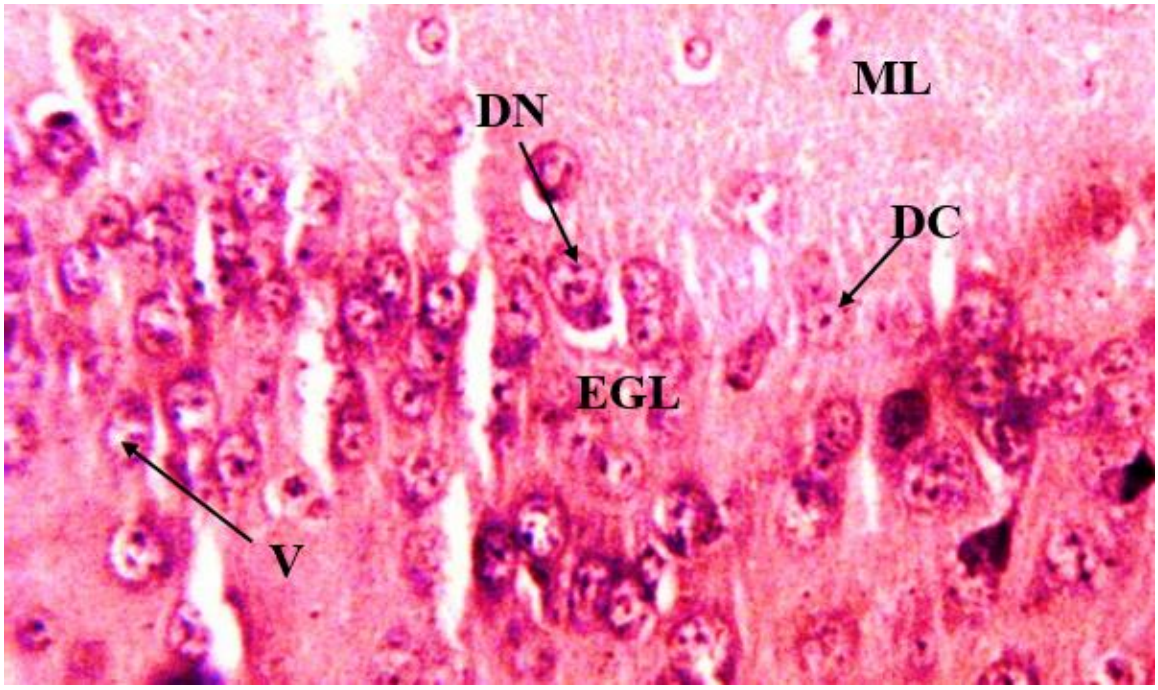


Plate XVI: Section of cerebral cortex of Group (Treated 2500mg/kg) showing an overview of cell architecture. DC- Degenerated cell, EGL- External Granular layer, ML- Molecular layer, DN- Disintegrated nuclei (H & E X 250).

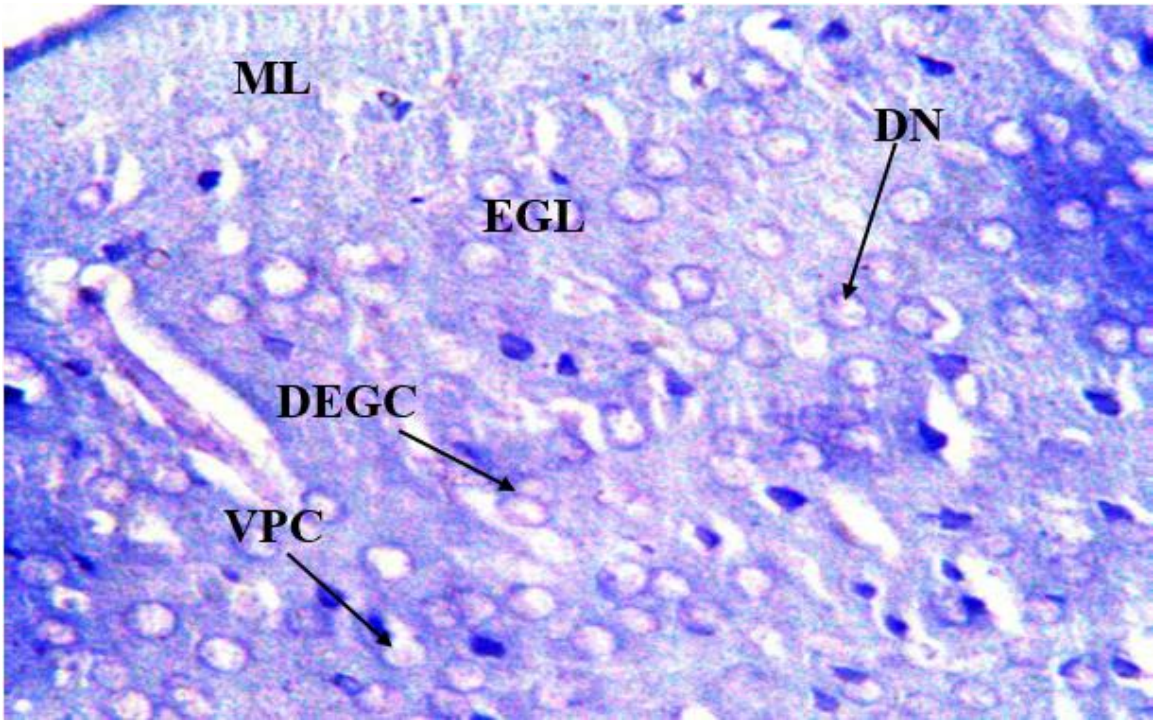


Plate XVII: Section of cerebral cortex showing distorted layer II cells. Where DEGC- Degenerated external granular cell, ML- Molecular cell, EGL- External Granular Layer, VPC- Vacuolated pyramidal cell, DN- Disintegrated nuclei (Cresyl Echt Violet X 250).

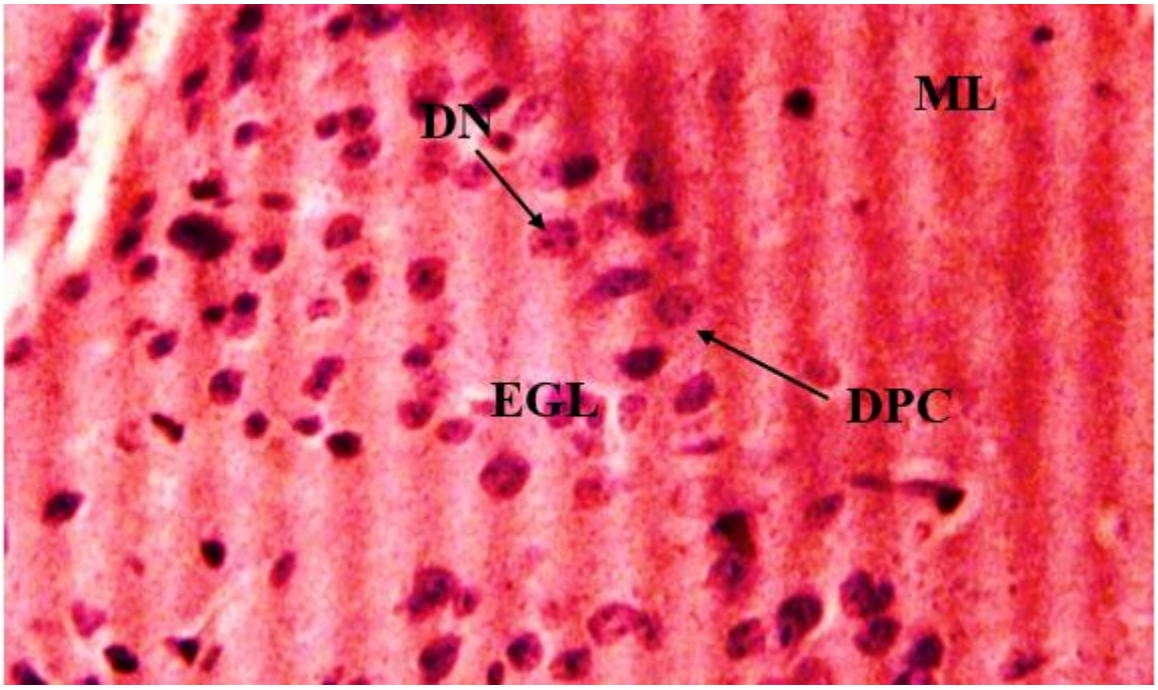


Plate XVIII: Section of cerebral cortex of Group 4 (Treated with 2500mg/kg) showing distorted cellular architecture. ML- Molecular layer, EGL- External granular layer characterized with reduced cell nucleus, DN- Disintegrated Nuclei (H & E X 250).

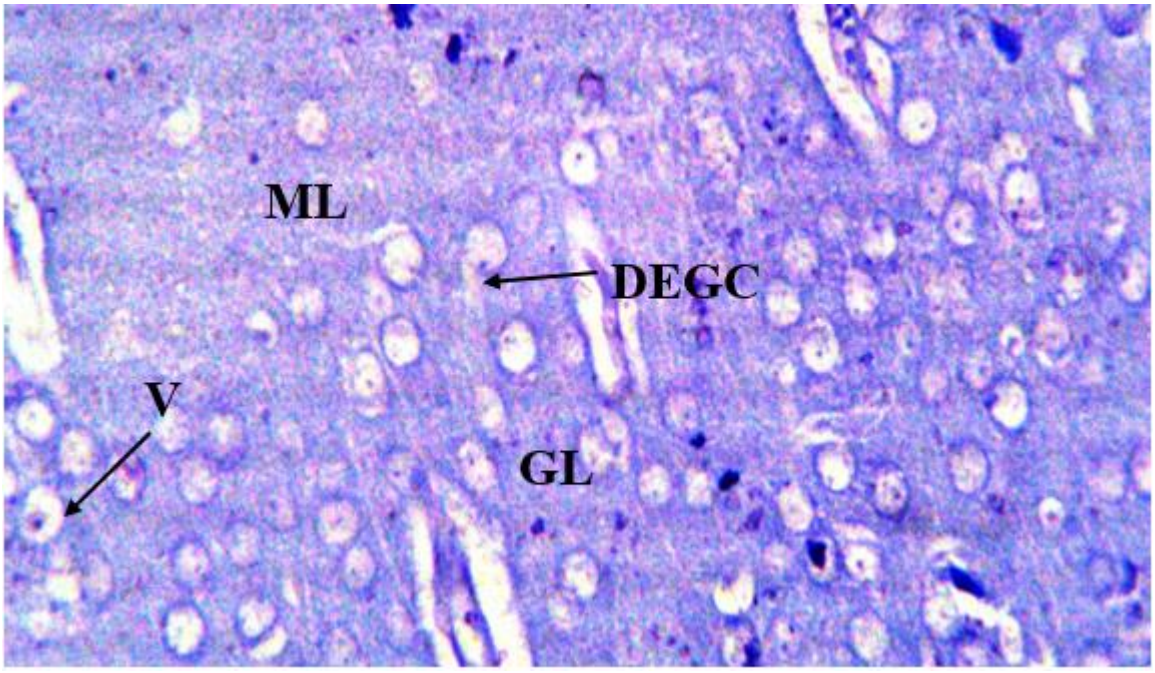


Plate XIX: Section of cerebral cortex of Group 4 (Treated Group 1250mg/kg) showing some affected cellular architecture. DEGC- Degenerated external granular cells, V- Vacuole, GL- Granular layer, ML- Molecular layer which shows normal cytoarchitecture (Cresyl Echt Violet X 250).

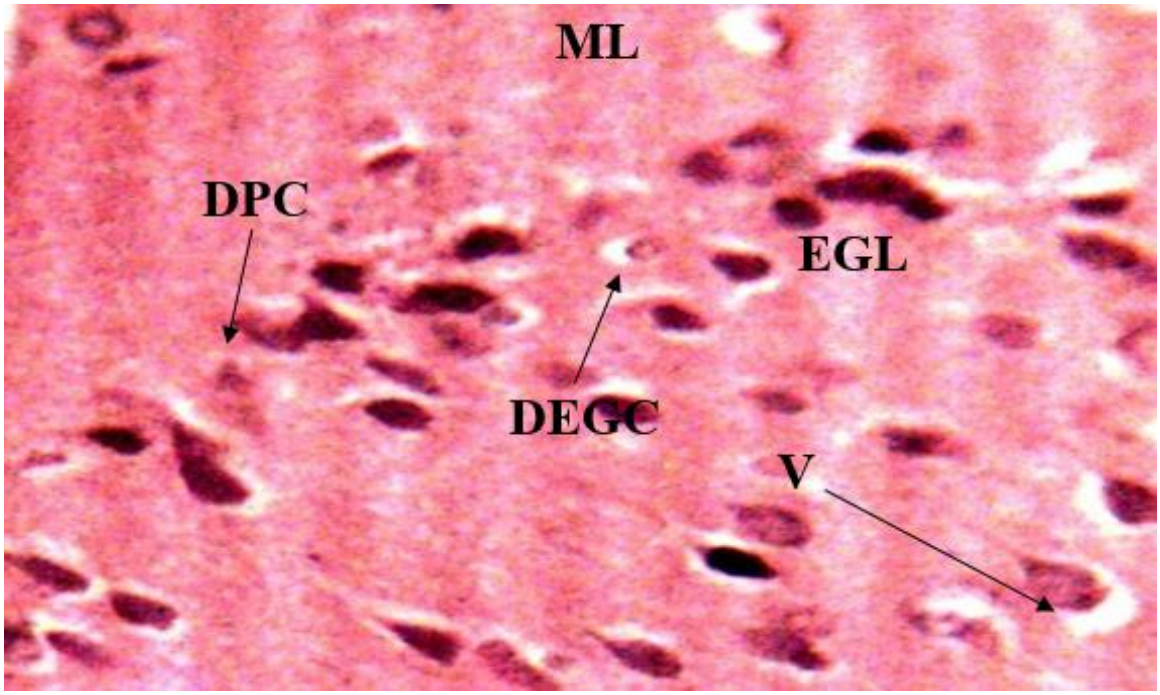


Plate XX: Section of cerebral cortex (Treated with Chloroquine 10mg/kg). ML- Molecular layer, EGL- External granular layer and DEGC- Degenerated external granular cell V- Vacuolation (H & E X 250).

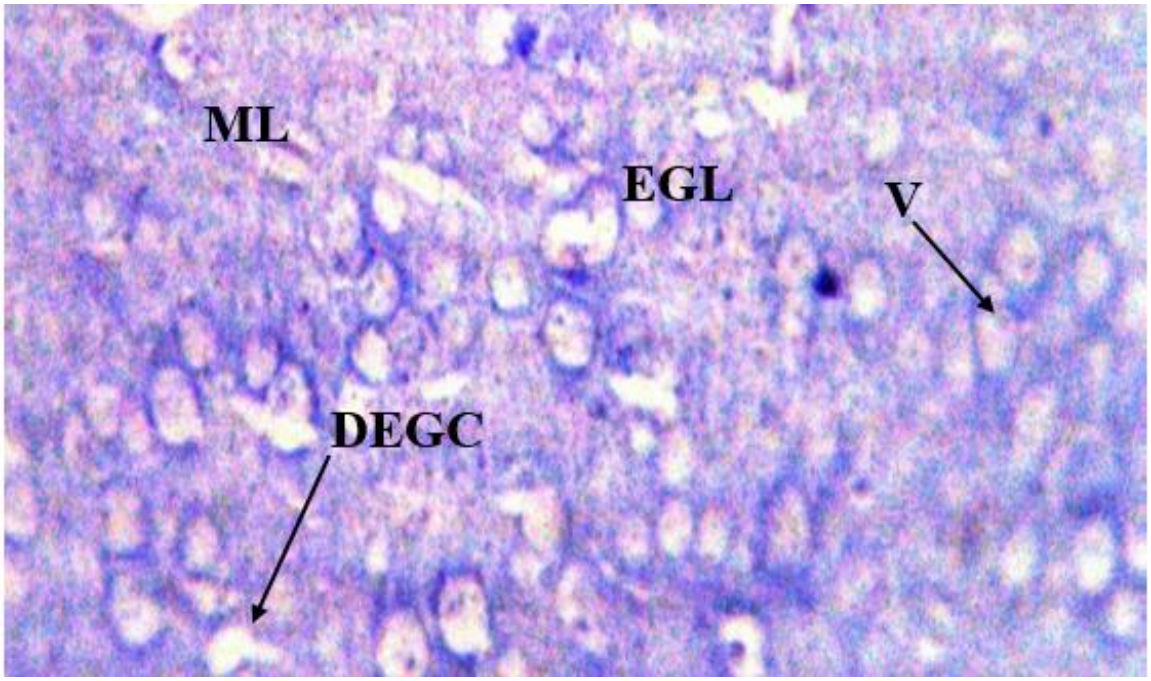


Plate XXI: Section of cerebral cortex (Treated with Chloroquine 10mg/kg). ML- Molecular layer, EGL- External granular layer and DEGC- Degenerated external granular cell V- Vacuolation (Cresyl Echt Violet X 250).

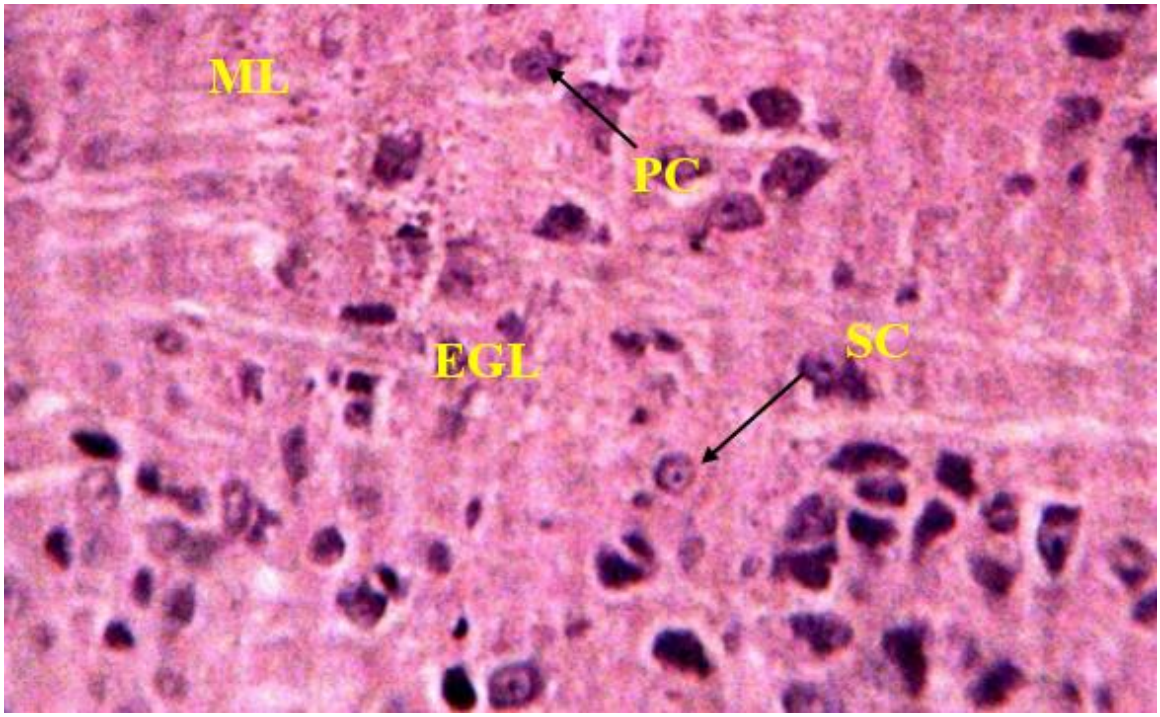


Plate XXII: Section of cerebral cortex of Group 1 (Control Group) showing normal architecture. EGL- External Granular layer, PC- Pyramidal cell, ML- Molecular layer, SC- Stellate cell (H & E X 250).

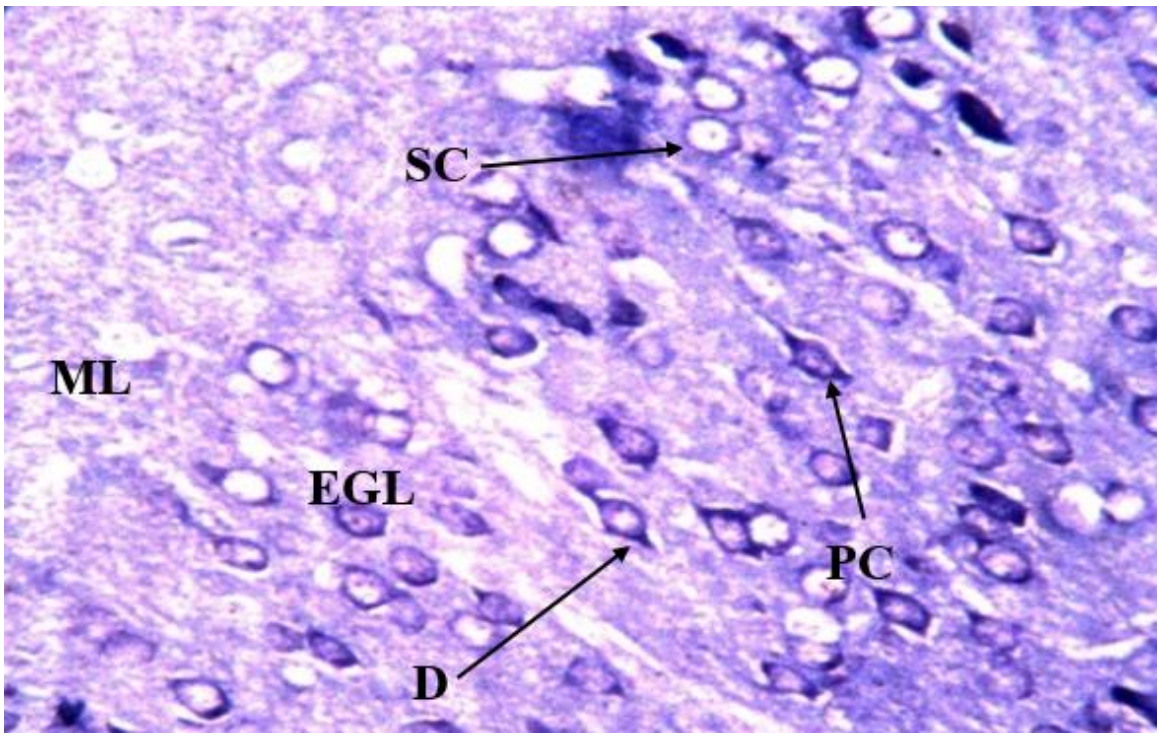


Plate XXIII: Section of cerebral cortex of Group 1 (Control Group) showing normal architecture. SC- Stellate cell, PC- Pyramidal cell, EGL- External Granular layer, ML- Molecular layer (Cresyl Echt Violet X 250).

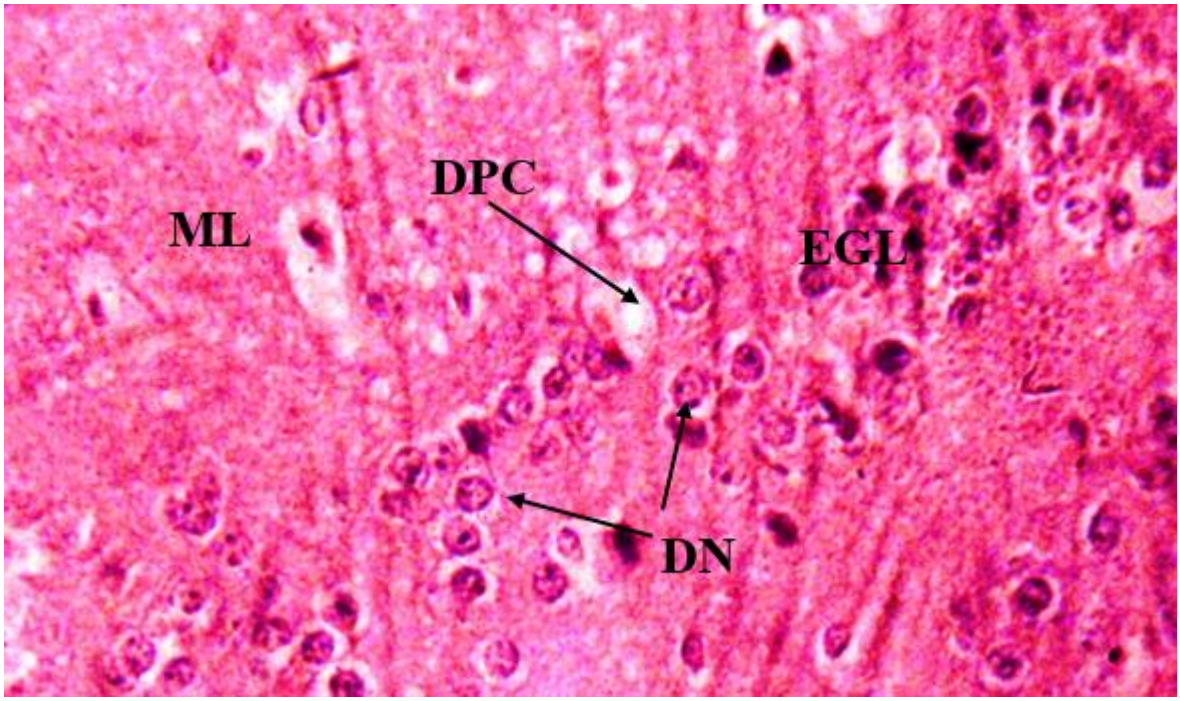


Plate XXIV: Section of cerebral cortex of Group 2 (Untreated Group). DPC- Degenerating Pyramidal cells, EGL- External Granular layer, ML- Molecular layer, DN- Disintegrated nuclei (H & E X 250).

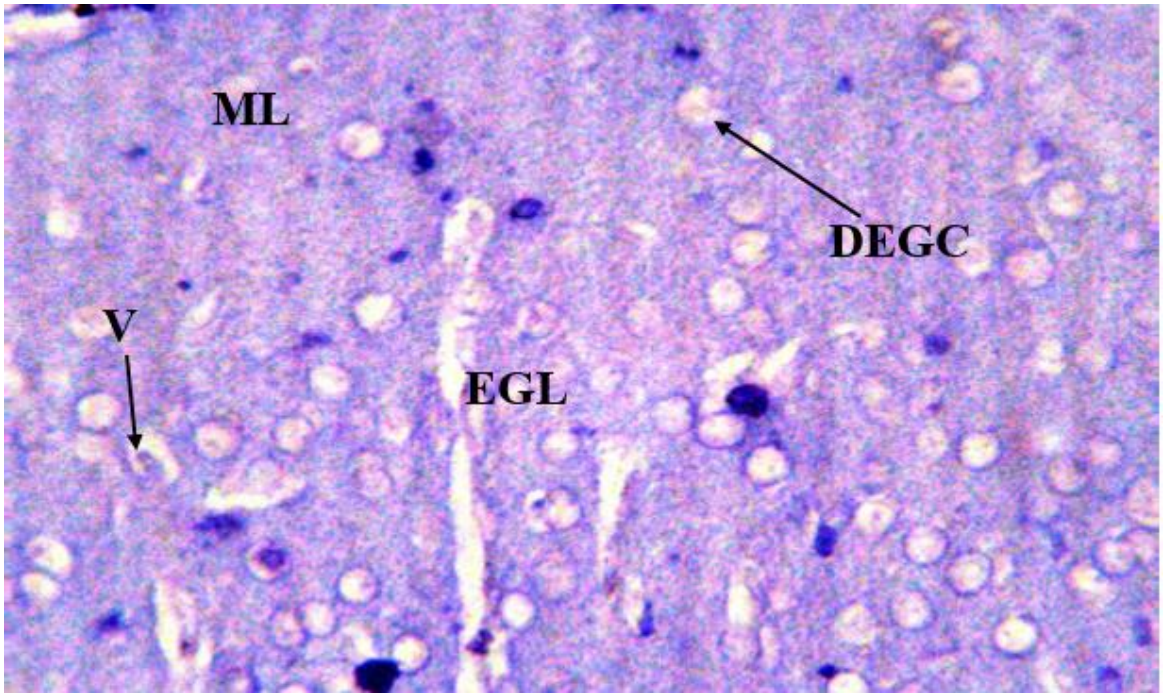


Plate XXV: Section of cerebral cortex of Group 2 (Untreated Group) showing an overview of cellular architecture. DEGC- Degenerating external granular cells (Stellate cells), EGL- External Granular layer, ML- Molecular layer, V- Vacuole (Cresyl Echt Violet X 250).

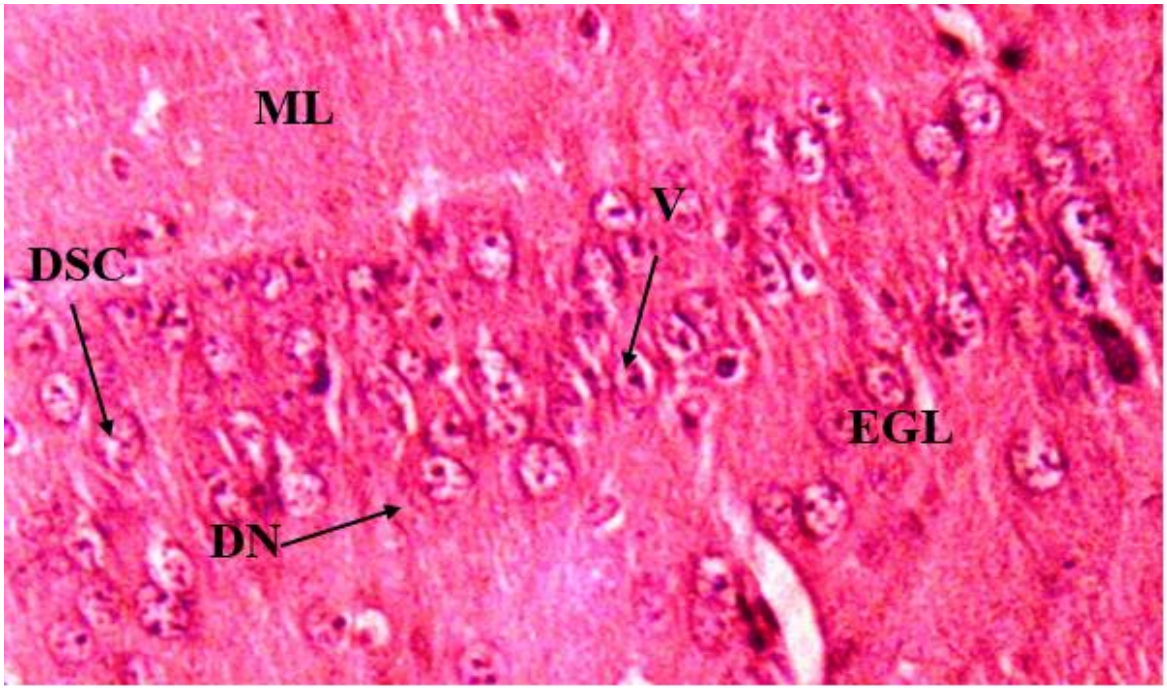


Plate XXVI: Section of cerebral cortex (Treated with 2500mg/kg). EGC- External granular cell with degenerating characteristics, EGL- External granular layer, ML- Molecular layer (H & E X 250).

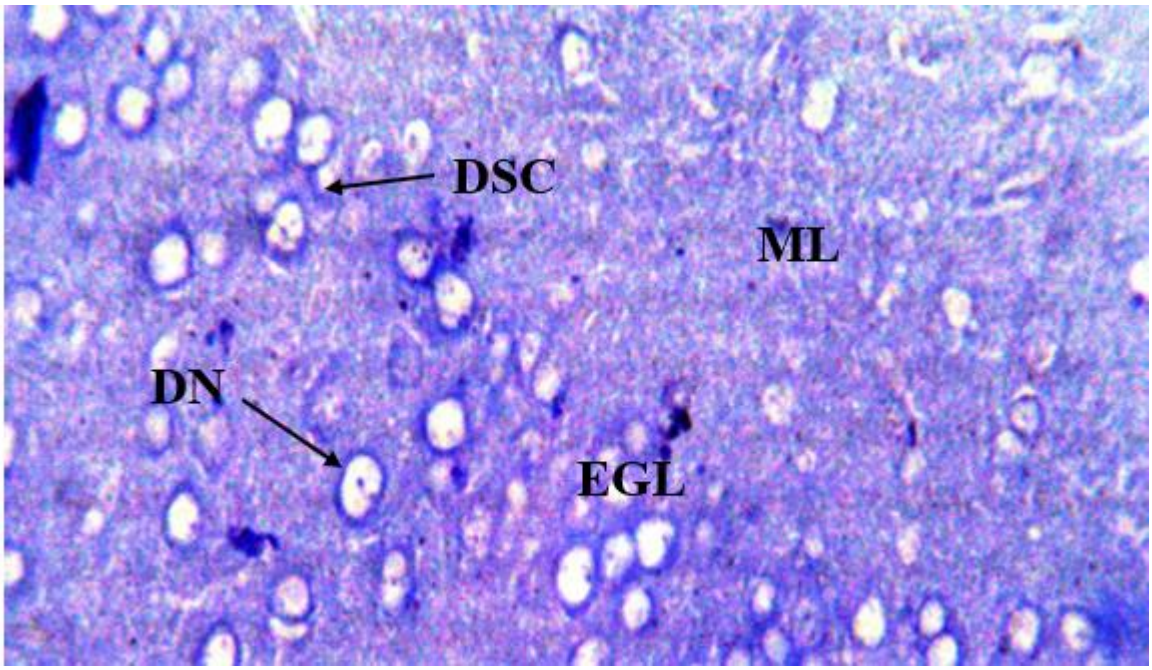


Plate XXVII: Section of cerebral cortex (Treated with 2500mg/kg) showing cells architecture. Where DSC- Degenerated Stellate cell, ML- Molecular cell, DN- Disintegrated nuclei, EGL- External Granular Layer (Cresyl Echt Violet X 250).

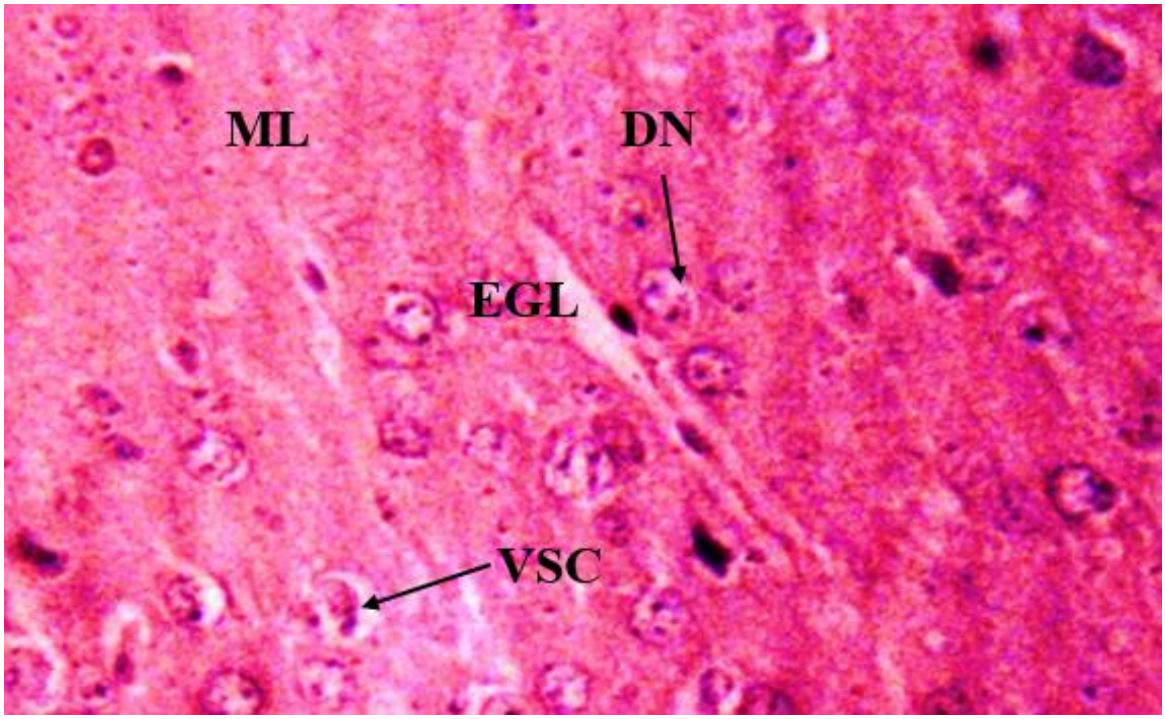


Plate XXVIII: Section of cerebral cortex (Treated with 1250mg/kg). DN- Disintegrated nuclei VSC- Vacuolated stellate cell with degenerating characteristics, EGL- External granular layer, ML- Molecular layer (H & E X 250).

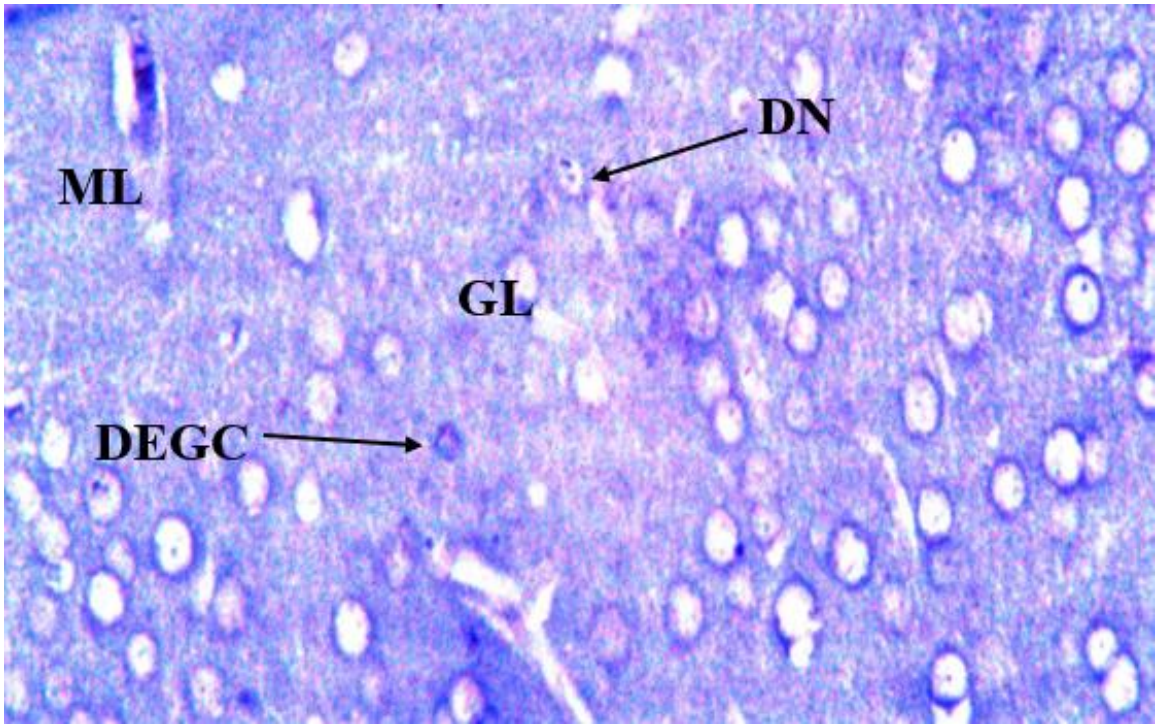


Plate XXIX: Section of cerebral cortex (Treated with 1250mg/kg). ML- Molecular layer, DN- Disintegrated nuclei characterized with no cell wall, GL- Granular layer (Cresyl Echt Violet X 250).

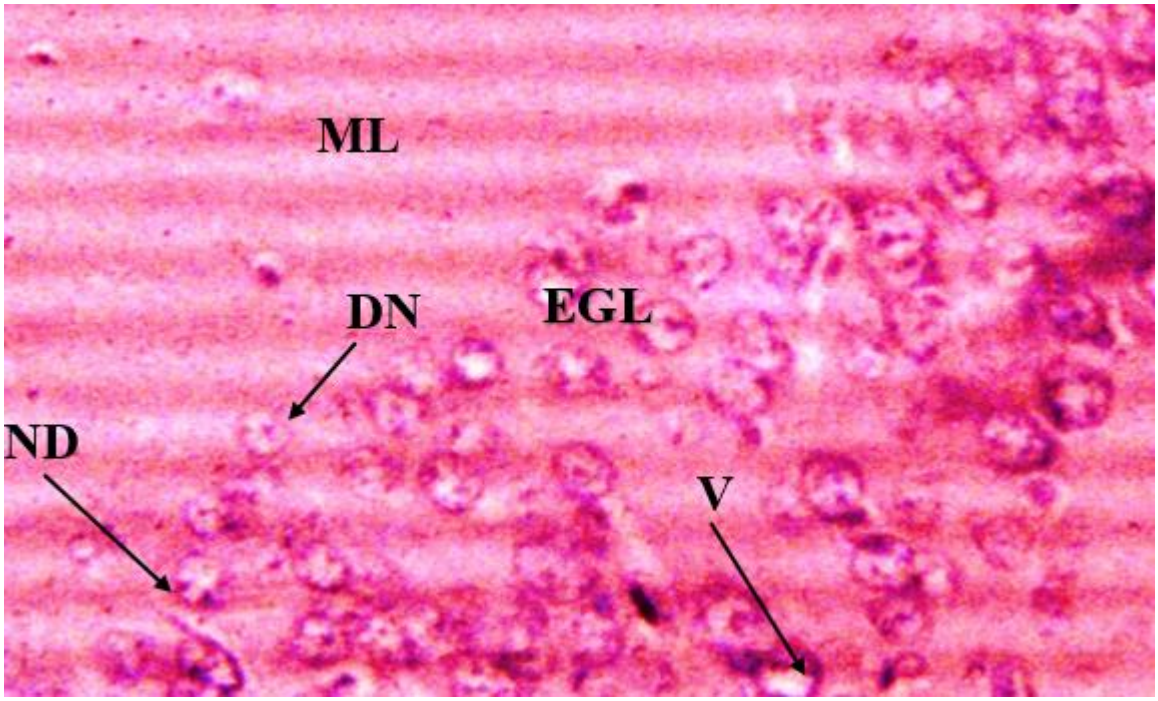


Plate XXX: Section of cerebral cortex (Treated with Chloroquine 10mg/kg) showing Nuclei displacement ND, ML- Molecular layer with normal cytoarchitecture V- Vacuole, DN- Disintegrated nuclei, EGL- External Granular layer (H & E X 250).

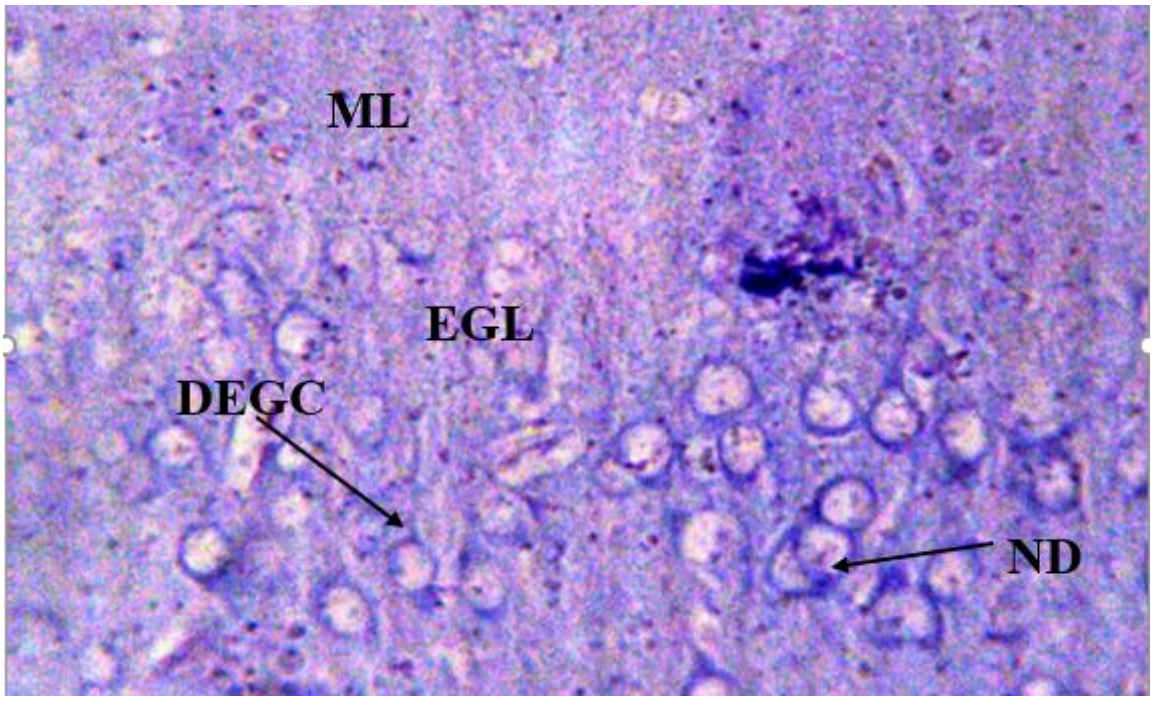


Plate XXXI: A is Section of cerebral cortex (Treated with Chloroquine10mg/kg) ML- Molecular layer, EGL- External granular layer and DEGC- Degenerated external granular cell (Cresyl Echt Violet X 250).

4.6.2 Histopathological studies of cerebellar cortex on a suppressive test, curative test and prophylactic test

In Suppressive test the cerebellar cortex of the mice in Group 1(Plate I) shows normal cytoarchitecture with normal cells in layer I and layer II. The first two layers of the cerebellar cortex of Group 2 (Plate II) reveal highly distorted cytoarchitecture of the molecular and Purkinje cells. The cerebellar cortex of the mice in Group 3 and Group 4 shows disorientation of cells architecture in the molecular and Purkinje layer. The cerebellar cortex of the Group 5 mice shows similar degenerative characteristics but not as severe as the Group 3 and 4.

In Curative test the cerebellar cortex with its three layers (Outer Molecular layer ML, middle Purkinje layer PL, and inner Granular layer GL) were examined and the cerebellar cortex of the mice in Group 1(Plate I) shows normal cytoarchitecture with normal cells in layer I and layer II. The first two layers of the cerebellar cortex of Group 2 (Plate II) reveal highly distorted cytoarchitecture of the molecular and Purkinje cells.

The cerebellar cortex of the mice in Group 3 and Group 4 shows disorientation of cells architecture in the molecular and Purkinje layer. The cerebellar cortex of the Group 5 mice which was treated with chloroquine shows similar degenerative characteristics but not as severe as the Group 3 and 4.

In the prophylactic test the cerebellar cortex of the mice in Group 1 (Plate XI) shows normal cytoarchitecture with normal cells in layer I and layer II. The first two layers of the cerebellar cortex of Group 2 (Plate XII) reveal highly distorted cytoarchitecture of the molecular and Purkinje cells. The cerebellar cortex of the mice in Group 3 and Group 4 shows disorientation of cells architecture in the molecular and Purkinje layer. The

cerebellar cortex of the Group 5 mice shows similar degenerative characteristics but not as severe as the Group 3 and 4

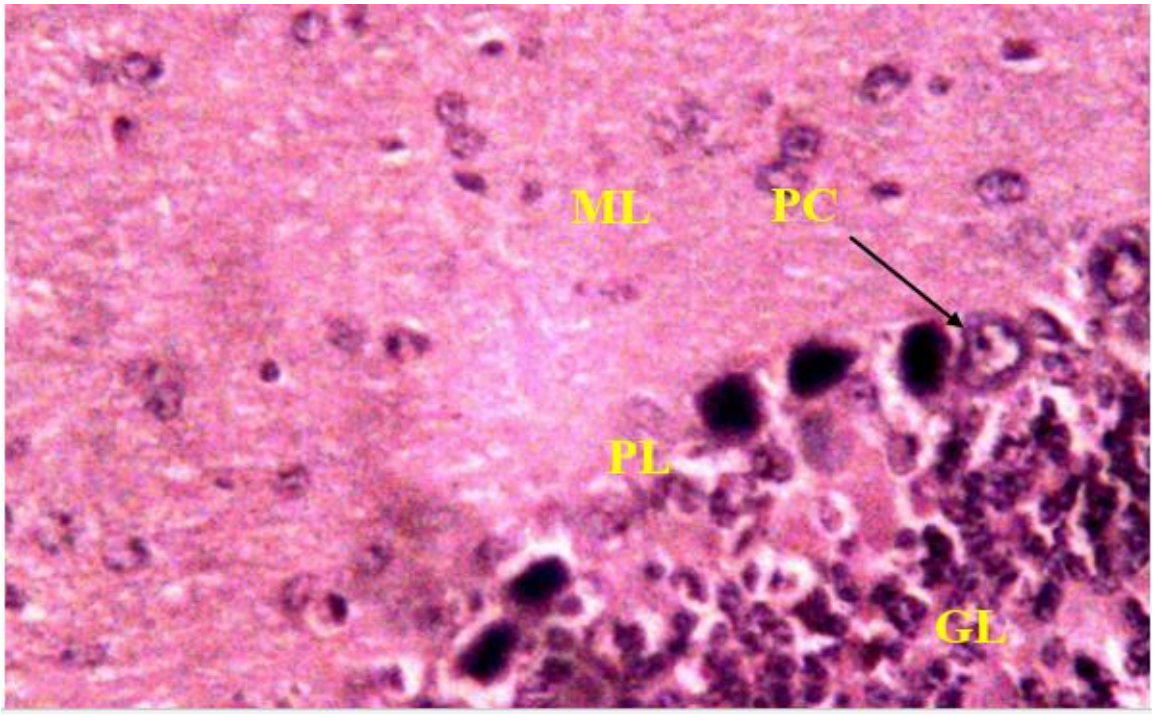


Plate XXXII: Section of cerebellum of Group 1(Control Group) showing ML- Molecular layer, PC – Purkinje cell, PL- Purkinje layer, GL- Granular layer (H & E stain X 250).

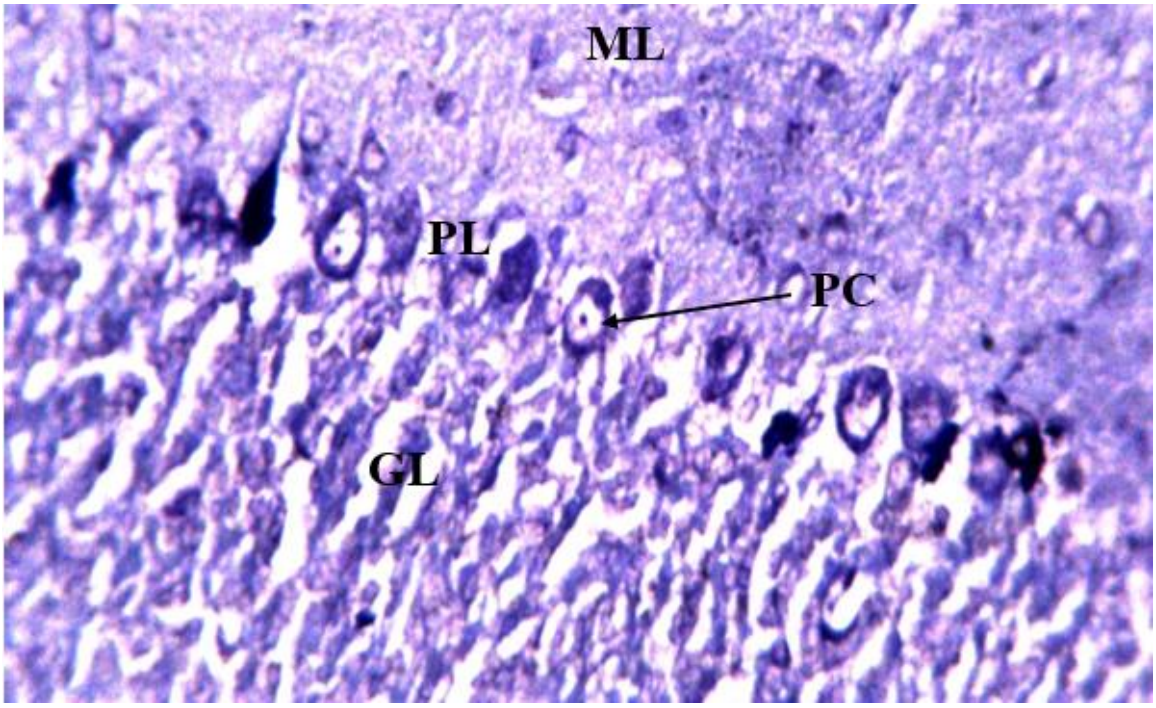


Plate XXXIII: Section of cerebellum of Group 1 (Control Group) showing ML- Molecular layer, PC – Purkinje cell, GL- Granular layer, PL- Purkinje layer (Cresyl Echt Violet, X 250).

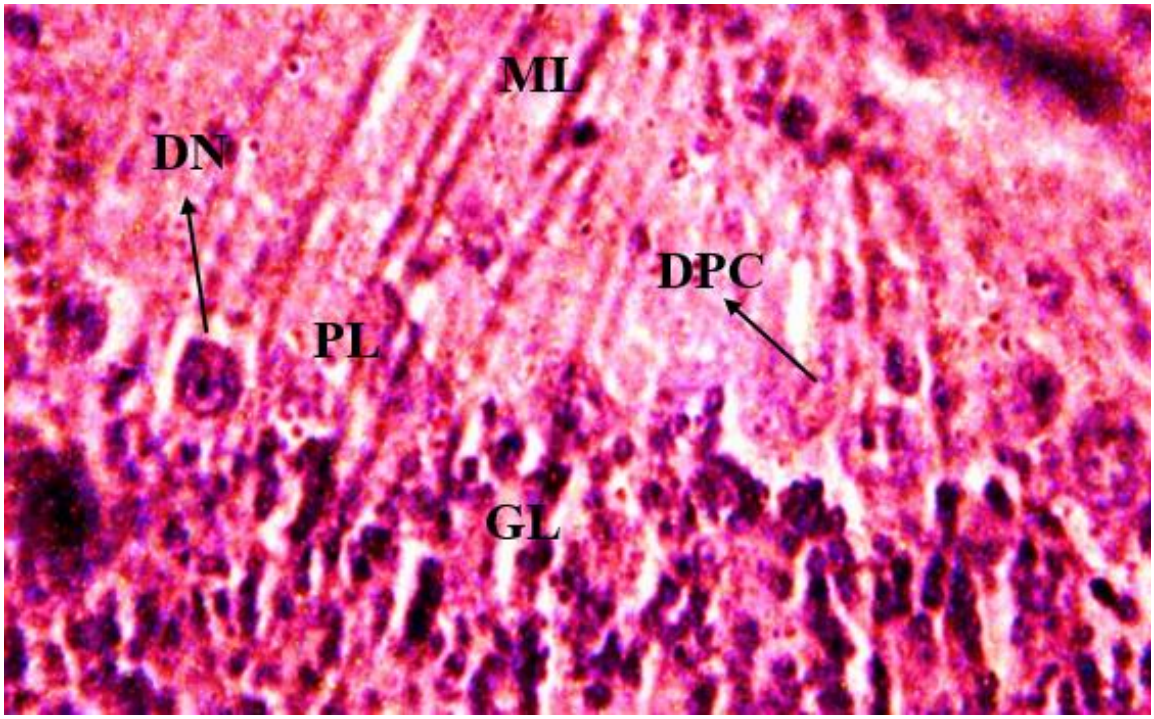


Plate XXXIV: Section of cerebellum of Group 2 (Untreated Group) showing ML- Molecular layer, DPC – Degenerating Purkinje cell, GL- Granular layer, DN- Disintegrated nuclei (H & E stain, X 250).

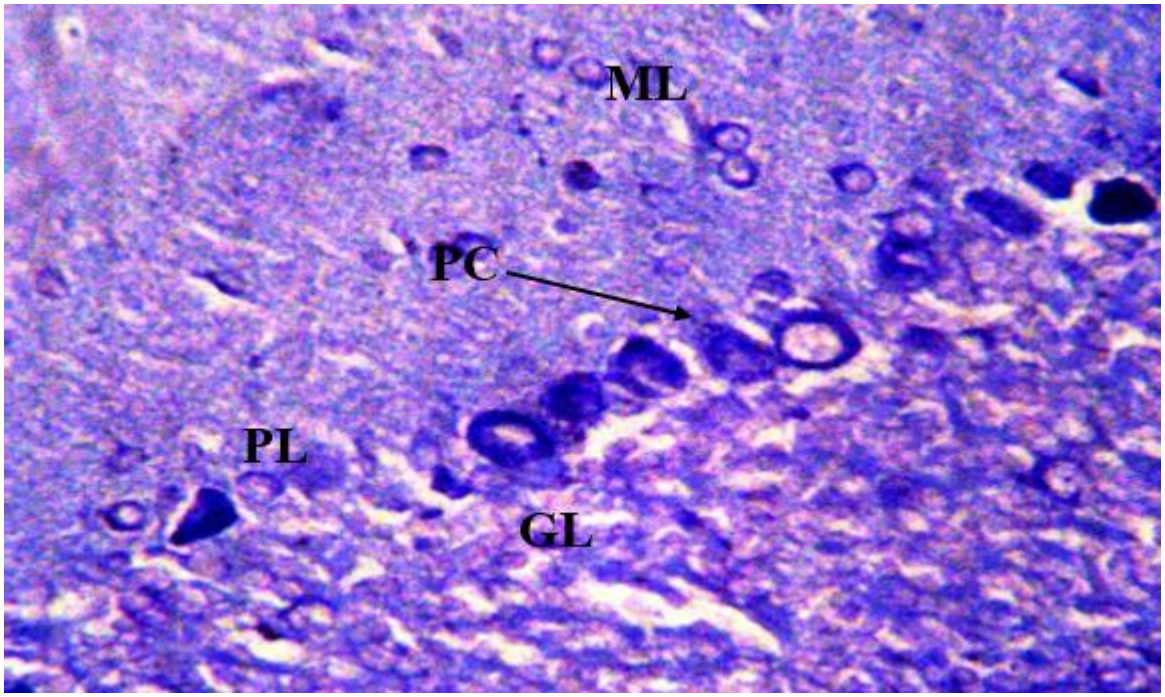


Plate XXXV: Section of cerebellum of Group 2 (Untreated Group) showing ML- Molecular layer, PC – Purkinje cell, PL- Purkinje layer, GL- Granular layer (Cresyl echt violet stain, X 250).

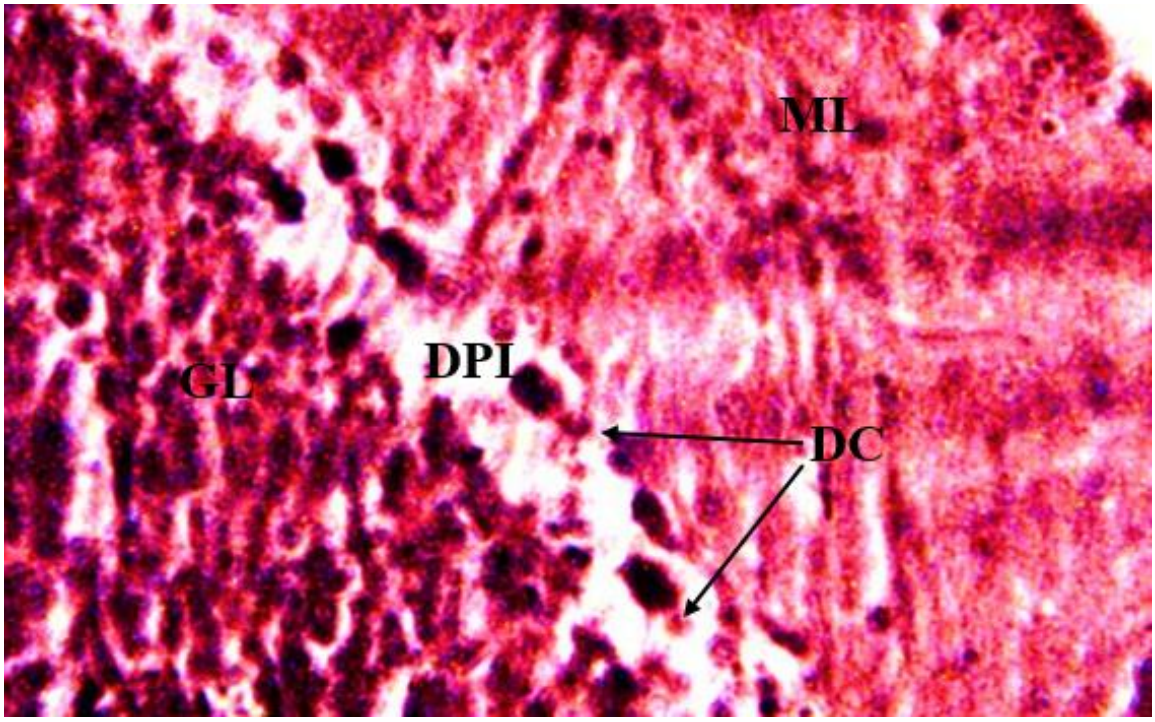


Plate XXXVI: Section of cerebellum of Group 3 (Treated Group 2500mg/kg) showing ML- Molecular layer, DC – Distorted cell, GL- Granular layer, DPL- Degenerating Purkinje layer (H and E stain, X 250).

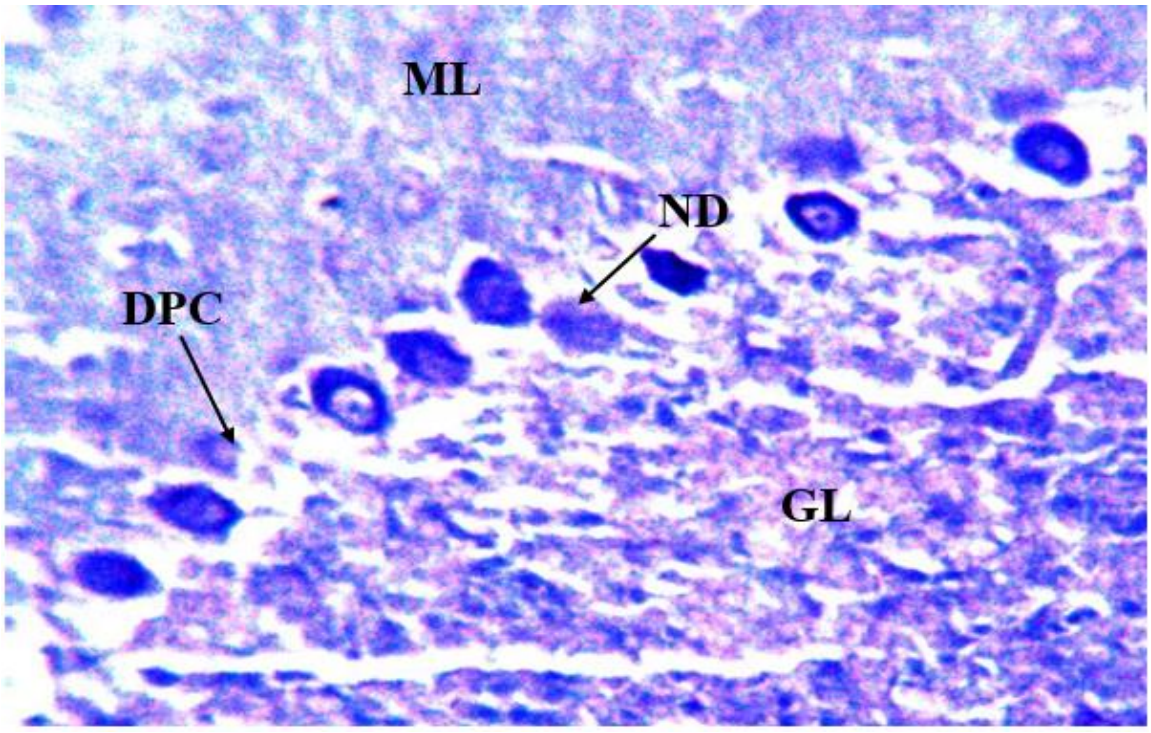


Plate XXXVII: Section of cerebellum of Group 3 (Treated Group 2500mg/kg) showing ML- Molecular layer, ND – Neurons displacement, GL- Granular layer, DPC- Degenerated Purkinje cell (Cresyl echt Violet stain, X 250).

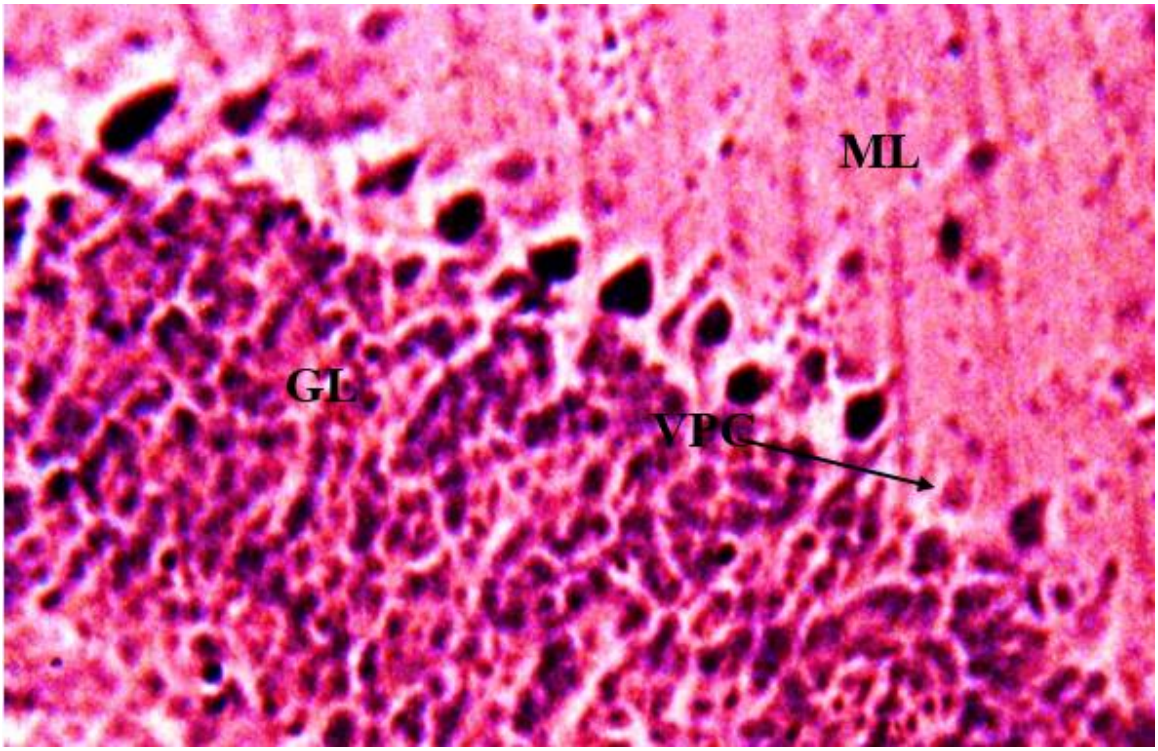


Plate XXXVIII: Section of cerebellum of Group 4 (Treated Group 1250mg/kg) showing ML- Molecular layer, VPC – Vacuolated Purkinje cell, PL- Purkinje layer (H and E X 250).

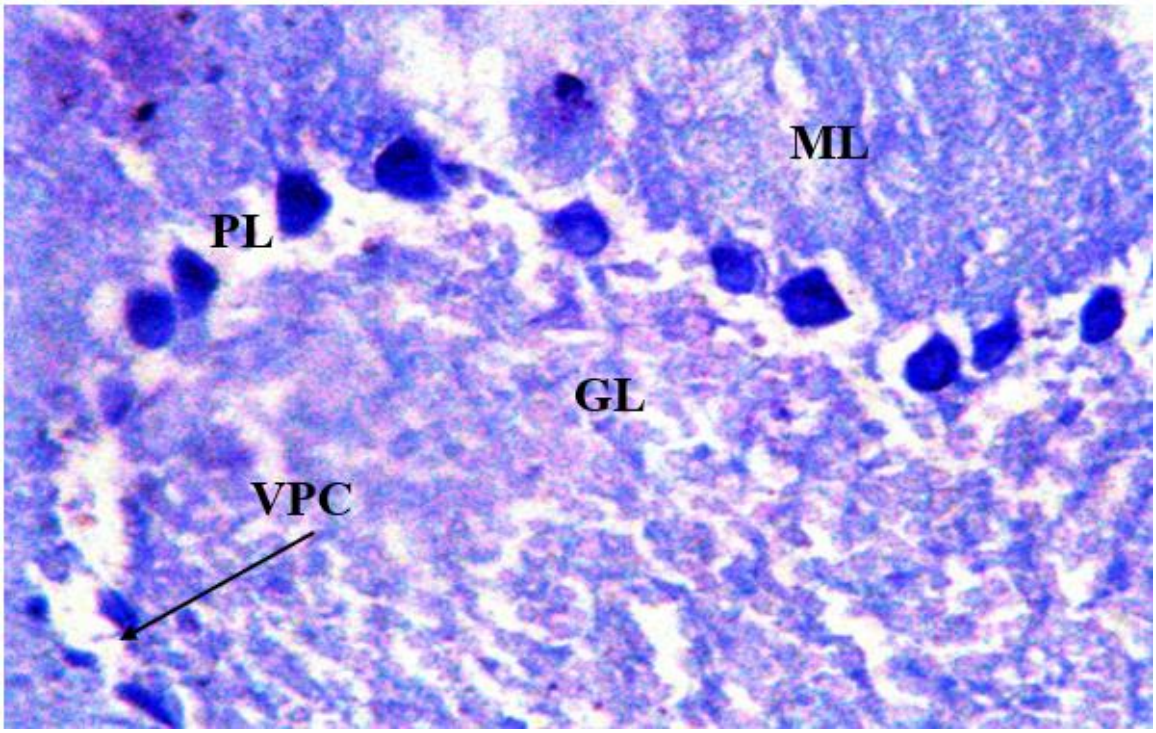


Plate XXXIX: Section of cerebellum (Treated Group 1250mg/kg) showing ML- Molecular layer, DPC – Degenerating Purkinje cell (Cresyl Echt Violet stain, X 250).

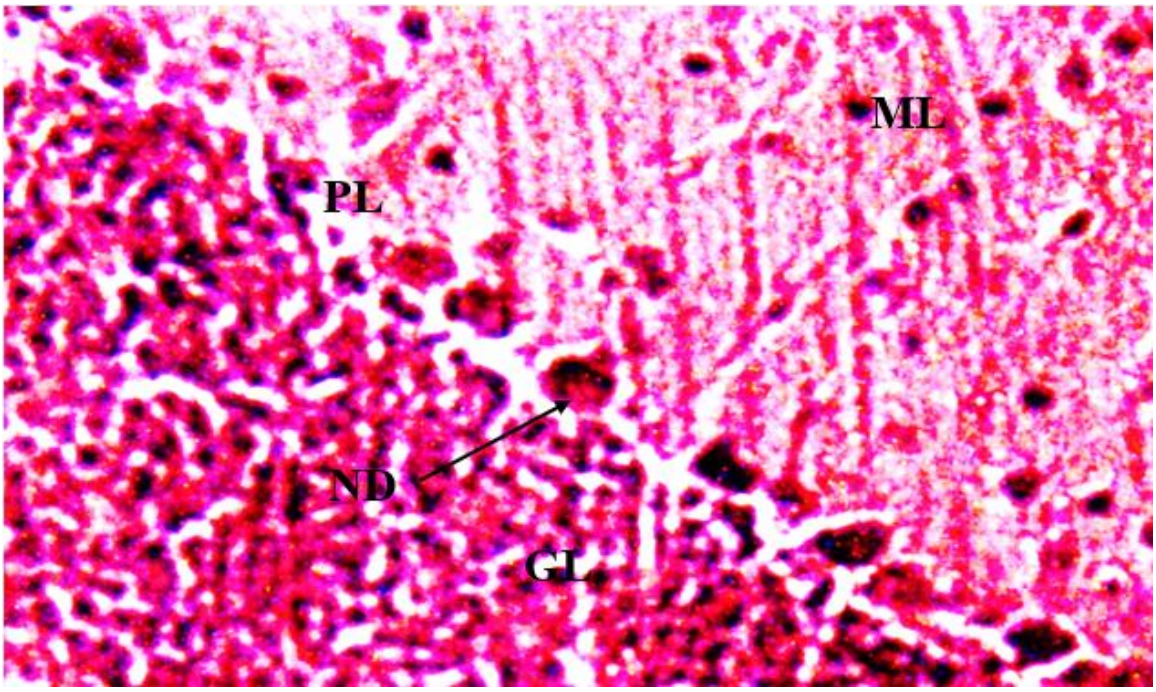


Plate XL: Section of cerebellum of Group 5 (Treated Chloroquine 10mg/kg) showing ML- Molecular layer, PC – Purkinje cell, GL- Granular layer, ND- Nuclei displacement (H and E stain, Mag. X 250).

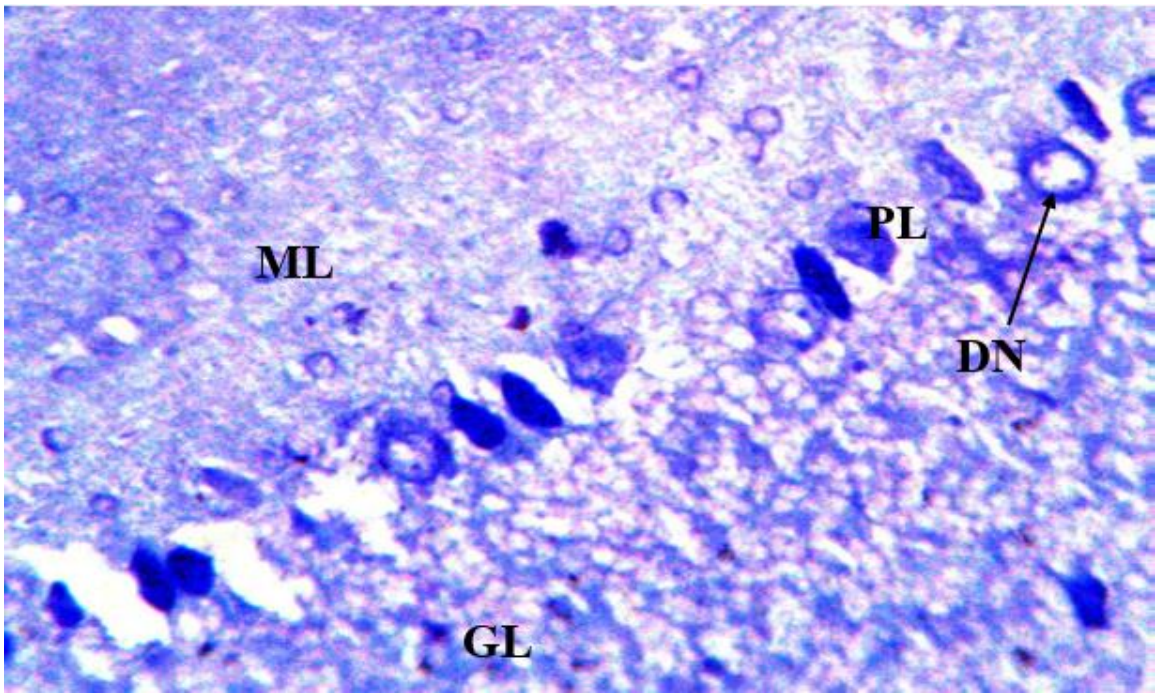


Plate XLI: Section of cerebellum of Group 5 (Treated Group 10mg/kg) showing ML- Molecular layer, PC – Purkinje cell, GL- Granular layer, DN- Disintegrated nuclei (Cresyl Echt Violet stain, X 250).

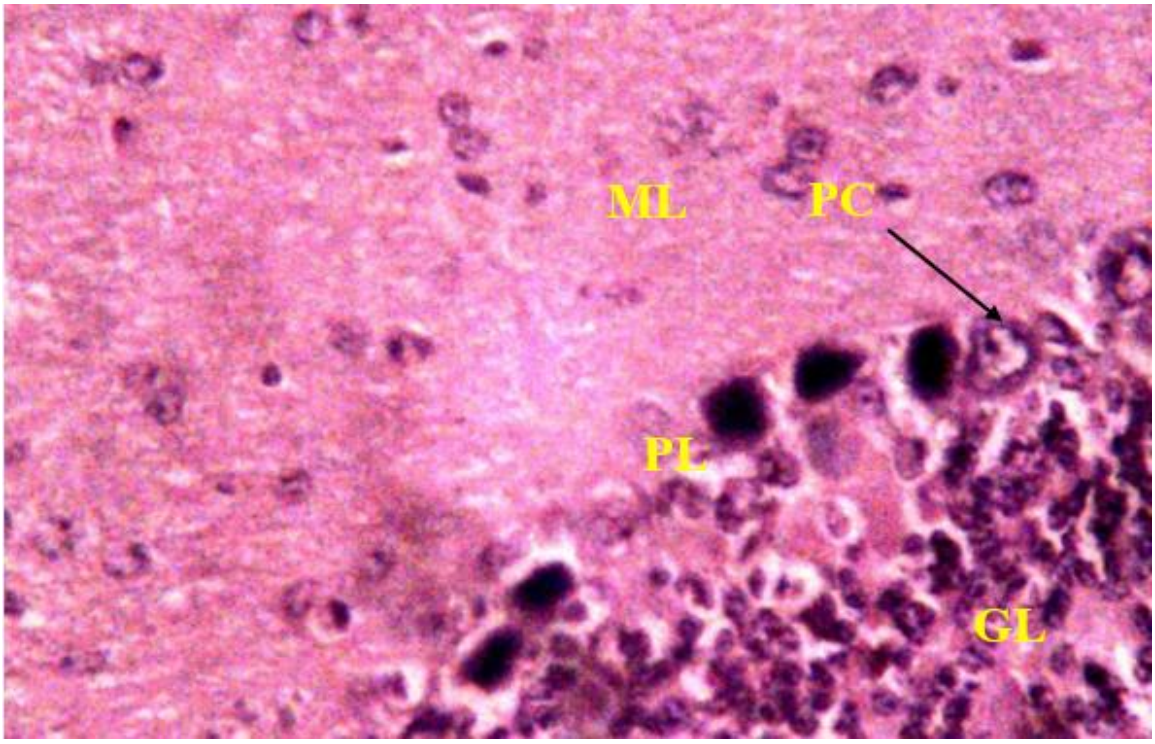


Plate XLII: Section of cerebellum of Group 1(Control Group) showing ML- Molecular layer, PC – Purkinje cell, PL- Purkinje layer, GL- Granular layer (H & E stain, X 250).

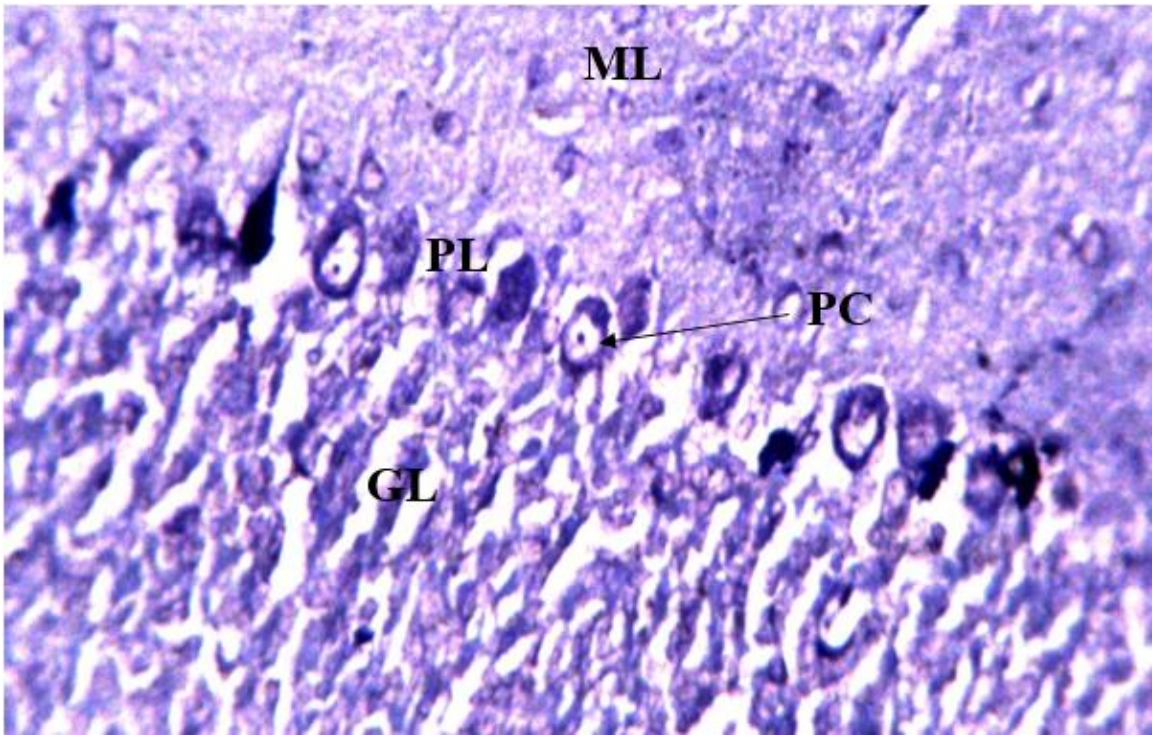


Plate XLIII: Section of cerebellum of Group 1(Control Group) showing ML- Molecular layer, PC – Purkinje cell, PL- Purkinje layer (Cresyl echt violet, X 250).

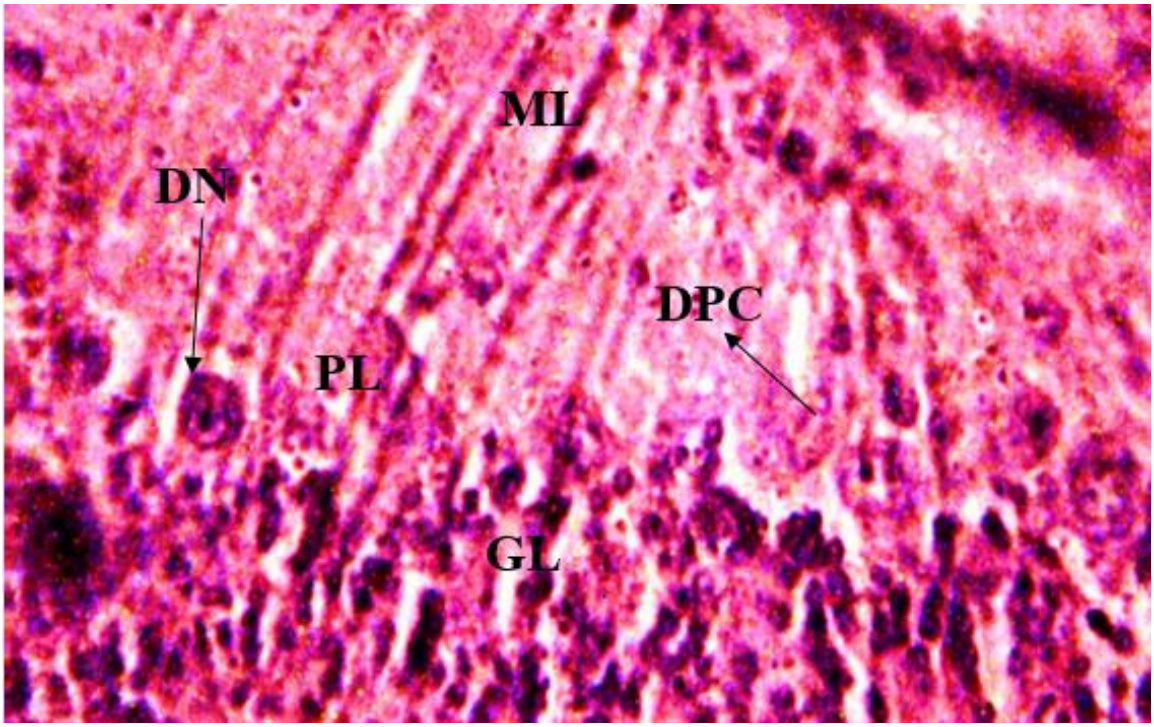


Plate XLIV: Section of cerebellum of Group 2 (Untreated Group) showing ML- Molecular layer, DPC – Degenerating Purkinje cell, GL- Granular layer, DN- Disintegrated nuclei (H & E stain, X 250).

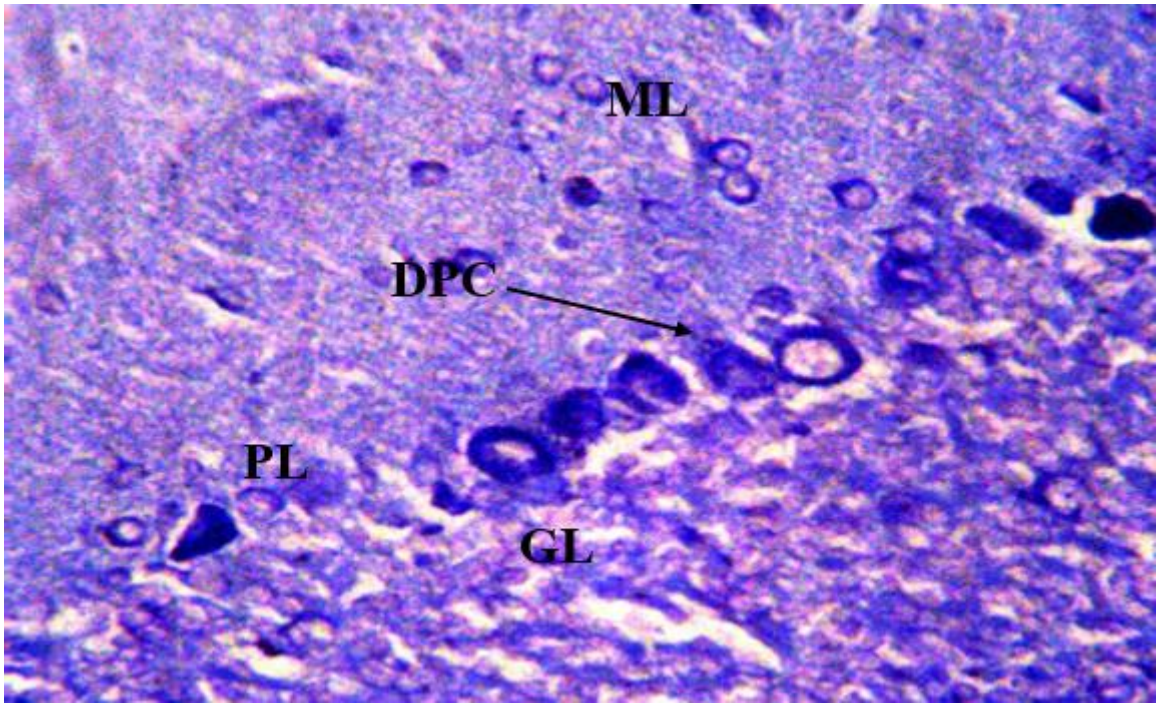
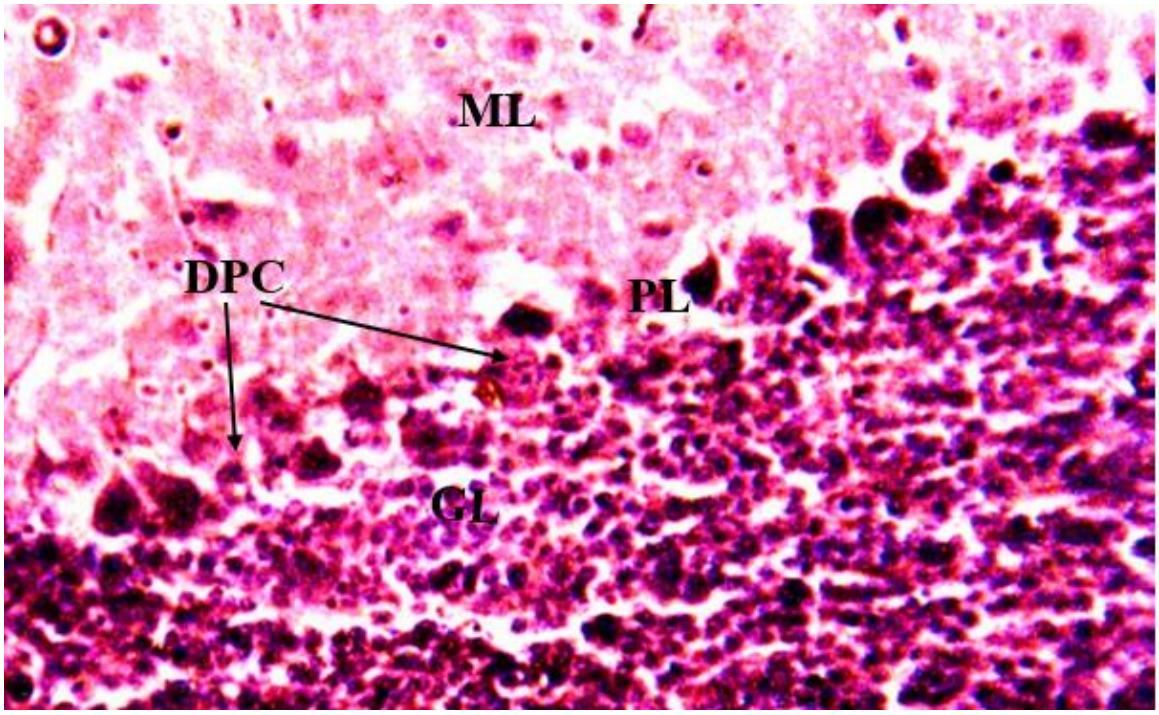


Plate XLV: Section of cerebellum of Group 2 (Untreated Group) showing ML- Molecular layer, DPC – Degenerating Purkinje cell, GL- Granular layer (Cresyl echt violet stain, X 250).



Plates XLVI: Section of cerebellum (Treated with 2500mg/kg) showing ML- Molecular layer, GL- Granular layer and DPC-degenerated Purkinje cell (H and E Stain. X 250).

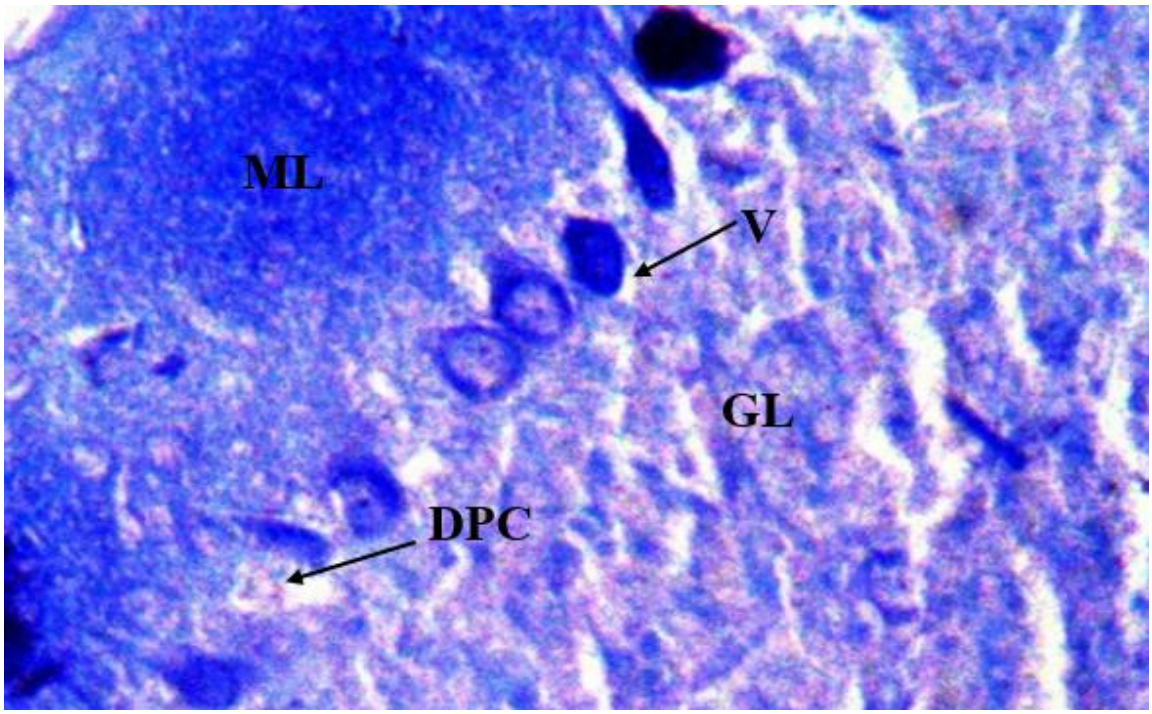


Plate XLVII: A section of cerebellum (Treated with 2500mg/kg) showing ML- Molecular layer, GL- Granular layer and DPC-degenerated Purkinje cell, V- Vacuole (Cresyl Echt Violet Stain X 250).

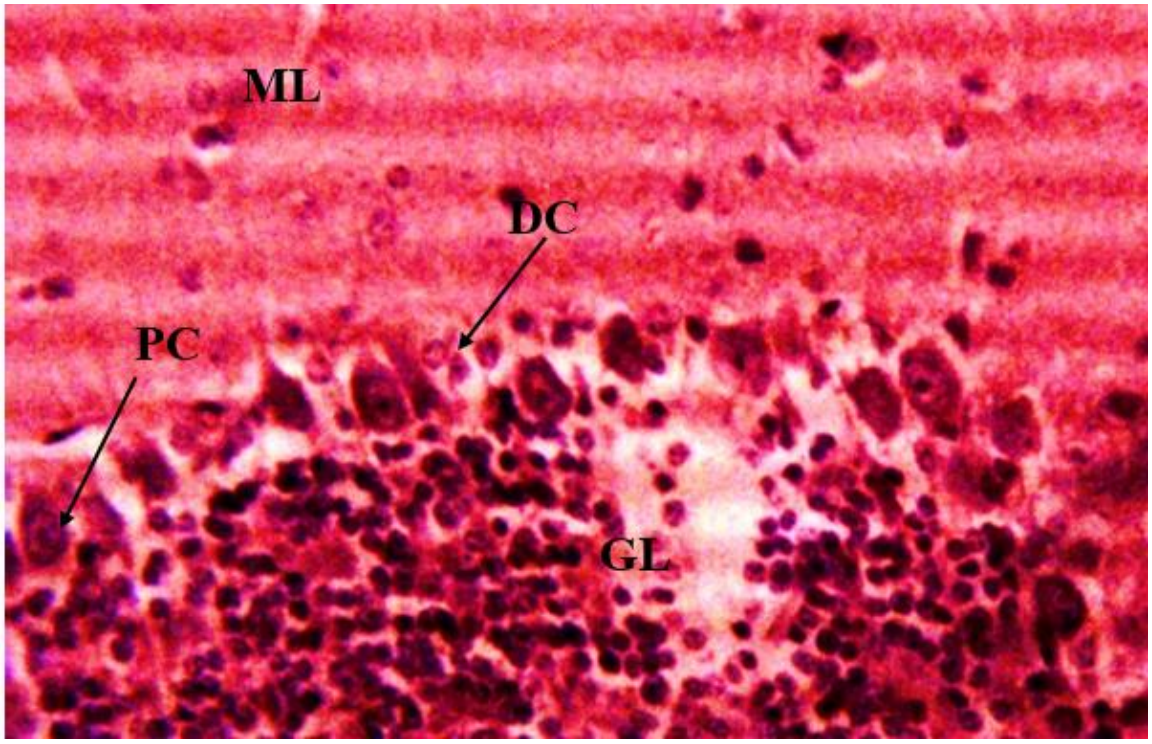


Plate XLVIII: A section of cerebellum (Treated with 1250mg/kg) showing ML- Molecular layer, PC – Purkinje cell, DC- Disintegrated cell, GL- Granular layer (H and E X 250).

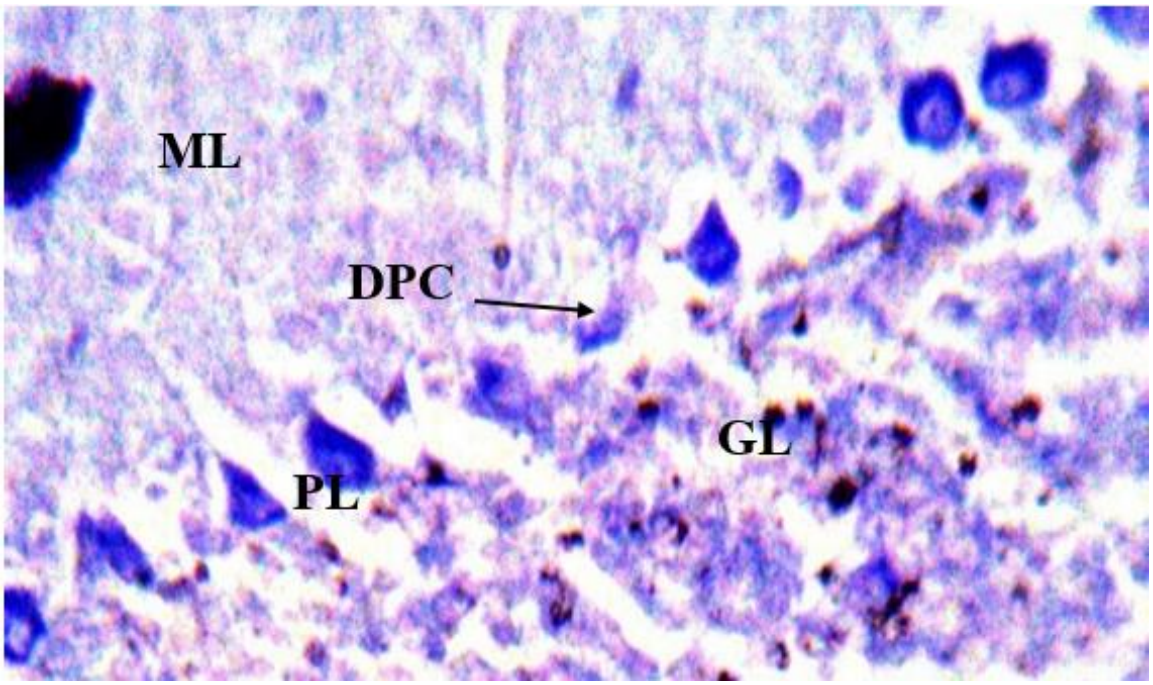


Plate XLIX: Section of cerebellum of Group 4 (Treated Group 1250mg/kg) showing ML- Molecular layer, DPC – Degenerated Purkinje cell, PL- Purkinje layer (Cresyl Echt Violet stain, X 250).

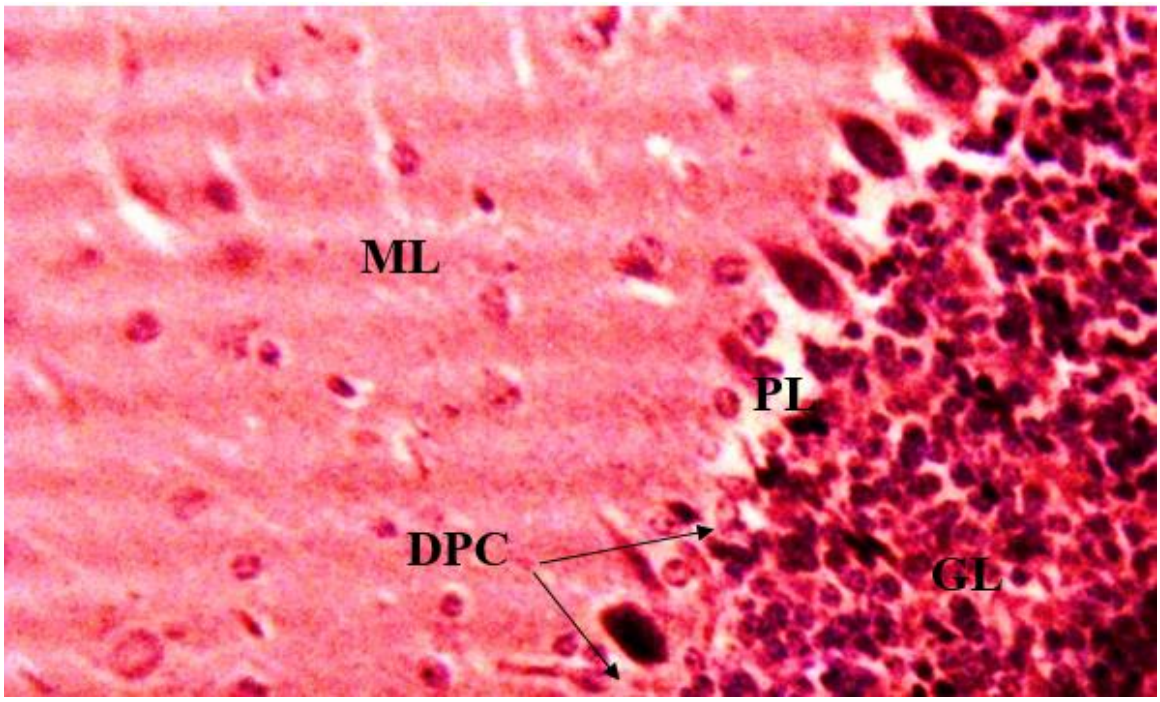


Plate L: Section of cerebellum (Treated with Chloroquine 10mg/kg) showing ML-Molecular layer, DPC-Degenerated Purkinje cell, GL- Granular layer (H and E X 250).

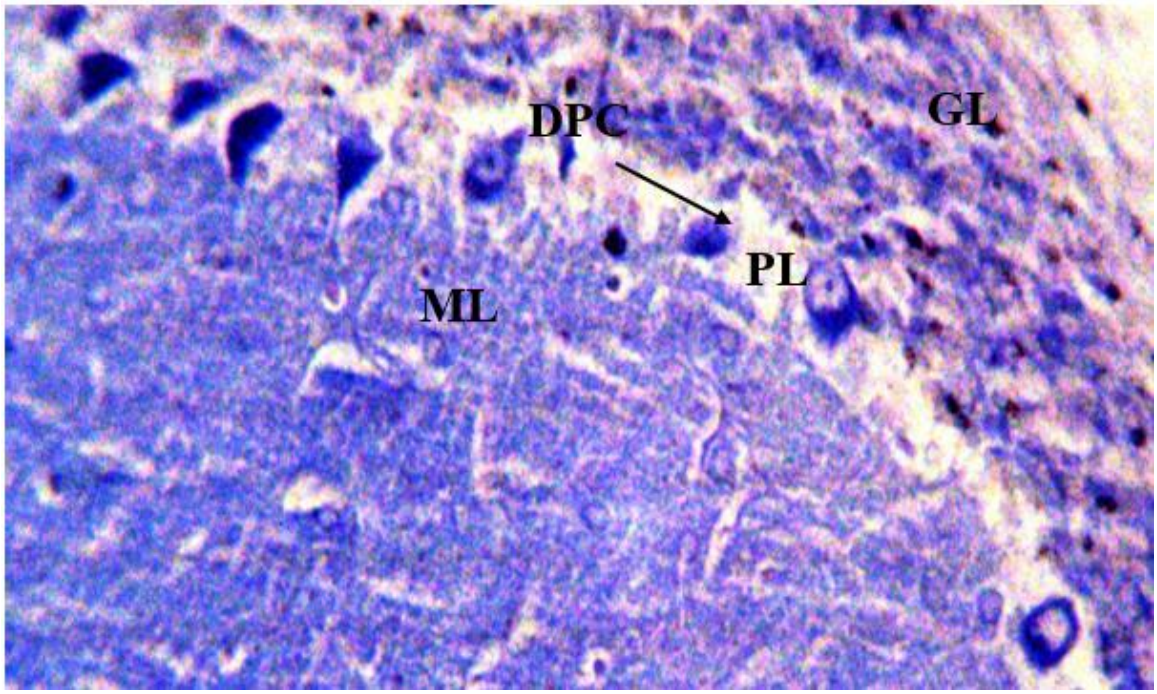


Plate LI: Section of cerebellum (Treated with Chloroquine 10mg/kg) showing ML-Molecular layer, DPC-Degenerating Purkinje cell, GL- Granular layer. PL- Purkinje layer (Cresyl Echt Violet Stain, X 250).

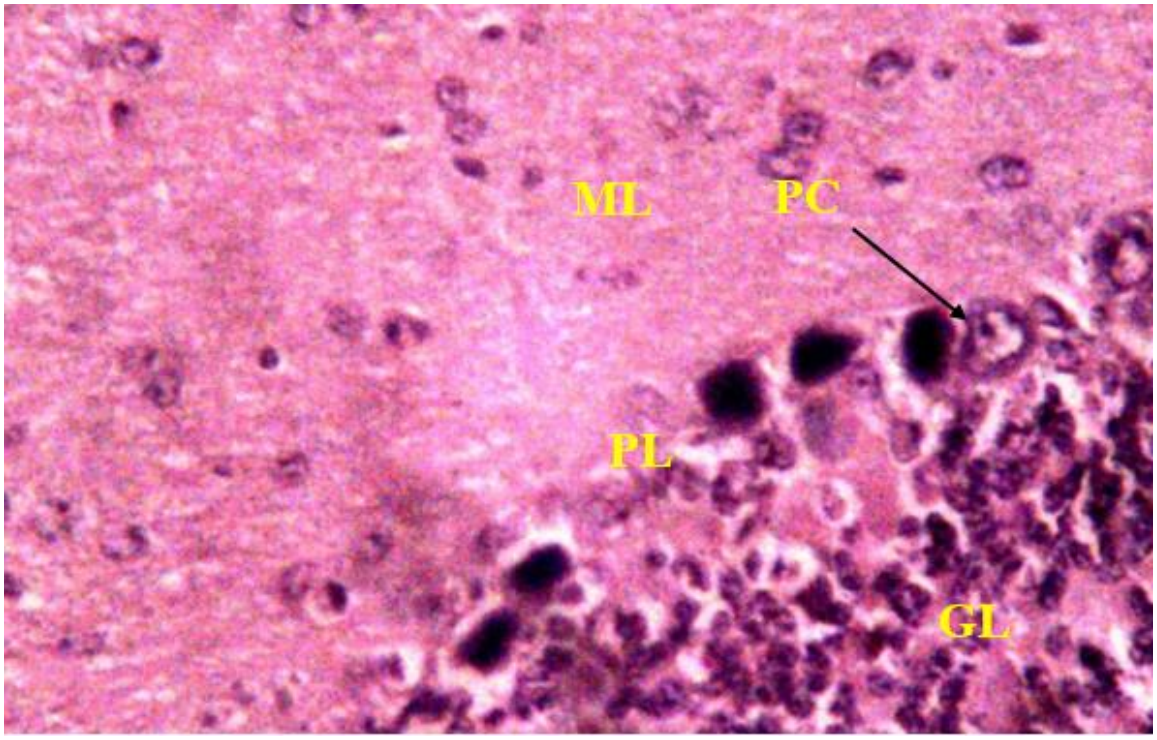


Plate LII: Section of cerebellum of Group 1(Control Group) showing ML- Molecular layer, PC – Purkinje cell, PL- Purkinje layer, GL- Granular layer (H & E stain, X 250).

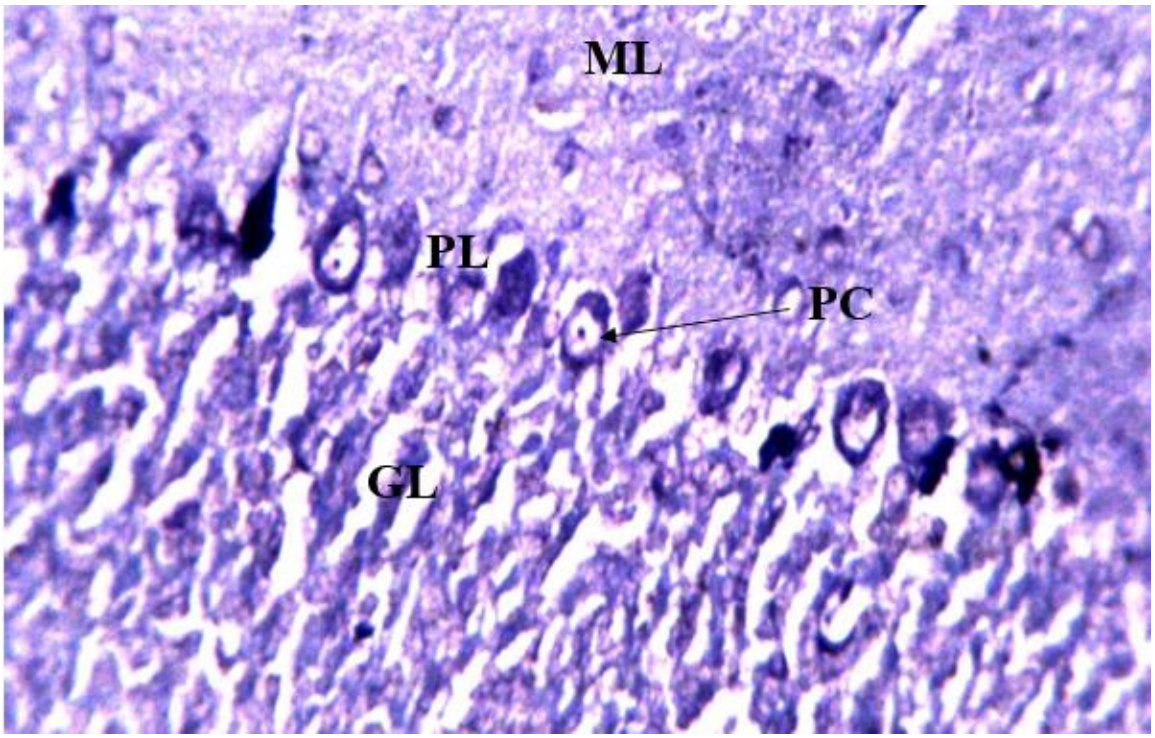


Plate LIII: Section of cerebellum of Group 1(Control Group) showing ML- Molecular layer, PC – Purkinje cell, GL- Granular layer (Cresyl echt violet, X 250).

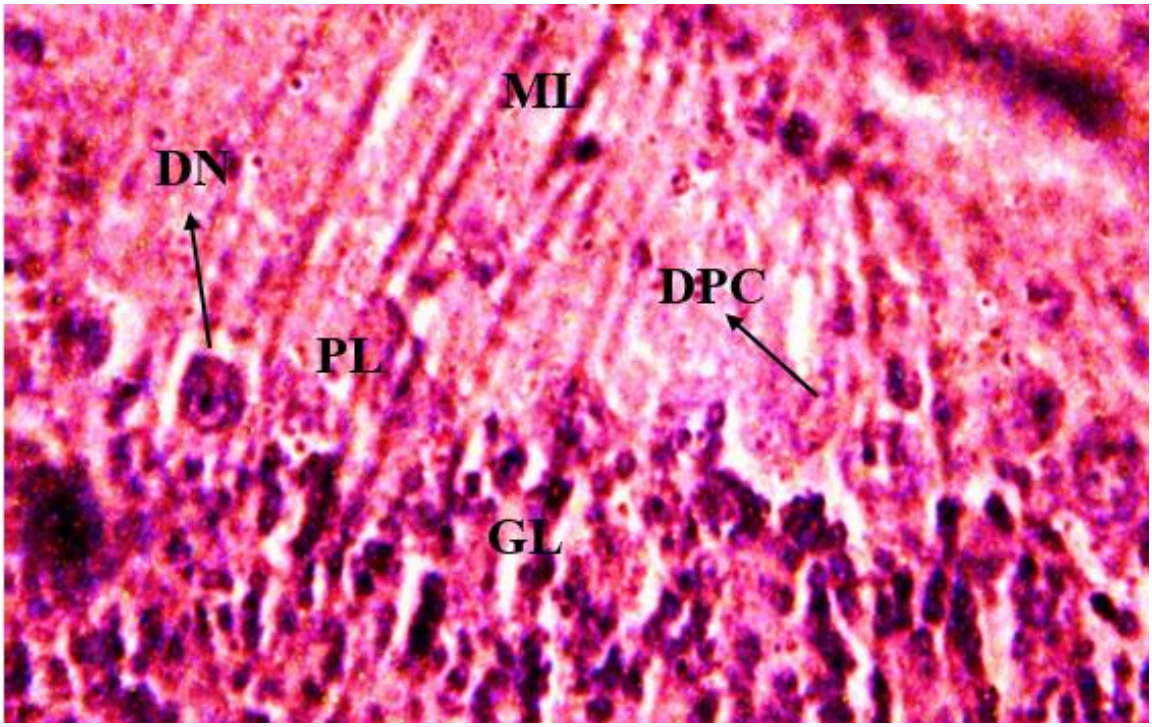


Plate LIV is a section of cerebellum of Group 2 (Untreated Group) showing ML- Molecular layer, DPC – Degenerating Purkinje cell, GL- Granular layer, DN- Disintegrated nuclei. (H & E stain, X 250).

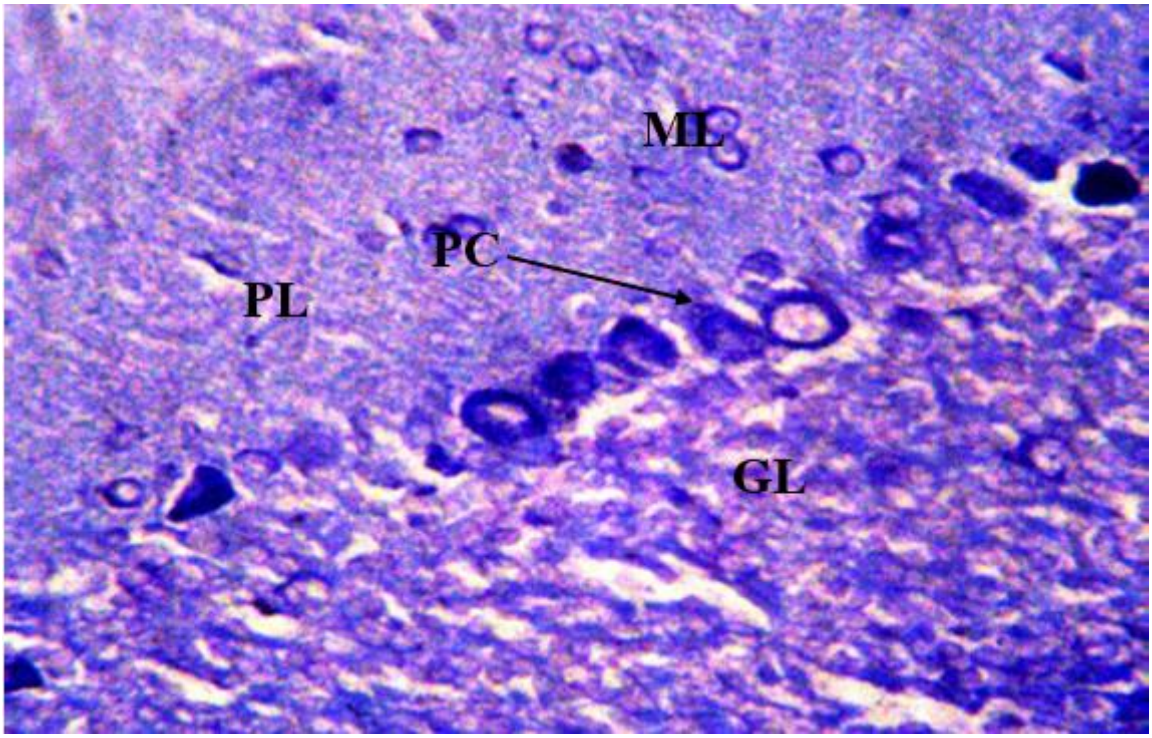


Plate LV: A section of cerebellum of Group 2 (Untreated Group) showing ML- Molecular layer, DPC – Degenerating Purkinje cell, GL- Granular layer (Cresyl Echt violet stain, X 250).

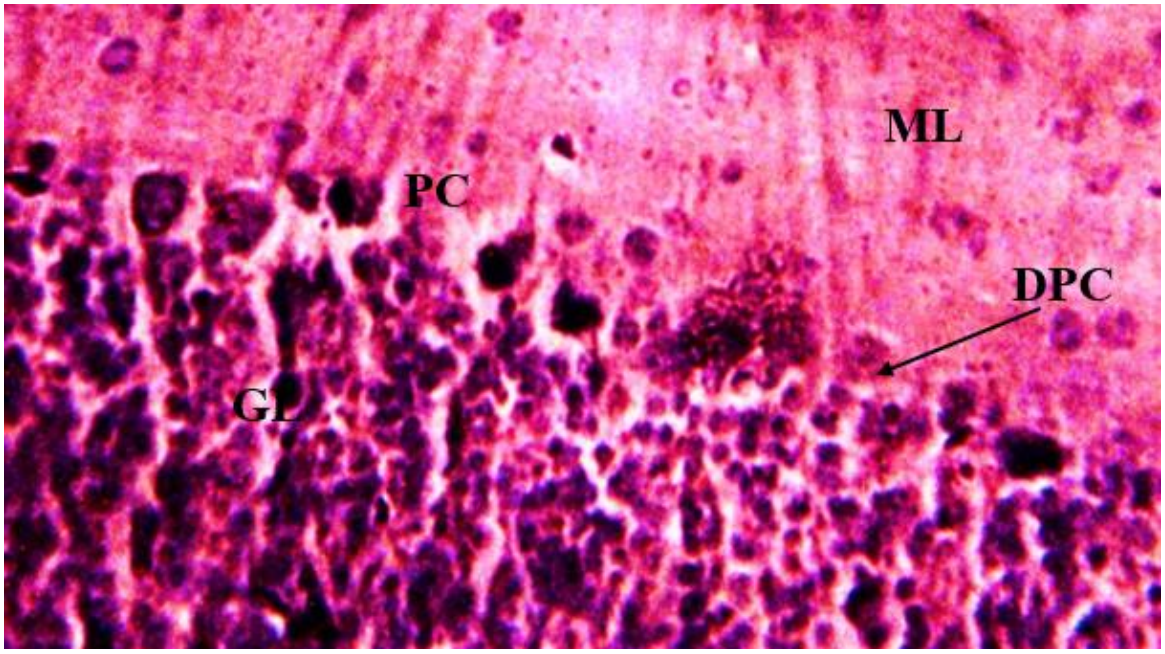


Plate LVI: Section of cerebellum (Treated with 2500mg/kg) showing ML- Molecular layer, DPC – Degenerating Purkinje cell (H and E stain, X 250).

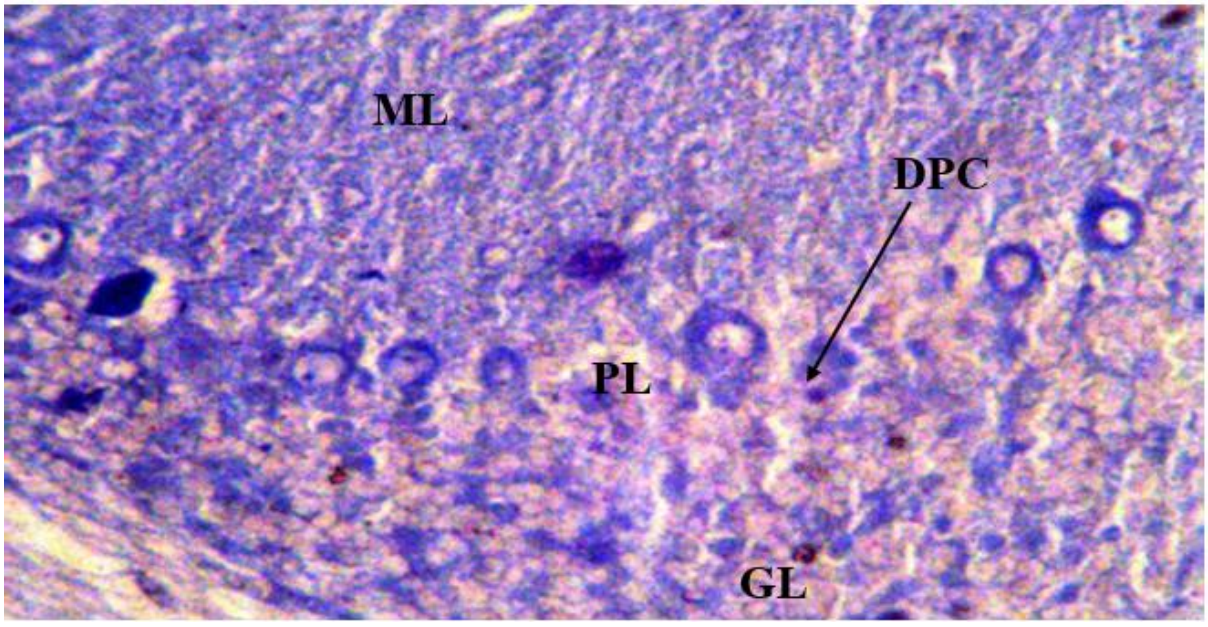


Plate LVII: section of cerebellum (Treated with 2500mg/kg) ML- Molecular layer, GL- Granular layer and DPC-degenerated Purkinje cell (Cresyl Echt Violet X 250).

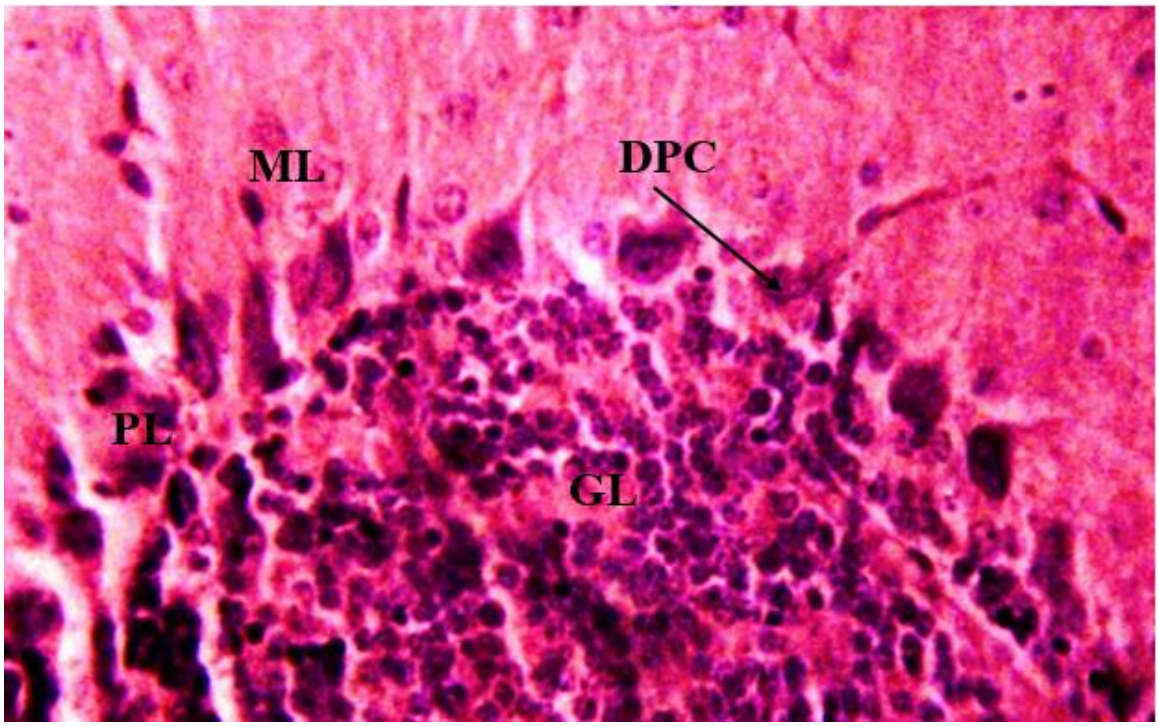


Plate LVIII: Section of cerebellum (Treated with 1250mg/kg) ML- Molecular layer, DPC – Degenerating Purkinje cell, GL- Granular layer (H and E X 250).

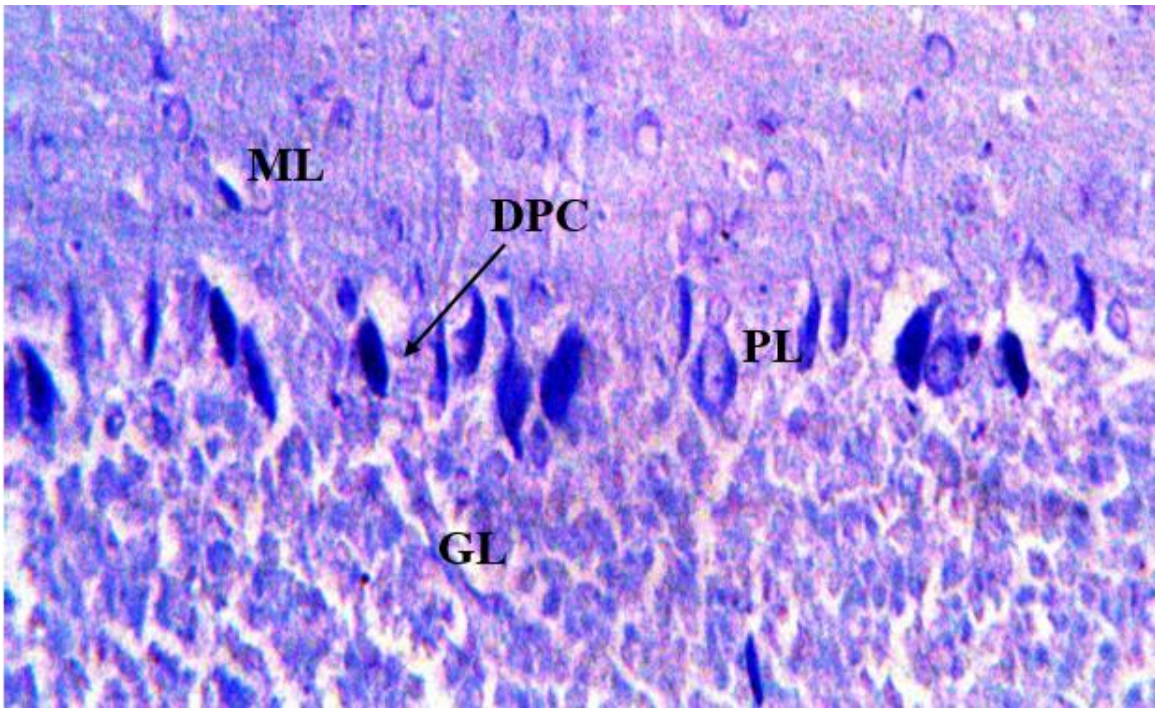


Plate LIX: Section of cerebellum (Treated with 1250mg/kg) ML- Molecular layer, PC – Purkinje cell, DC- Degenerated cell, GL- Granular layer (Cresyl Echt Violet X 250).

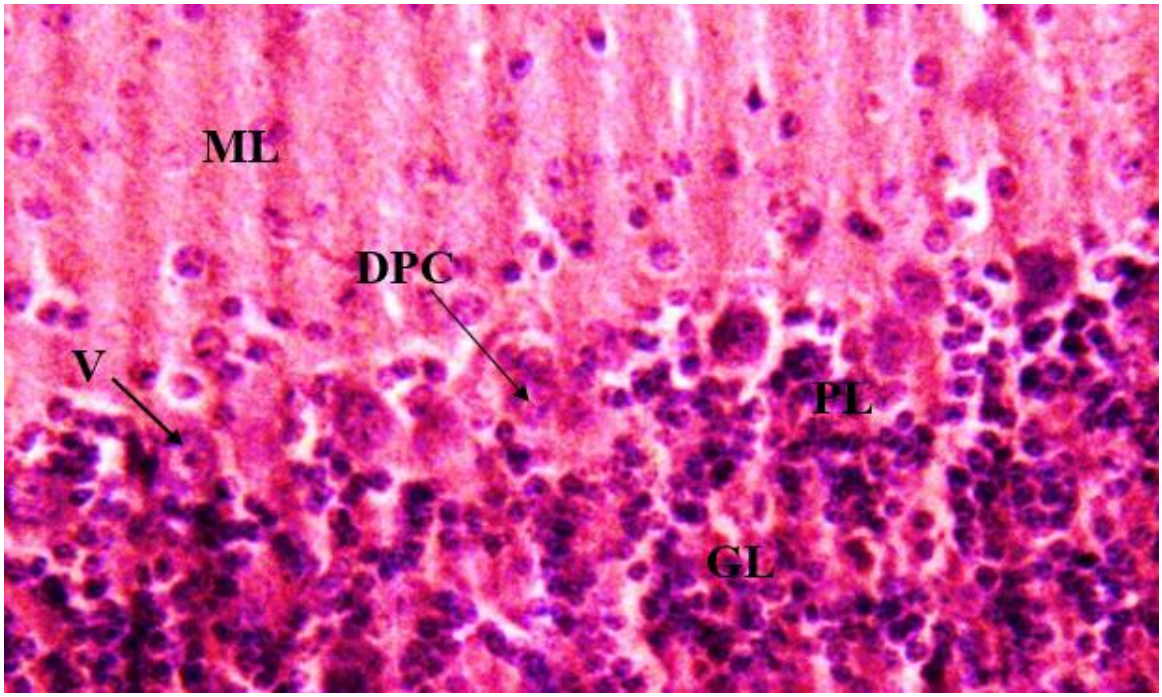


Plate LX: Section of cerebellum (Treated with Chloroquine10mg/kg) ML- Molecular layer, DPC – Degenerating Purkinje cell, GL- Granular layer, V- Vacuole (H and E X 250).

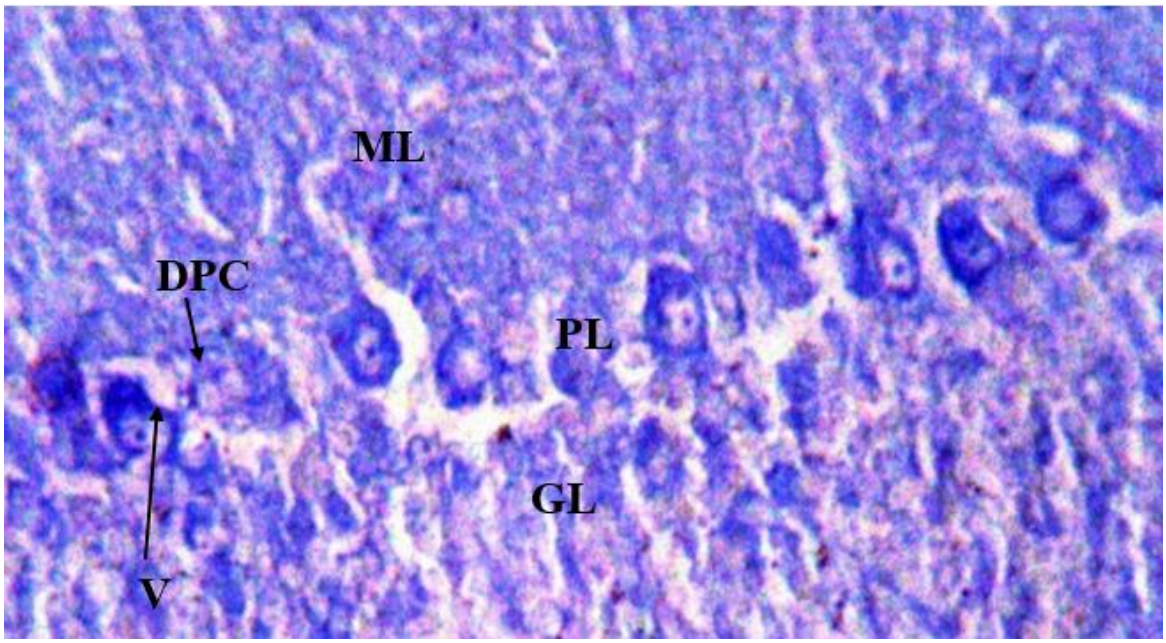


Plate LXI: Section of cerebellum (Treated with Chloroquine10mg/kg) showing ML- Molecular layer, DPC-Degenerated Purkinje cell, GL- Granular layer (Cresyl Echt Violet X 250).

CHAPTER FIVE

5.0 DISCUSSION

The widely used medicinal plants have formed the basis of health care throughout the world since the earlier days of humanity and have considerable importance (Ebong *et al*., 2008). Plant materials remain an important source of medicinal products in the fight against malaria (Aderounmu, 2007). Since widely used antimalarial drugs such as quinine and artermisinin were isolated from plants (Rosenthal, 2001), and because of the increased resistance and lack of accessibility to existing affordable drugs, the pharmacognostic investigations of plants for the establishment of complementary medicine for malaria within traditional plants is necessary (Wang *et al*., 2007). Thus, knowledge of uses and side effects of medicinal plants provide a vital contribution to human health care. *Vernonia* species are the sources of many local medicines (Amole *et al*., 2006), people living in areas, where *V. amygdalina* grows use the plant for treating malaria and malaria symptoms.

Even though medicinal plants are ASSUMED to be safe, many of them are potentially toxic (Ajaiyeoba *et al*., 2006) therefore, evaluating the safety level of the herbal medicine is necessary for the determination of the safe dose that can be used for treatment (Verma and Singh, 2008). According to Lorke's method 1983 lethal dose has serve as a base for the establishment of the safe dose for treatment. The absence of serious acute toxic symptoms such as mortality, impaired movement, restlessness, reduced motor activity etc. within 24 hours and survival of mice after oral administration up to a dose of 2000 mg/kg body weight of the extract for a two week indicates that the estimated oral median lethal dose (LD_{50}) is not toxic and the extract at 4000 mg/kg body weight is non-lethal (CDER, 1996).

Consequently, for the present study where lorke's method 1983 was used oral administration of the ethanolic extract of *Vernonia amygdalina* at the dose of 2000 mg/kg

for the acute toxicity did not cause sign of toxicity like hyperactivity, twitching, rigidity, irritability, jumping, sleep, sedation, abnormal secretion, death within 24 hours, while in the highest dose of 5000mg/kg also shows no toxicity sign. Therefore, the currents study of plants leaf can be considered as safe at a maximum dose for up to 5000mg/kg, according to lorke's method 1983. The main antimalarial mode of action of terpinoids, quinolines and artemisinin derivatives whose structures are totally different with those of quinoline alkaloids has been established to be their inhibition of haemin polymerisation through their binding with haemin (Kayembe *et al*., 2012). The in-vivo model was employed for this study because it takes into account the possible prodrug effect and possible involvement of the immune system in eradication of infection (Waako *et al*., 2010).

P. berghei was used in the prediction of treatment outcomes (Dikasso *et al*., 2006) and hence it was an appropriate parasite for the study. Moreover, several conventional antimalarial agents such as chloroquine, halofantrine, mefloquine and more recently artemisinin derivatives have been identified using rodent model of malaria (Madara *et al*., 2010).

The four-day suppressive test, which mainly evaluates the antimalarial activity of patient on early infections, while Rane's test, which evaluates the curative capability of extracts on established infections in patient, and prophylactic test (repository test) which evaluate the prophylactic capability of the patient extract prior to infection to prevent the upcoming disease are commonly used for antimalarial drug screening.

The extracts were considered active when parasitaemia was reduced by > 30% (Carvalho *et al*., 1990). Accordingly, the current study result showed that the ethanolic extracts of *Vernonia amygdalina* were dose dependent suppressive effect against *P. berghei* in all the three models of antimalarial test.

In Four-day suppressive model the ethanolic extract shows a significant level of ($p< 0.001$) suppression of 64.5%, 67.6% at the dose of 2500mg/kg, 1250mg/kg when compare the standard drug (control). This is in accordance to (Temsegen *et al*., 2015) where aqueous and hydromethanol leaf extract showed a significant ($p< 0.0001$) chemo suppression of 68.8% and 69.2% at the dose of 600 mg/kg respectively when compared to the normal control. The current study showed a decrement in suppressive effect than the previous report, which might be the solvent used by the present study had a poor extraction effect.

The extracts of *V. amygdalina* suppressed the growth of *P. berghei* malaria in the Curative design (Rane's test), with highest malaria suppression recorded at the dose of 2500 mg/kg. The percentage decrease in parasitaemia was not comparable to 10 mg/kg Chloroquine, as Chloroquine had the lowest suppression. This suggests that *V. amygdalina* leaf extract can suppress parasite growth if given orally for curative purposes. The oral treatment with *V. amygdalina* on Day 7 revealed that the ethanolic extracts had reduced the level of parasitaemia to 45.6% and 50.6%, respectively (table 4.4). In addition, in curative test of the current study, the effect of the ethanolic extract of *V. amygdalina* on the established malaria infection had shown a significant ($p<0.015$) at day 7 with parasitaemia suppression with maximum of 50.6 % at the dose of 1250mg/kg, respectively (Table 4.4).

Other study was also done on *V. amygdalina* plant against this rodent parasite, (Anoka, 2008), which reported that the aqueous leaf extract of *V.amygdalina* were found to have a significant ($P < 0.05$) and caused a 73.9% curative effect at the dose of 200mg/kg i.p (intraperitoneal). The slight result difference might be due to route of administration. In addition, Iwalokun (2008), which showed 83.3 and 100 % cure rate at the dose of Chloroquine10mg plus *V. amygdalina* 62.5 and Chloroquine10mg/kg plus V. amygdalina125mg/kg respectively. The synergetic effect of chloroquine with

V.amygdalina might be the reason for the difference between the previous study and the present study.

The present study also showed the prophylactic effect of ethanolic extract of *V. amygdalina* with significant (P<0.057) parasitaemia suppression effect at the dose of 1250 mg/kg with 72.7% respectively as compared to normal control group. While the standard drug, pyrimethamine, showed the highest effect (76.2%, P<0.057) at the dose of 1.2 mg/kg as compared to normal control group.

Even if much data has not been obtained on the repository effect on *V. amygdalina* other report during a repository study with the use of medicinal plant extract by (Ebiloma et al., 2012), in the treatment of mice infected with *P. berghei* with aqueous leaf extract of *Morinda lucida,* with 70.18% at the dose of 800mg/kg activity were recorded. Study done by Petros and Daniel (2012) who also checked the prophylactic effect of other traditional medicinal plant extract against *P. berghei* also exhibited a significance (75.6%, P < 0.05) effect at the dose of 600mg/kg.

CHAPTER SIX

6.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

6.1 Conclusion

The spread of resistant malaria parasites to the available antimalarial drugs call for a new chemotherapeutic agent to control the disease. Medicinal plants are constantly screened for bioactivity in our quest for the discovery of new and effective therapeutic agents. The results of this study could help encourage more identification and validation of natural products which has shown antiparasitic properties thus facilitating the development of a new generation of antimalarials.

In conclusion, the results obtained from current study indicated that;

i. Both the safety and efficacy test of the ethanolic leaf extracts of of *V. amygdalina* suggested that the plant is not toxic and did not cause acute toxicity symptoms up to the highest dose (5000mg/kg) in toxicity studies and (2500mg/kg) administered to the experimental mice.
ii. The antimalarial test results of this study showed that the ethanolic extracts of the plant material possessed a suppressive antimalarial activity. The ethanolic extracts showed antimalarial effects, thus it justifies the traditional usage as malaria remedy in Africa locality. The ethanolic plant extract produced statistically significant parasitaemia suppression in dose dependent manner during a standard 4-days suppressive test

iii. The ethanolic extracts of the leaves of *V. amygdalina* shown a promising curative and suppressive anti-malarial activities using *Plasmodium berghei* infected mice in a dose-dependent manner.

iv. In general, antimalarial activities as well as the lack of toxicity of the extracts found in the present study may partly confirm the claim by traditional practitioners for the use of the plants against malaria and support the ethnobotanical use of the plant in the treatment of malaria.

6.2 Recommendations

i. Further pharmacological screening with bioassay guided chemical fractionations of the ethanolic extracts of the study plant would permit the isolation and identification of antimalarial active compounds.

ii. It is recommended that further study should be conducted considering the other parts of the brain with the relevant developmental structure to attain efficacy in regulating anti-plasmodial activity in postnatal episodes of malaria.

iii. It is recommended that, the plant extracts should be evaluated together with some of the other antimalarial drugs currently used in order to find out synergistic effects on their ability to reverse development of resistance.

iv. It is also recommended that the mass population should develop the habit of using bitter leaf in their culinary practices so that they can gain the benefits associated with the medicinal plant (*Vernonia amygdalina).*

6.3 Contribution to Knowledge

i. Establishment of the trend that the *Plasmodium berghei* parasite can affect the cerebral and cerebellar cortices of young mice as a function of varying time and

durations thus providing more fact to the pathophysiology of the severe malaria (Cerebral Malaria CM) more prevalent in the young ones.

ii. Suppressive effect of ethanol leaf extract of *Vernonia amgydalina* was eminent and establish through the parasite quantification and suppression percentage via inverse proportionality. It has a significant difference of ($p< 0.001$), suppression of 74.4%, 67.6% at the dose of 2500mg/kg and 1250mg/kg of bodyweight respectively with the percentage parasitaemia of the dose 2500mg/kg (2.84±0.05) and 1250mg/kg (3.28±0.12) compared to the inoculated untreated group which has parasitaemia as high as (4.44±0.16).

iii. Ethanol leaf extract of *Vernonia amgydalina* ameliorate the effect of *Plasmodium berghei* parasite in the curative test with the level of parasitaemia suppression to be 59.5% and 45.6% at the dose of 2500mg/kg and 1250mg/kg, with significant level of ($p< 0.015$). The percentage parasitaemia of the 2500mg.kg and 1250mg.kg dose of the extract is as low as (2.48±0.22) and (3.76±0.35) compare to the inoculated group has parasite load of (4.34±0.57).

iv. That the extract possesses prophylactic activity and shows reduction in parasite load though without significant difference between the groups. However, there is no relationship observed between the percentage parasitaemia quantification and the histological observation.

v. The ameliorative effect observed in the suppressive and curative test were significant and dose dependent. Also, the histological changes in the cerebral and cerebellar cortices shows relationship between the observations in treated group compare to *P. berghei* group.

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