THE EFFECTS OF SUBACUTE EXPOSURE TO MOSQUITO COIL SMOKE ON NEURO-BEHAVIOUR, THE HIPPOCAMPUS, CEREBRAL AND CEREBELLAR CORTICES OF ADULT WISTAR RATS

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BY

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FEBRUARY, 2017

DECLARATION

I hereby declare that the research work reported in this thesis entitled "**TheEffects of Sub-Acute Exposure to Mosquito Coil Smoke on Neuro-behaviour, The Hippocampus, Cerebral and Cerebellar Cortices of Adult Wistar Rats**" was carried out by me in the Department of Human Anatomy, Faculty of Medicine, A.B.U Zaria, under the supervision of Prof. S.S Adebisi and Dr. A.A Buraimoh. The information obtained from the literatures have been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree in any institution.

_____ _____

Mahmood Usman Date

CERTIFICATION

This thesis entitled "T**he effects of Sub-Acute Exposure to Mosquito Coil Smoke on Neuro-behaviour, The Hippocampus, Cerebral and Cerebellar Cortices of Adult Wistar Rats**" by Mahmood Usman meet the regulation governing the Master of Science (M.Sc.) degree in Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of Prof. S.S. Adebisi and Dr. A.A. Buraimoh. It is therefore approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to my parents for their support and prayers towards the actualization of my dreams.

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ABSTRACT

Mosquito coil is the preferred anti-mosquito product in low income communities because it is cheap and readily available. Burning mosquito coils indoors generates smoke that can repel mosquitoes effectively. However, the smoke contains pollutants of health concern such as carbon monoxide, acenaphthene, penanthrene and benzo(a)pyrene. This study was designed to determine the effect of exposure to mosquito coil smoke on memory, learning, oxidative stress and histological changes. Twenty-eight (28) adult male Wistar rats were randomly grouped into four (4) with group I serving as control and exposed to ambient air while the experimental group II was exposed to mosquito coil smoke for 1hour,group III for 2hours and group IV for 3hoursin a partially ventilated chamber for twenty-eight (28) consecutive days. Analysis of the results collected showed statistical significant difference in short-term memory (33.7±14.7, 21.9±4.0, 28.2±10.4 and 36.1±6) at $p<0.05$ in the smoke exposed groups. No statistically significant difference was noticed in learning and motor activity ($p>0.05$). Interestingly, significant difference was recorded in the oxidative stress maker and in the trace elements analysis which could have resulted in the histopathological changes observed in the study. This resultsof this study showed that exposure to mosquito coil smoke causes deficit in short term memory and leads to oxidative stress in adult Wistar rats by altering some trace elements.

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ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
AC	Aggregation of cells
AChE	Acetylcholinesterase
ANOVA	Analysis of Variance
BHT	Dibutylhydroxide toludene
CA	Cornu Ammonis
CAT	Catalase
CNS	Central Nervous System
CO	Carbon monoxide
CO_2	Carbon dioxide
Cu	Cupper
DNA	Deoxyribonucleic acid
DPC	Degenerating pyramidal cell
DSC	Degenerating Stellate cell
EEG	Electro encephalograph
EHD	Environmental Health Department
EPA	Environmental Protection Agency
Fe	Iron
GABA	Gamma Amino Butyric Acid
GSH	Glutathion
H and E	Heamatoxylin and Eosin
LPO	lipid peroxide
MDA	Malondialdehyde
Mn	Manganese

NMDARs	N-methyl D aspartate receptors
PAH	Polyaliphatic hydrocarbon
PBO	Piperonyl btoxide
PC	pyramidal cell
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SC	Stellate cell
SEM	Standard error of mean
SOD	Superoxide dismutase
SPSS	Statistical Product and Service Solution
USEPA	United State Environmental Protection Agency
UV	Ultra violet
V	Vacoulation
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme
Zn	Zinc

CHAPTER ONE

1.0 Introduction

In most urban and rural areas of Africa, Asia and South America, mosquito populations are menacing throughout the year, except for some attenuation during summer and winter. Mosquitoes transmit diseases such as malaria, dengue fever, hemorrhagic fever etc. (Sharma, 2001). Malaria and other mosquito-borne diseases serves as a major health problem in these tropical areas, Insecticides like mosquito coil usage has increased in recent decades with an annual world consumption estimated at 32 billion coils as at the year 2000 (Mshelia *et al*., 2013). General abuse and wanton overuse of these mosquito coils and other insecticides in the control of mosquitoes pose a serious public health challenge, especially innocuous and chronic inhalation of the fumes and consumption of produce that may have been laced inadvertently by chemical constituent of the insecticide (Mshelia *et al*., 2013).

Mosquito coils are the preferred anti-mosquito products in low income communities because it is cheap, easy to use and readily available, these coils are burned indoors as a common practice in many households in Africa, Asia and South America including Nigeria. (Garba *et al*., 2007). In 1999, fire that swept through a three-story dormitory building at a summer camp in South Korea killing 23 people including 19 children was caused by a spark from an ignited mosquito coil (Charles, 2006).

Mosquito coil is a mosquito repelling incense,widely known as an efficient mosquito repellent usually shaped into a spiral and made from a dried paste of Pyrethrum powder (McKean, 2005). The active ingredients found in mosquito coils can be any or some of the following; Pyrethrum, Pyrethrin and Allethrin. Other ingredients include

Dibutylhydroxyl toluene (BHT), Piperonyl butoxide (PBO), aromatic and aliphatic hydrocarbons (Sharma, 2001).

World Health Organization pesticide evaluation scheme (WHOPES) stated in 1996 that all pesticides are toxic to humans to some degree (WHO, 1996). Every year, hundreds of millions of cases of insect-borne diseases occur, representing a major threat to global public health. Vector-borne diseases account for around 17% of the estimated global burden of infectious diseases. Environmental changes together with operational, financial and managerial problems, pesticide resistance and increasing population mobility have contributed to increases in the prevalence of many of these diseases in recent decades (Harsimran, and Harsh,2014; Akunna *et al* 2013).

Epidemiologic studies show an association between Long-term exposure to mosquito coil smoke and asthma and persistent wheeze in children (Azizi and Henry, 1991; Fagbule and Ekanem, 1994). Studies of toxicological effects of mosquito coil smoke on Rats shows focal debilitation of the tracheal epithelium metaplasia cells and morphological alteration of the alveolar macrophages (Liu and Wong, 1987). Biochemical analysis of serum obtained from rats exposed to mosquito coil smoke showed significant increase in serum levels of urea and creatinine, indicating functional damage to the kidney (Panda 1999). Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet while creatinine is more specific to the kidney since kidney damage is the only significant factor that increases serum creatinine level (Cheesbrough, 1998).

Exposure to mosquito coil smoke shows decrease in the protein biosynthetic activity of the liver, which could affect capacity of serum protein-mediated transport of various substance (Okine *et al*., 2004). Exposure to mosquito coil smokehas been reported to challenge the immune system in experimental rat model resulting in decreased neutrophil,lymphocyte and body weight (Garba *et al*., 2007). Learning and memory tasks that are dependent upon motor or sensorimotor function may also be disrupted by pyrethroids at levels well below those that induce severe neurotoxicity (Wolansky and Harrill, 2007). Other mosquito repellents that are not Pyrethroid based have been shown to cause neuronal degeneration in the brain leading to impairment of learning and memory (Abdel-Rahman *et al*., 2001; Sudakin and Trevathan, 2003).

Oxidative stress is the imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (deDiego *et al*., 2009). Increase in erythrocyte lipid peroxidation is correlated with the inhibition in erythrocyte AChE activity and so erythrocyte AChE can be a marker enzyme in pyrethroid toxicity (Kale *et al*., 2009). Despite the fact that mosquito coil smoke may have many potential adverse health effects, large populations in developing countries still use mosquito coils in their daily lives (Liu *et al*., 2003). However, despite the reported adverse effects, there is paucity of information on the toxicological effects of mosquito coil smoke on learning and memory. Thus research in this area is pertinent.

1.1 Statement of Research Problem

Burning mosquito coils indoors generates smoke that can repel mosquitoes effectively. This practice is currently used in numerous households around the globe, especially in

Africa, Asia and South America (Liu *et al*., 2003). However, the smoke may contain pollutants of health concern such as carbon dioxide (CO_2) and carbon monoxide (CO) yet there is scarcity of information on its effect on learning and memory.

1.2 Justification of the Study

There are evidence that inhalation of mosquito coil smoke can lead to depression, confusion and cognitive impairment. However, little is known of its effect on learning and memory. The study seeks to investigate the deficit in learning and memory following exposure to mosquito coil smoke. This study would help elucidate the detrimental effects of mosquito coil smoke exposure on learning and memory in experimental animals.

1.3 Scope of the Study

This study was limited to the general histology, histochemistry, behavioural changes and degenerative changes in the hippocampus, cerebellar and cerebral cortices and alteration in some trace elements.

1.4 Aims and Objectives of the Study

The aims of the study are to investigate the effects of exposure to mosquito coil smoke on Neurobehaviour, Histological and Histochemical changes on brain tissues of Adult Wistar rats.

1.4.1 Objectives

i. to investigate the effects of mosquito coil smoke on short-term memory in adult Wistar rats using object recognition test.

ii. to evaluate the effects of mosquito coil smoke on spatial learning and memory in adult Wistar rats using Morris water maze.

iii. to examine the effects of mosquito coil smoke on the motor activity of adult Wistar rats using Montoya staircase test.

iv. to find out the histopathological changes in hippocampus, cerebellar and cerebral cortices of adult Wistar rats following exposure to mosquito coil smoke using H and E stain and Cresyl Fast Violet stain.

v. to study the activities of some oxidative stress biomarkers in the brain tissue of the adult Wistar rats following exposure to mosquito coil smoke.

vi. to evaluate the possible effects of mosquito coil smoke on some trace elements Copper (Cu), Iron (Fe), Zinc (Zn) and Manganese (Mn) in the brain tissue.

1.5 Study Hypothesis (Alternate Hypothesis)

Exposure to mosquito coil smoke does have a neurotoxic effect on adult Wistar rats.

CHAPTER TWO

2.0 Literature Review

2.1 Mosquito Coil

Mosquito coils are widely used as mosquito repellent. The major active ingredients of most mosquito coils are pyrethrins (allethrin) accounting for about 0.2 – 0.4% of the coil mass (Akunna *et al.*, 2013). When a Mosquito coil is burnt, the insecticides evaporate (pyrethrin, Polyaliphatic hydrocarbon, aldehyde etc.) with smoke, preventing mosquitoes from entering the room and harm those already in the room. The remaining components of mosquito coils include organic fillers, binders, dyes and other additives capable of burning well without flame. The combustion of these remaining components generates large amounts of submicrometer particles and gaseous pollutants such as acenaphthene, penanthrene, benzo(a)pyrene etc. These particles can be coated with a wide range of organic compound generated through incomplete combustion of mosquito coil base materials (Abubakar and Hassan, 2006).



Figure 2.1. Mosquito coil

2.2 Active ingredients

Pyrethrins and Pyrethroids are the active ingredients in mosquito coils, their usage has increased in the recent decades with the decline in the use of organophosphate pesticides, and has introduced newer issues, example residential usage of insecticides containing pyrethrins and pyrethroids which may lead to urban runoff and potentially exposing aquatic life to harmful levels in water and sediment (USEPA, 2013).

Pyrethrins are botanical insecticides derived from Chrysanthemum flowers, a plant most commonly found in Africa and Australia. It works by altering nerve function that causes paralysis in the target insect, and eventually resulting in death (USEPA, 2013). Pyrethrins, is a single ingredient in a pesticide containing six components that have insecticidal activity which are; pyrethrin 1, pyrethrin 2, cinerin 1, cinerin 2, jasmolin 1 and jasmolin 2(USEPA, 2013). Pyrethroids on the other hand are synthetic chemical insecticides whose chemical structure are adopted from the chemical structure of the pyrethrins and act in the same manner as pyrethrins. Pyrethroids are modified to increase their stability in the sunlight (USEPA, 2013). Pyrethroids includes, Allethrin stereoisomers, Bifenthrin, Cypermethrin, Tetramethrin, Tralomethrin, etc. (USEPA, 2013).

2.3 Pyrethroids site of action

The main site of action of the pyrethroids is the sodium channel, which is kept open for long period of time, causing prolonged sodium current to flow, leading to hyper-excitation of the nervous system (Sharma, 2001). Synthetic pyrethroids, like allethrin cause sub-normal or super-normal excitability by altering the sodium ion channel opening time (Sharma, 2001).

2.4 Exposure to Mosquito Coils Smoke

Exposure to allethrin-based mosquito coil for 16 weeks showed a significant decrease in the absolute and relative testicular weight of the models, with a significant reduction in sperm count and motility. In addition, the animals exposed to mosquito coil demonstrated a reduction of basal seminiferous epithelial cells, marked testicular atrophy, germinal aplasia and hypospermatozoa formation. There was a significant, progressive and clear degenerative change in their seminiferous tubule characterized by vacuolization of the interstitium and devoid of spermatozoa (Akunna *et al.,* 2013).

Mosquito coils with the active ingredient d-allethrin (0.2% w/w), was tested in a laboratory against mosquitoes of different ages. Results showed adequate knockdown ofmosquitoes, but with low mortalities for mosquitoes below 10days of age. The susceptibility of mosquitoes increased with age as indicated (Yap *et al.,* 1996). The study also indicates that bloodengorged mosquitoes were more difficult to be knocked down, but easier to kill than non-blood engorgedmosquitoes. Nevertheless, the number of eggs oviposited by females' mosquitoes, egghatchability, pupation rate and adult emergence rate were not affected if the mosquitoes were still able toblood-engorge (Yap *et al.,* 1996).

2.5 Memory

Memory is an organism's ability to store, retain and recall information and experience (Hacking, 1996; Mark, 2007; Michael, 2009). During the late nineteenth and early twentieth century, scientist have put memory within the paradigm of cognitive psychology (Hacking, 1996). In recent decades, it has become one of the principal

pillars of a branch of science called cognitive neuroscience, an interdisciplinary link between cognitive psychology and neuroscience (Brockmeier, 2010).

2.5.1 Types of memory

2.5.1.1 *Sensory memory*

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

2.5.1.2 *Short-term memory*

Short-term memory involves the hippocampus and allows recall for a period ofseveral seconds to a minute without rehearsal. Its capacity is also very limited(Mandler, 1967). Memory capacity can be increased through a process calledchunking. For example, in recalling a ten-digit telephone number, a person couldchunk the digits into three groups: first, the area code such as 215, then a three-digitchunk 123 and lastly a four-digit chunk 4567 (Brockmeier, 2010). This method ofremembering telephone numbers is far more effective than attempting to remember astring of 10 digits. This is because of the ability to chunk the information intomeaningful groups of numbers. Short-term memory is believed to rely mostly on anacoustic code for storing information, and to a lesser extent a visual code (Alberimi, 2001).

2.5.1.3 *Long-term memory*

Long-term memory involves the posterior cingulate gyrus and can store much larger quantities of information for potentially unlimited duration, sometimes a whole life span (Alberimi, 2001). Its capacity is immeasurably large. For example, given a random seven-digit number we may remember it for only a few seconds before forgetting, suggesting it was stored in our short-term memory. On the other hand, a person can remember telephone numbers for many years through repetition, this information is said to be stored in long-term memory (Tyler *et al.,* 1979). This type of memories, are maintained by more stable and permanent changes in neural connections widely spread throughout the brain. The hippocampus is essential for learning new information and the consolidation of information from short-term to long-term memory, although it does not seem to store information itself (Gross, 1993). Without the hippocampus, new memories are unable to be stored into long-term memory, and there will be a very short attention span (Gross, 1993; Mark, 2007)

2.6 The Central Nervous System

The Central Nervous System (CNS) is composed of the brain and spinal cord and is surrounded by bone mainly the skull and vertebrae (Saladin, 2010). Fluid and tissue alsoinsulate the brain and spinal cord (Saladin, 2010). The brain is composed of three parts: the cerebrum, the cerebellum and the brain stem (Saladin, 2010).

2.6.1 Hippocampus

2.6.2 Morphology and formation of the hippocampus

Anatomically, the hippocampus is an elaboration of the edge of the cerebral cortex (Gray, 2000, Chris, 2008). In rodent, the hippocampus, a horseshoe shape paired structure, with one hippocampus located in the left brain hemisphere and the

other in the right hemisphere (Gross, 1993). The hippocampus, the dentate gyrus and the sibiculum collectively make up the hippocampal formation, which is said to be part of the limbic system as shown in Figure 2.2. The hippocampus and the dentate gyrus belong to the allocortex and has simplified laminar pattern compared with the neocortex (Chris, 2008).

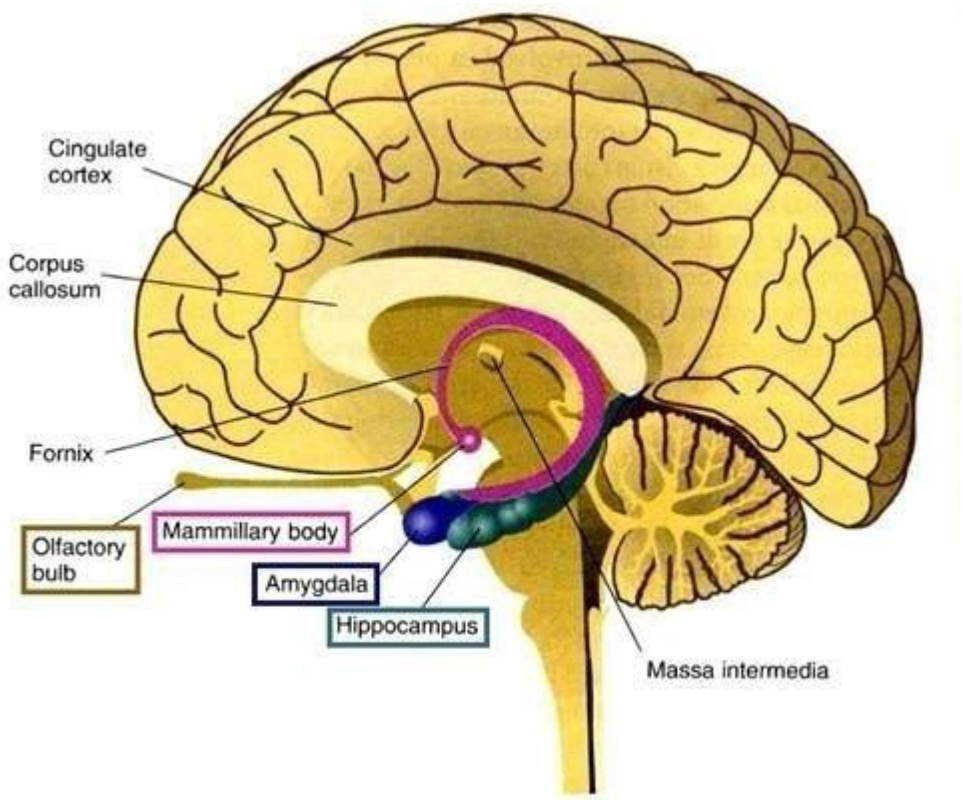


Figure 2.2: Hippocampus and associated structures of the limbic system (Chris, 2008).

2.6.3 The cells and layers of hippocampus

The hippocampus is composed of multiple subfields. Though terminology varies among authors, the terms most frequently used are dentate gyrus and the cornu ammonis literally "Amun's horns", abbreviated CA. The dentate gyrus contains the fascia dentata and the hilus, while CA is differentiated into fields CA1, CA2, and CA3

(Chris, 2008). The horse shoe appearance of the hippocampus is caused by cell density differentials and the existence of varying degrees of neuronal fibers as shown in Figure 2.3. The CA regions are also structured depth-wise in clearly defined strata (Hiroaki, 2007; Philippe, 2007; Annabelle 2009).

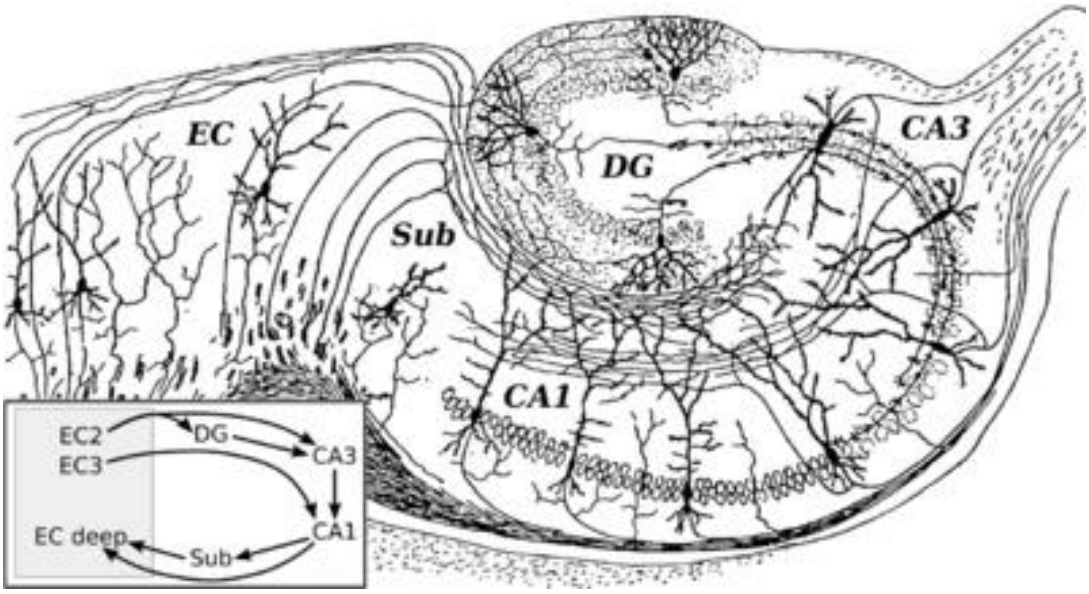


Figure 2.3: Cells and layers of the hippocampus (Annabelle, 2009).

The alveus is the deepest layer and contains the axons from pyramidal neurons passing on towards the fimbria/fornix, one of the major outputs of the hippocampus. Stratum oriens is the next layer superficial to the alveus. The cell bodies of inhibitory basket cells and horizontal trilaminar cells, named for their axons innervating the three layers- the oriens, pyramidal, and radiatum are located in this stratum (Annabelle, 2009). The basal dendrites of pyramidal neurons are also found here, where they receive input from other pyramidal cells, septal fibers and commissural fibers from the contra lateral hippocampus usually recurrent connections, especially in CA3 and CA2 regions (Annabelle, 2009).

Stratum pyramidale contains the cell bodies of the pyramidal neurons, which are the principal excitatory neurons of the hippocampus. This stratum tends to be one of the more visible strata to the naked eye. In region CA3, this stratum contains synapses from the mossy fibres that course through stratum lucidum. This stratum also contains the cell bodies of many interneurons, including axo-axonic cells, bistratified cells, and radial trilaminar cells (Chris, 2008). Stratum lucidum is one of the thinnest strata in the hippocampus and only found in the CA3 region (Annabelle, 2009). Mossy fibers from the dentate gyrus granule cells course through this stratum in CA3, though synapses from these fibres can be found in stratum pyramidale (Annabelle, 2009).

Stratum radiatum like stratum oriens, contains septal and commissural fibers. It also contains Schaffer collateral fibers, which are the projection forward from CA3 to CA1 (Annabelle, 2009). Some interneurons that can be found in more superficial layers can also be found here, including basket cells, bistratified cells, and radial trilaminar cells. Stratum lacunosum is a thin stratum that too contains Schaffer collateral fibers, but it also contains perforant path fibers from the superficial layers of entorhinal cortex (Annabelle, 2009). Due to its smallsize, it is often grouped together with stratum moleculare into a single stratum called stratum lacunosum-moleculare (Chris, 2008). Stratum moleculare is the most superficial stratum in the hippocampus. Here the perforant path fibers form synapses onto the distal, apical dendrites of pyramidal cells.

The hippocampal sulcus or fissure is a cell-free region that separates the CA1 field from the dentate gyrus. Because the phase of recorded theta rhythm varies systematically through the strata, the fissure is often used as a fixed reference point for recording EEG as it is easily identifiable (Annabelle, 2009).

2.6.4 The dentate gyrus

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

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

2.6.5 Functions of hippocampus

The hippocampus is believed to play important role in several functions of the body. The

hippocampus is the part of the brain that is involved in memory formation, organization and storage (Annabelle, 2009). Hippocampus is a limbic system structure that is particularly important informing new memories and connecting emotions and senses,such as smell and sound, tomemories. The hippocampus acts as a memory indexer bysending memories out to theappropriate part of the cerebral hemisphere for long-termstorage and retrieving themwhen necessary (Annabelle, 2009).

2.7 Cerebellum

2.7.1 Morphology of the cerebellum

The cerebellum is the largest part of the hindbrain. It is dorsal to the pons andmedulla, 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, whichcontain afferent and efferent fibres (Gray, 2000). The cerebellum occupies the posterior cranialfossa, where it is covered by the tantrum cerebelli. It is roughly spherical, butsomewhat constricted in its median region, and flattened - its greatest diameter istransverse (Clerke, 1996). In other words, the cerebellum is located at the bottom ofthe brain, with the large mass of the cerebral cortex above and the portion of thebrainstem called the pons in front of it (Clerke, 1996). It is separated from theoverlying cerebrum by a layer of leathery dura mater. All of its connections with theother parts of the brain travel through the pons (Clerke, 1996).

2.7.2 Cellular layers of the cerebellum

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

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 enlargementcalled a dendritic claw (Linas *et al.*, 2004). These

enlargements are sites of excitatoryinput from mossy fibers and inhibitory inputfrom Golgi cells (Linas *et al.*, 2004).

II. **The Purkinje layer** lies in the middle, forming a narrow zone that contains only the cell bodies of Purkinje cells (Linas *et al.*, 2004). They are distinguished by the shape of the dendritic tree. The dendrites branch very profusely, but are severely flattened in a plane perpendicular to the cerebellar folds. Thus, the dendrites of a Purkinje cell form a dense planar net, through which parallel fibers pass at right angles (Linas *et al.*, 2004; Bell, 2008). The dendrites are covered with dendritic spines, each of which receives synaptic input from a parallel 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. (Clerke, 1996; Gray, 2000). Purkinje cell dendritic trees extend towards the surface and spreadout in a plane perpendicular to the long axis of the cerebellar folia. Purkinje cell dendrites are flattened. This outermost layer of the cerebellar cortex also contains two types of inhibitory interneurons, stellate cells, and basket cells (Linas *et al.*, 2004).

2.7.3 Functions of the cerebellum

The cerebellum performs the following function:

i. Coordination of Movement; the cerebellum controls the timing and pattern of muscle activation during movement.

ii. Maintenance of Equilibrium in conjunction with the vestibular system.

iii. Regulation of Muscle Tone and modulation of spinal cord and brain stem mechanisms involved in postural control (Linas *et al.,* 2004).

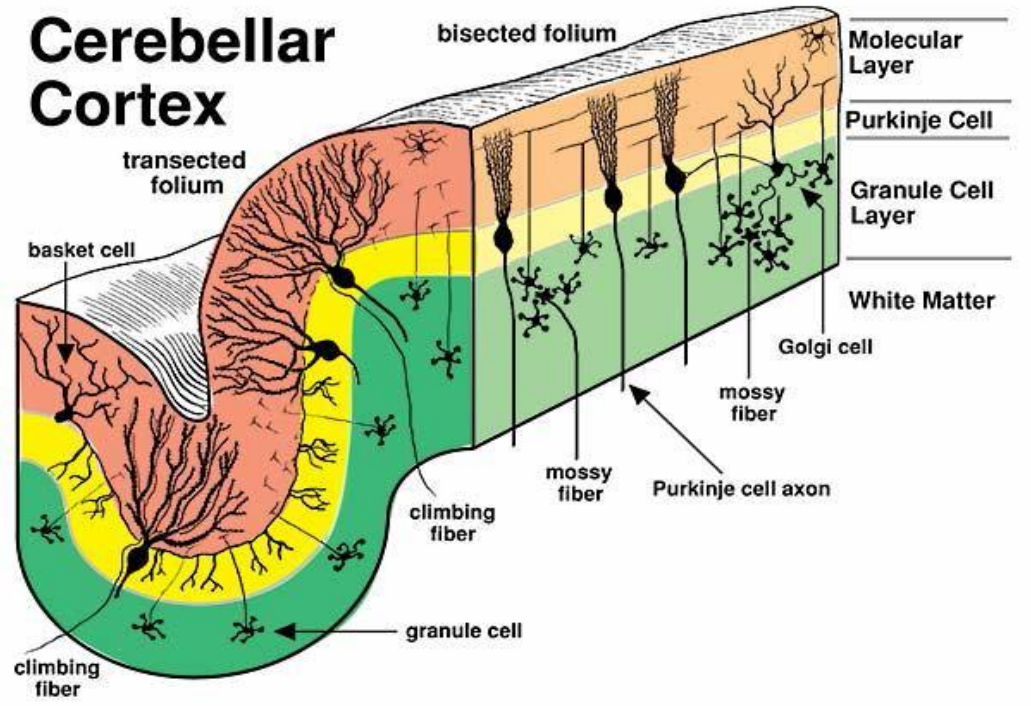


Figure 2.4: Cellular layers of the cerebellum showing the outer molecular layer (brown), the purkinje cell layer (yellow) and the granule cell layer (green) (Linas *et al.,* 2004).

2.8 The Cerebrum

2.8.1 The morphology of the cerebrum

The cerebrum comprises a large portion of the brain. It lies in front or on top of the brainstem and in humans is the largest and best-developed of the five major divisions of the brain (Kandel *et al.,* 2000). The cerebral cortex is the largest part of the brain; it is formedby grey matter and cerebral hemisphere on the surface as shown in Figure 2.6. The cortex is convoluted with projections called sulci and gyri. The cerebral cortex is

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

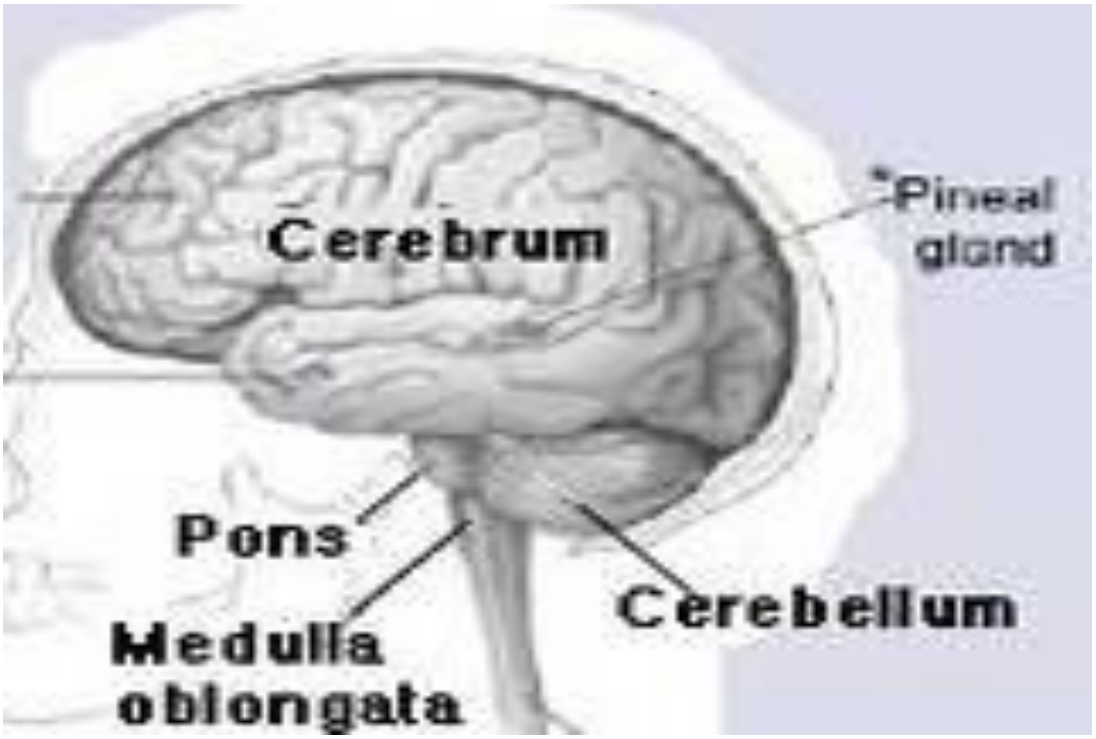


Figure 2.5: the structure of the brain showing the cerebrum (Kandel*et al.*,2000)

2.8.2 Cellular layers of the cerebral cortex

The neurons of the cerebral cortex vary in size, shapes, lengths, branching patterns and orientation of their processes (Kandel *et al.*, 2000). These neuronal cells are named based on their specific characteristic features which demarcates them from each other. The neurons include the following:

The pyramidal cells: They are most abundant cervical neurons in the cortex. All others are referred to as non-pyramidal neurons. About 2/3 (two third) of the cerebral cortex

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

The stellate cells: They are small and multipolar and form 1/3 (one third) of the total population of neurons of the cortex under low magnification. They look like granular cells. They are therefore been termed granular neurons (cells) by earlier workers. Stellates cell are of various types depending on their bodies and on the pattern of their ramification processes. Their axons are short and end within the cortex and their processes extend chiefly in radical direction within the cortex in some case horizontally. Some Stellates cells may be uniform rather than Stellates, with one process arising at either end. Stellates neurons are classified as spiny and non-spiny neuron. The spiny Stellates cells use glutamate as neurotransmitter, while in most non-spiny Stellates cells it is Gamma Amino Butyric Acid (GABA). The neurons of the cerebral cortex are grouped into six main layers, from outside to inside namely as shown in Figure 2.7, according to Kandel *et al*. (2000); Shipp (2007) and Saladin (2010).

Layer I: The Molecular layer, which contains few scattered neurons and consists mainly of extensions of apical dendritic tufts of pyramidal neurons and

horizontallyoriented 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 (Nieuwenhuys, 2011).

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 mediumsize pyramidal neurons, as well as non-pyramidal neurons with vertically-oriented intracortical axons. Layers I through III are the main target of inter-hemispheric corticocortical afferents, and layer III is the principal source of corticocortical efferents (Nieuwenhuys, 2011; 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 efferents, as such there are large pyramidal cells which give rise to axons leaving the cortex and

running down through the basal ganglia, the brain stem and the spinal cord (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 fibers to the thalamus, establishing a very precise reciprocal interconnection between the cortex and the thalamus. These connections are both excitatory and inhibitory. Neurons send excitatory fibers to neurons in the thalamus and also from collateral to other ones via the thalamic reticular nucleus that inhibit these thalamus neurons or ones adjacent to them. Since the inhibitory output is reduced by cholinergic input to the cerebral cortex, this provides the brainstem with adjustable "gain control for the relay of lemnsical inputs(Kandel *et al.*,2000).



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

2.8.3 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 (Kandel *et al*., 2000). These variations are as follow

I. 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.

II. In the granular cortex, the granular cortex is highly developed while the pyramidal and ganglion layer are poorly developed or absent. This type of cortex is mostly present in sensory area including the post-central gyrus (Kandel *et al.,* 2000).

2.8.4 Functions associated with cerebrum

The cerebrum is the most highly developed part of the human brain and is responsible for determining intelligence**,** motor function, planning and organization**, t**ouch sensation, thinking, perceiving, producing and understanding language. Most informationprocessing 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 pre-central 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:

Wistar rats, Mosquito coil, Light Microscope, Ultrospec Plus Spectrophotometry,Amscope, Computer, Tissue Processing Reagents, partially ventilated chamber, Cages,Animal feed, distilled water, weighing balance, sacrificing chamber, Ketamine, diazepam, Bouin's fluid, 2ml syringes, Gloves, dissecting kit, Dissecting tray.

3.2Experimental Design

A total of twenty-eight (28) Adult male Wistar rats were purchased from the Human Physiology Department, Faculty of Medicine, Ahmadu Bello University, Zaria, Kaduna, Nigeria. The animalsweightabout 110g-120g, they were caged and allowed toacclimatize for two (2) weeks in the animal house of the Department of Human Anatomy,Ahmadu Bello University, Zaria. Animals were fed twice a day with Growers mesh purchased from Mae-Syl Agrochemical Company at 22 Sokoto road, Samaru Zaria, Kaduna State, Nigeria.

Animals were weight and randomly divided into four (4) groups of seven animals each.The exposure of the animals to the mosquito coil lasted for a period of twenty-eight (28) consecutive days.Rambo mosquito coil manufactured by Gongoni Company limited,89A Sharada Industrial Estate, Phase III, Kano, Nigeria was purchased from Samaru market, Zaria, Kaduna State, Nigeria.

3.3 Experimental Procedure

Animals were grouped into four (4) groups of seven (7) animals per group as GI, GII, GIII and GIV. Group GI served as the control and was exposed only to fresh air. Groups GII, GIII and GIV were exposed to mosquito coil smoke in a partially ventilated chamber of 30cm x 30cm x 45cm dimension for 1 hour, 2 hours and 3 hours respectively for the period of twenty-eight (28) consecutive days.

Groups	Number of animals	Exposure time	Duration
GI (ambient air)	7		28 days
GII (mosquito coil smoke)	7	1 hour	28 days
GIII (mosquito coil smoke)	7	2 hours	28 days
GIV (mosquito coil smoke)	7	3 hours	28 days

Table 3.1 Animal grouping, exposure and duration

Animals were exposed to mosquito coil smoke between 8:00am – 11:00am daily.

3.4 Neurobehavioral Studies

3.4.1 Short-term memory using Novel object recognition

Novel object recognition was used to test for short-term memory according to the method of Ennanceur and Delacour (1988) and modified by Moscardo E. *et al*, (2012). The rats were exposed to two objects and were allowed to explore these objects then one of the objects was replacedwith a novelobject. If memory is functioning normally, the rats would have spent more time exploring the novel object than the familiar object. If exploration of all objects is the same, this was interpreted as a memory deficit.



Figure 3.1.Showing a rat exploring a novel object during abject recognition test.

3.4.2 Spatial learning and memory test using Morris water maze.

Morris water maze was used to test for spatial learning and memory according to method of Morris (1984), which was further modified by Charles (2006), Mark (2007) and Liu *et al,* (2011). According to this method, a platform was submerged beneath the surface of the water which served as an escape route. The animal task is to find the hidden platform. The animal starting point was changed from time to time, so as to build a cohesive spatial representation of the pool in order to fine the hidden platform during training trials and the latency to find the platform location was recorded both during the training and experimental period as shown in Figure 3.2. Animals were placed in a circular pool of clear water which was partitioned into four quadrants. Each animal was placed in the pool and was allowed to swim from one quadrant to the other in search of the escape route as shown in figure 3.2 below. The time taken by each animal to locate the platform which served as the escape route was recoded as the latency period in seconds.

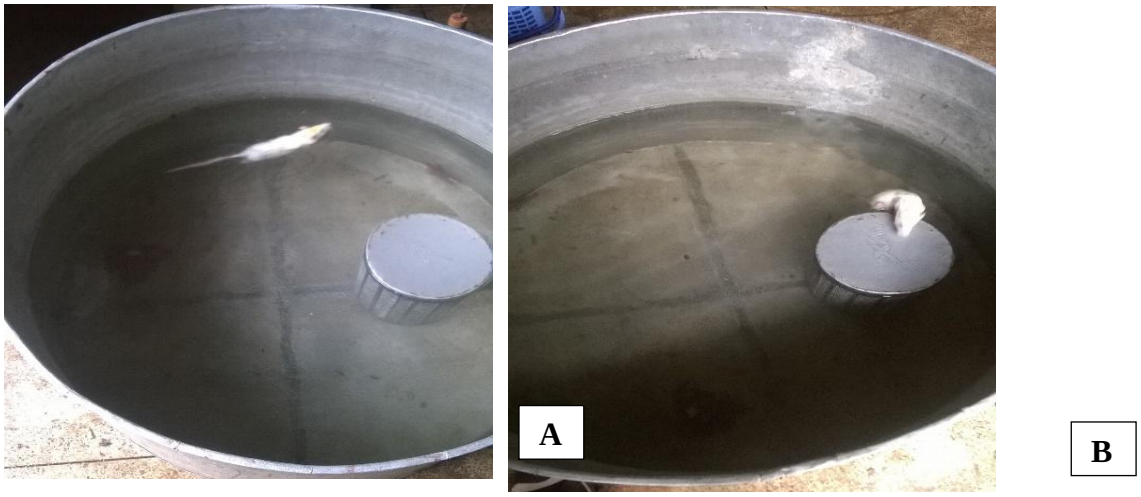


Figure 3.2 Morris water maze pool showing: (A) the Rat looking for the platform (B) Rat getting on to the platform

3.4.3Montoya staircase test method

The Montoya staircase test method was used to study the locomotor function and skills in motor co-ordination. This method helps in determining the effects of a test substance on motor function abilities (Montoya *et al*, 1990 and 1991). The tasks involved forepaw reaching for food pellets located in the well at the top of the staircase and the time taken to explore the activity cage and reach the food pellets was recorded. The test was carried out as a measure of motor coordination shown by muscle action in exploring the staircase and motor and locomotor function shown by the independent forelimb ability to reach out and grasp food pellet in the staircase.

3.4.2.1 *Training time*

The animals were first trained to recognize that there were food pellets in the well at the top of the Montoya staircase for four consecutive days. The animals were able to familiarise themselves with the Montoya staircase and then the animals were deprived of food for 18 hours in order to facilitate their search for food. During the test, each animal was placed on the foot of the staircase which enabled them to climb up the stairs and reach the food pellets. Each test session lasted for 2 minutes, at the end of which the time taken to climb up from the foot of the stairs was recorded and grasping abilities of each animal was also observed as shown in Figure 3.3 below.

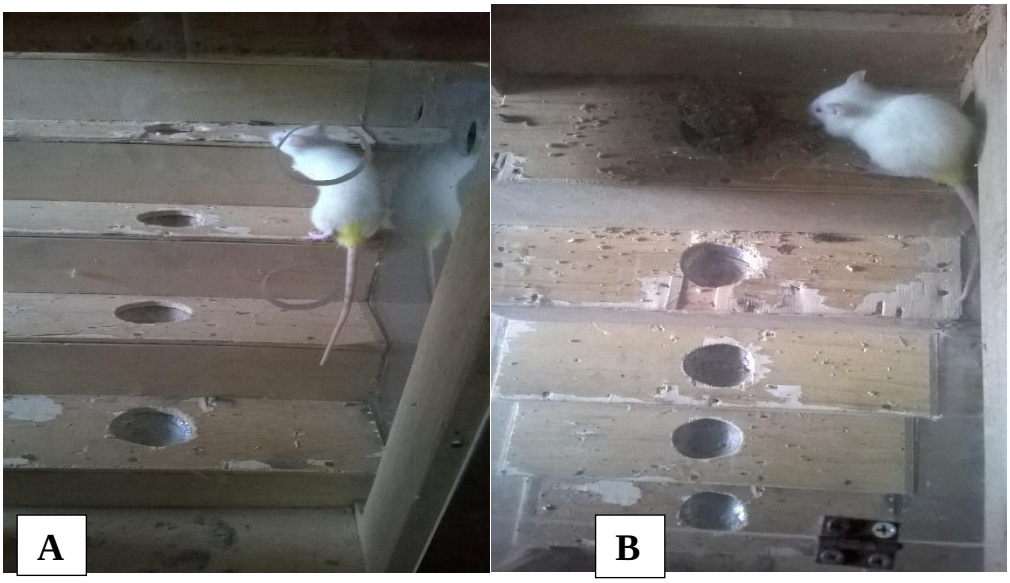


Figure 3.3. Montoya staircase test showing: (A**)** The Rat climbing the staircase and (B) the Rat at the top of the stairs.

3.5 Animal Sacrifice

At the end of 24 hours after the last exposure, the animals were anesthetized using ketamine/diazepam cocktail (0.9mg/kg body weight) and the brain tissues were removed by opening through the sutures of the skull and the brain was then fixed in Bouin's fluid for fast fixation.

3.6 Tissue Processing

The tissues were allowed to stand in the fixative and then taken to Histopathology Department laboratory, Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. The tissues were processed for routine H and E technique and special stain called Cresyl Violet stain.

3.6.1H and E staining method

The methods of H and E staining technique was carried out by de-waxing the tissue in two changes of xylene for three (3) minutes each, hydrated by passing them through descending grades of alcohol (100%, 95%, 90%, and 70%) for three minutes each, and then stained in Harris haematoxyline for ten minutes, washed in tap water to remove excess stain. The slides were then flooded with acid alcohol for few seconds for differentiation and then washed in tap water again. The slides were then blued in Scott's tap water for five minutes and counter stained with Eosin for three minutes. The sections were rinsed in tap water, and then dehydrated in ascending grades of alcohol, cleared in xylene. Tissues were then mounted with cover slips using a mounting media. Sections of the tissues were viewed under light microscope and photomicrographs were taken using digital Am scope (MD 900) photomicroscope.

3.6.2 Cresyl fast violet staining (Nissl stain)

Cresyl Violet stain was used to stain Nissl substance in the cytoplasm of the neurons. This technique employs basic aniline dye to stain RNA blue and was used to highlight important structural features of the neurons. The nissl substance (rough endoplasmic reticulum) appeared dark blue due to the staining of the ribosomal RNA, giving the cytoplasm a mottled appearance. DNA present in the nucleus will be stained a similar colour (Jones, 1996).

3.6.3 Atomic absorption spectrophotometry

Varian Fast Sequential Atomic Absorption Spectrophotometry with model numberAA240FS was used to quantify the amount of Iron, Copper, Zinc and Manganese in thecerebrum and cerebellum of theWistar rats. This was done at Multi-user ScienceResearch laboratory, Chemistry Department, Faculty of Physical Sciences, Ahmadu Bello University Zaria. The tissues were homogenised with Phosphate Buffer Solution (PBS) prior to the meta-analysis. It was then digested with nitric acid, heated and allowed to cool, this was done to remove organic matrix from the tissue homogenate (EHD, 2002).

Subsequent to digestion, the samples were diluted with deionised water and aqueous samplecontaining the metal analyte was aspirated into an air-acetylene flame, causing evaporation of the solvent and vaporization of the free metal atoms (USEPA, 2013). This process is called atomization. A light source (hollow cathode lamp) operating in the UV-visible spectral region was used to cause electronic excitation of the metal atoms, and the absorbance is measured with a conventional UV-visible dispersive spectrometer with photomultiplier detector (USEPA, 2013). The narrow spectral lines of

atomic samples necessitate the use of a line source as well as a high-resolution monochromator. This helps to prevent interference from adjacent spectral lines of other atomic species present in the sample matrix (USEPA, 2013). In this experiment, AAS in conjunction with flame atomization was used to determine specific metals (Fe, Zn, Cu, Mn) in aqueous sample. The availability of a spectrometer equipped with a lamp turret (allowing several line sources to be used in sequential fashion) facilitated the measurement of multiple metals in the sample (USEPA, 2013).

3.6.4Data analysis

The results were analysed using the Statistical Products and Service Solution (SPSS) *Version* 20 and the results expressed as mean ± SEM. Statistical significance between means were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to see where the significance lies.A p-value < 0.05 was considered statistically significant.

CHAPTER FOUR

4.0 Result

4.1 Physical Observation

There was no visible change noticed in the control group few hours after exposure to fresh air. The animals were active. Scratching of the nose, running nose and watery stool was observed in all the experimental groups few hours after exposure to mosquito coil smoke. The experimental animals were observed to be more active during the first two weeks of mosquito coil smoke exposure.

4.2 Body Weight Changes

The result shows non-significant decrease in weight in group III compared to the control (Group I) during the first and second week of the experiment, while there was no significant increase in weight in groups II and IV when compared with control $p>0.05$. The weight of all the treated groups (II, III and IV) was not significantly higher than that of the control in the third and fourth weeks $p>0.05$ as shown in the table 4.1 below.

Table 4.1 Effect of sub-acute exposure to mosquito coil smoke on body weight of Wistar rats.

Group	N	Weight (g)			
		Week 1	Week 2	Week 3	Week 4
I (control)	7	103.3±4.0	114.4±3.6	120.2±4.4	128.3±4.6
II (1hour smoke exposed)	7	108.3±4.6	122.9±5.3	132.0±7.0	147.4±6.8
III (2hours smoke exposed)	7	94.3±5.3	107.3±5.6	125.6±5.6	140.6±5.5
IV (3hours smoke exposed)	7	106.4±4.8	124.1±5.8	143.1±6.1	151.3±5.2

Data presented as Mean ±SEM
n= Number of rats in a group = 7

4.3 Short-Term Memory Test Using Novel Object Recognition Test

The result of the short-term memory test using Novel object recognition test as shown in Table 4.2 below, shows non-significant higher preference score for novel object in the control (group I) when compared with other groups (II, III and IV) in the first and fourth weeks. However, a significant higher preference scores were recorded in control when compared with groups II (33.7±14.7) and III (21.9±4.0) in the second week and groups III (28.2±10.4) and IV (36.1±11.6) during the third week of the experiment at $p<0.05$.

4.4 Spatial Learning and Memory Test Using Morris Water Maze Test

The result of spatial learning and memory test using Morris water maze as shown in Table 4.3 below shows non-significant difference in mean time taken to complete Morris water maze test between the control (group 1) and mosquito coil smoke exposed groups II, III and IV $p>0.05$.

4.5 Motor Activity Test Using Montoya Staircase Test

The result of the motor activity using Montoya staircase method recorded non-significant difference in mean time taken for the animals to reach to the top of the staircase between the control (group I) and mosquito coil smoke exposed groups II, III and IV $p>0.05$, as shown in Table 4.4.

Table 4.2 The effect of sub-acute exposure to mosquito coil smoke on short-term memory of Wistar rats using Novel object recognition test.

Group	N	Preference Score (%)			
		Week 1	Week 2	Week 3	Week 4
I (control)	7	61.2±8.6	73.3±4.6	75.8±3.6	73.2±7.8
II (1hour of exposure)	7	45.4±9.8	33.7±14.7*	50.3±6.4	47.3±16.9
III (2hours of exposure)	7	62.2±7.9	21.9±4.0*	28.2±10.4**	38.6±3.7
IV (3hours of exposure)	7	38.2±4.1	46±4.4	36.1±11.6**	34.3±4.6

Data presented as Mean ±SEM

n= Number of rats in a group = 7

* Symbol indicating significant difference at P< 0.01 compared to the group I (control).

** Symbol indicating significant difference at P< 0.02 compared to the group I (control).

Table 4.3 The effect of sub-acute exposure to mosquito coil smoke on learning and memory in adult Wistar rats using Morris water maze test.

Group	N	Mean time taken (Second)			
		Week 1	Week 2	Week 3	Week 4
I (control)	7	21.3±6.9	25.3±8.6	15.2±3.0	19.9±3.2
II (1hour of exposure)	7	21.2±5.6	24.4±5.3	26.0±9.1	32.9±5.9
III (2hours of exposure)	7	33.8±9.9	17.2±1.0	19.7±5.9	24.9±6.4
IV (3hours of exposure)	7	21.9±4.9	12.6±0.3	15.5±2.5	14.4±4.9

Data presented as Mean ±SEM
n= Number of rats in a group = 7

Table 4.4The effect of sub-acute exposure to mosquito coil smoke on motor activity of adult Wistar rats using Montoya stair case.

Group	N	Mean time taken (Second)			
		Week 1	Week 2	Week 3	Week 4
I (control)	7	76.7±24.7	93.2±26.8	66.4±25.7	61.7±23.1
II (1hour of exposure)	7	39.1±27.2	40.6±18.5	48.3±25.3	45.1±25.3
III (2hours of exposure)	7	33.1±16.4	26.8±8.3	60.6±26.4	45±26.0
IV (3hours of exposure)	7	40.3±26.6	53.1±22.5	120±0.0	94.7±25.3

Data presented as Mean ±SEM
n= Number of rats in a group = 7

4.6 Oxidative Stress Biomarkers

The result of possible effects of sub-acute exposure to mosquito coil smoke on lipid peroxidation and antioxidant enzyme activities of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) as shown in table 4.5 below shows non-statistically significant increase in the lipid peroxidation (LPO) level between the animals in the control (group I) as compared to other groups (II, III and IV). Also, non-significant decrease was recorded in glutathione (GSH) activity between control and other groups II, III and IV. However, statistically significant decrease was recorded in Catalase (CAT) activity between control and all other groups II (67.7±6.8), III (70.6±8.8) and IV (61.1±3.8) $p<0.05$. Also statistically significant decrease was recorded in Superoxide dismutase (SOD) activity (2.4±1.3) between control and group IV animals $p<0.05$.

Table 4.5 The effects of sub-acute exposure to mosquito coils smoke on oxidative stress biomarkers.

Enzyme biomarkers	**Group I**	**Group II**	**Group III**	**Group IV**
MDA	15.2±1.05	15.7±0.64	15.4±1.1	15.9±0.58
SOD	6±0.20	5.1±0.73	2.9±0.89	2.4±0.77*
CAT	95.5±6.65	67.7±3.93**	70.6±5.13**	61.1±2.13**
GSH	33.5±1.8	31.2±0.82	31.9±2.66	28.6±1.21

Data presented as Mean ±SEM

* Symbol indicating significant difference at P< 0.02 compared to the group I (control).

** Symbol indicating significant difference at P< 0.002 compared to the group I (control).

4.7 Atomic Absorption Spectrometry (AAS)

The result of the trace element analysis using AAS in cerebrum and cerebellum of the adult male Wistar rats showed the presence of Copper (Cu), Iron (Fe) Zinc (Zn) and Manganese (Mn)in µg/g of brain tissue.

Iron has the highest amount in 71µg/g weight of the brain tissue in both the cerebrum and cerebellum of the young adult male Wistar rats. The amount of Fe in the cerebrum of the mosquito coil smoke exposed groups (II, III and IV), shows time dependent very significant depletion when compared with the amount found in the control (group I) statistical significance $p< 0.05$. Similarly, in the cerebellum, the amount of Fe was very significantly depleted in the smoke exposed groups III and IV, but elevated in group II when compared with the control. Statistical significance $p<0.05$.

In cerebrum, the amount of Cu, Zn and Mn were all depleted very significantly when compared with the control with only time dependency recorded in the amount of Cu across the groups (II, III and IV), while Zn and Mn shows no such time dependency as shown in table 4.6 below. In cerebellum, similar depletion in the amounts of Cu, Zn and Mn was recorded with no time dependency across the groups but all being more depleted in group IV than in either group II or III as shown in table 4.7 below. Statistical significance $p<0.05$.

Table 4.6 The effects of sub-acute exposure to mosquito coil smoke on some essential trace elements in the cerebrum.

	Group I	Group II	Group III	Group IV
Cu	6.8±0.15	5.4±0.15*	4.7±0.13*	2.5±0.18*
Fe	71±0.31	64.3±0.41*	60±1.0*	48.6±0.62*
Zn	49.7±0.89	35.7±0.18*	39.9±0.35*	28.9±0.93*
Mn	11.5±0.83	9.1±0.43*	5.2±0.19*	8.3±0.81*

Data presented as Mean ±SEM

* Symbol indicating significant difference at P< 0.001 compared to the group I (control).

Table 4.7 The effects of sub-acute exposure to mosquito coil smoke on some essential trace elements in the cerebellum.

	Group I	Group II	Group III	Group IV
Cu	12.1±027	7.4±0.02*	3.5±0.11*	4.3±0.11*
Fe	68.2±1.3	110.7±0.30*	28.3±0.52*	48.5±0.15*
Zn	47.2±0.00	41.4±0.19*	41.5±0.24*	30.9±0.14*
Mn	12.8±0.25	7.2±0.07*	7.7±0.02*	5.1±0.01*

Data presented as Mean ±SEM

* Symbol indicating significant difference at $P< 0.05$ compared to the group I (control).

4.8 Histological Studies

The result from histological observations showed varying degrees of histopathological presentations in the mosquito coil smoke exposed groups. The cytoarchitecture of the cerebral and cerebellar cortices were explored using Haematoxylin and Eosin (H&E) and Cresyl Fast Violet staining techniques to enable an in-depth histopathological evaluation of the coil smoke exposure on the tissues at varying durations of exposure in adult male Wistar rats.

4.8.1 Histology of cerebral cortex

Photomicrograph of cerebral cortex of Wistar rat from control (group I) using H and E, in Plate I showed normal cellular architecture revealing different types of neural cells including pyramidal cells and stellates cells. Plate II shows a section of the cerebral cortex of Wistar rats (group II) exposed to mosquito coil smoke for one (1) hours daily for twenty-eight (28) days showing some degenerating pyramidal cells. Plate III shows a section of cerebral cortex of Wistar rats (group III) exposed to mosquito coil smoke for the duration of two (2) hours daily for twenty-eight (28) days with some degenerating pyramidal and stellate cells. Plate IV is showing aggregation of cells and vacoulations in the tissue of (group IV) Wistar rats exposed to mosquito coil smoke for three (3) hours daily for twenty-eight days (28).

4.8.1 Histology of cerebellar cortex

The cerebellum also stained with H and E shows normal architecture of Purkinje cells in the Purkinje layer, the molecular layer and the granular layer appearing normal as shown in plate V for the control (group I) exposed only to normal air. Plate VI shows

similar normal cerebellar architecture with the Purkinje cells seemingly normal in Wistar rats (group II) exposed to the smoke for 1 hour for 28 days. Plate VII shows cerebellum of Wistar (group III) rats exposed to the smoke for 2 hours for 28 days with degenerated Purkinje cells in the Purkinje layer. Plate VIII shows cerebellum of Wistar rats (group IV) exposed to the smoke for 3 hours for 28 days also with degenerated Purkinje cells.

4.8.3 Histology of hippocampus

The results of the histological study of hippocampus using H and E showed normal architecture of the hippocampus in the control (group I) rats exposed only to normal atmospheric air as seen in plate IX with pyramidal cells and pyramidal layer appearing normal. Plate X shows photomicrograph of hippocampus of Wistar rat (group II) exposed to mosquito coil smoke for 1 hour for 28 days also showing normal pyramidal cell layer. Plate XI shows hippocampus of Wistar rat (group III) exposed to the smoke for 2 hours through 28 days with some degenerating pyramidal cells and disturbed pyramidal cell layer. Plate XII shows hippocampus of rats (group IV) exposed to the smoke for 3 hours through 28 days with degenerating pyramidal cells and disoriented pyramidal cell layer.

4.8.4 Cresyl Fast Violet

Plates XIII to XXIV demonstrated the cerebrum, cerebellum and hippocampus using cresyl fast violet. Plate XIII is the Control Group showing nissl substanceand finely arranged cortical cells while the smoke exposed groups exhibited a varying levels of Nissl substance condensation and distortion in the nuclear outline. Plate XVII is the photomicrograph of cerebellum from the control group showing finely arranged

purkinje cells with nissl substance but in the mosquito coil smoke exposed groups, (Plate XVIII to XX)there were also varying groups of condensation and shrinkage of cell. Similar pattern of nissl substances condensation were exhibited in the hippocampus of the mosquito coil smoke exposed groups(Plate XXII to XXIV) when compared with the control (Plate XXI).

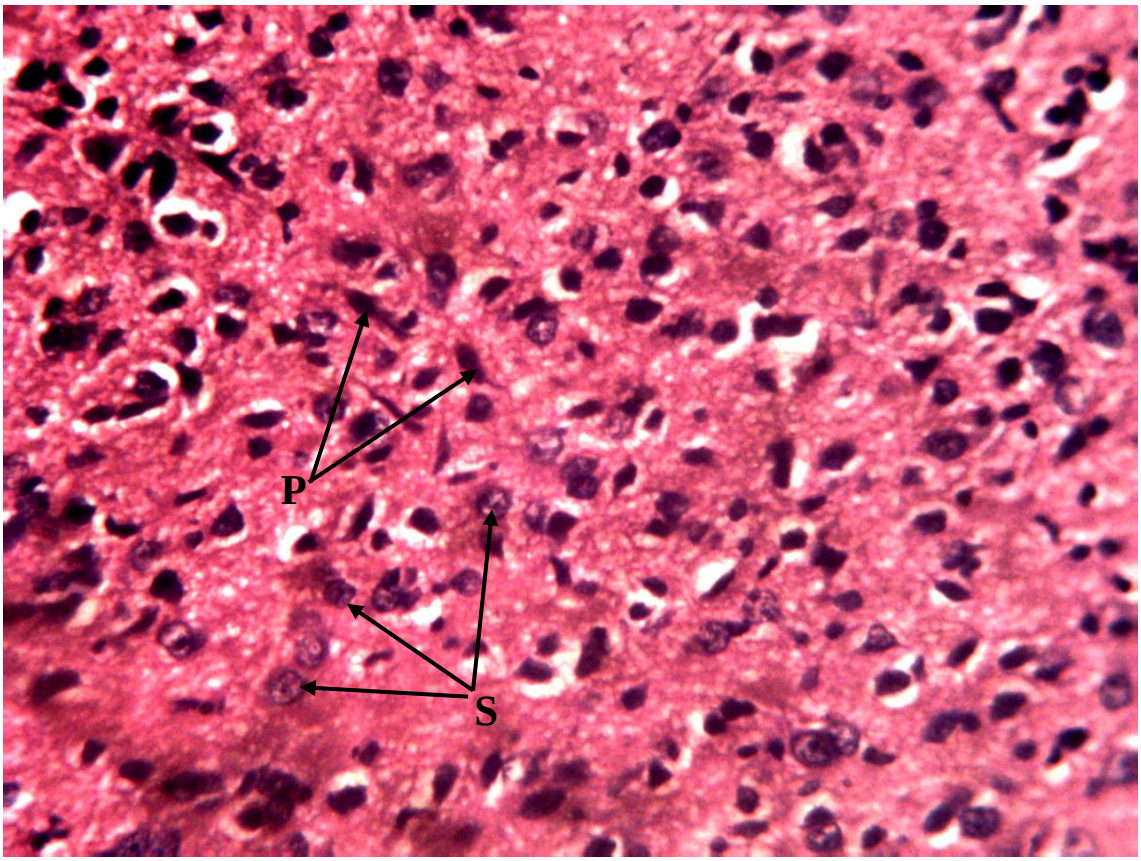


Plate I: A cross section of cerebral cortex of Wistar rat for control (group I) exposed to fresh air showing normal architecture with Pyramidal (P) cells and Stellate (S) cells (H and E X250).

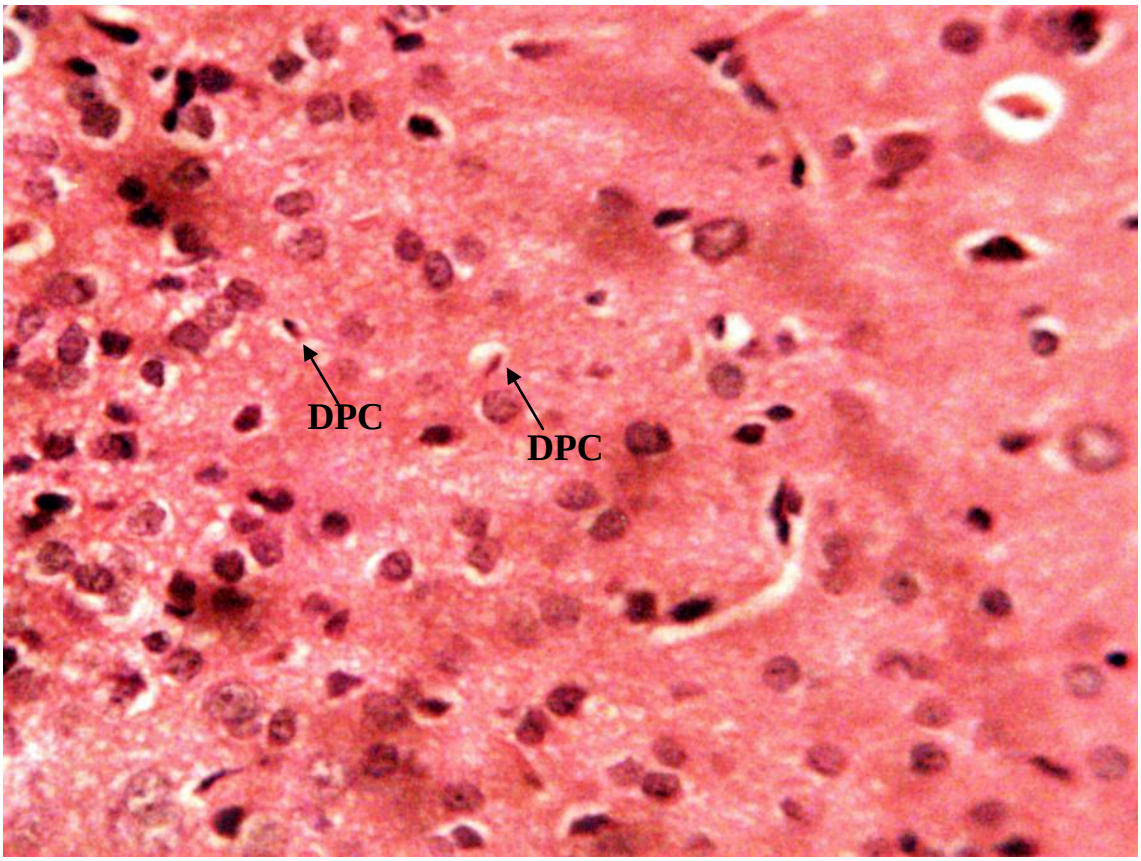


Plate II: A crosssection of cerebral cortex of Wistar rat (group II) exposed to mosquito coil smoke for 1 hour through 28 days showing some degenerating Pyramidal cells (DPC) (H and E X250).

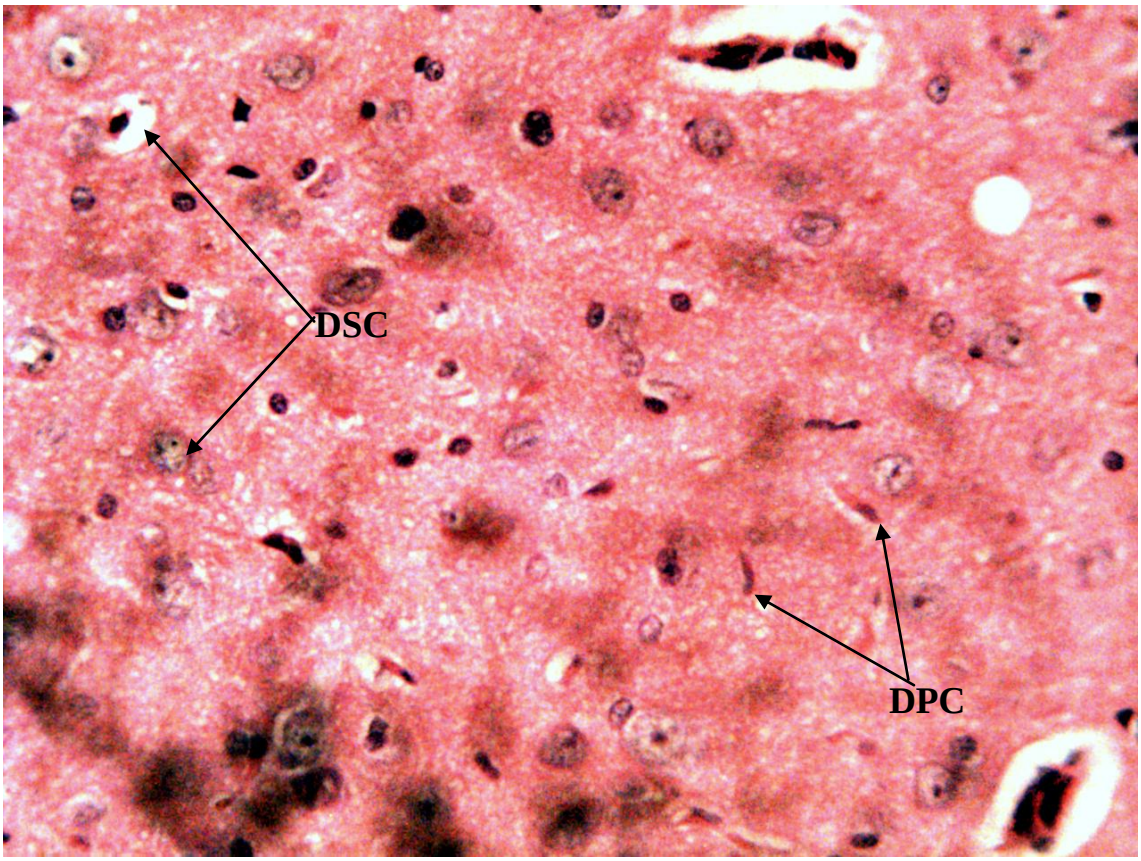


Plate III: A crosssection of cerebral cortex of Wistar rat (group III) exposed to mosquito coil smoke for 2 hours through 28 days showing some degenerating Pyramidal cells (DPC) and some degenerating Stellate cells (DSC) (H and E X250).

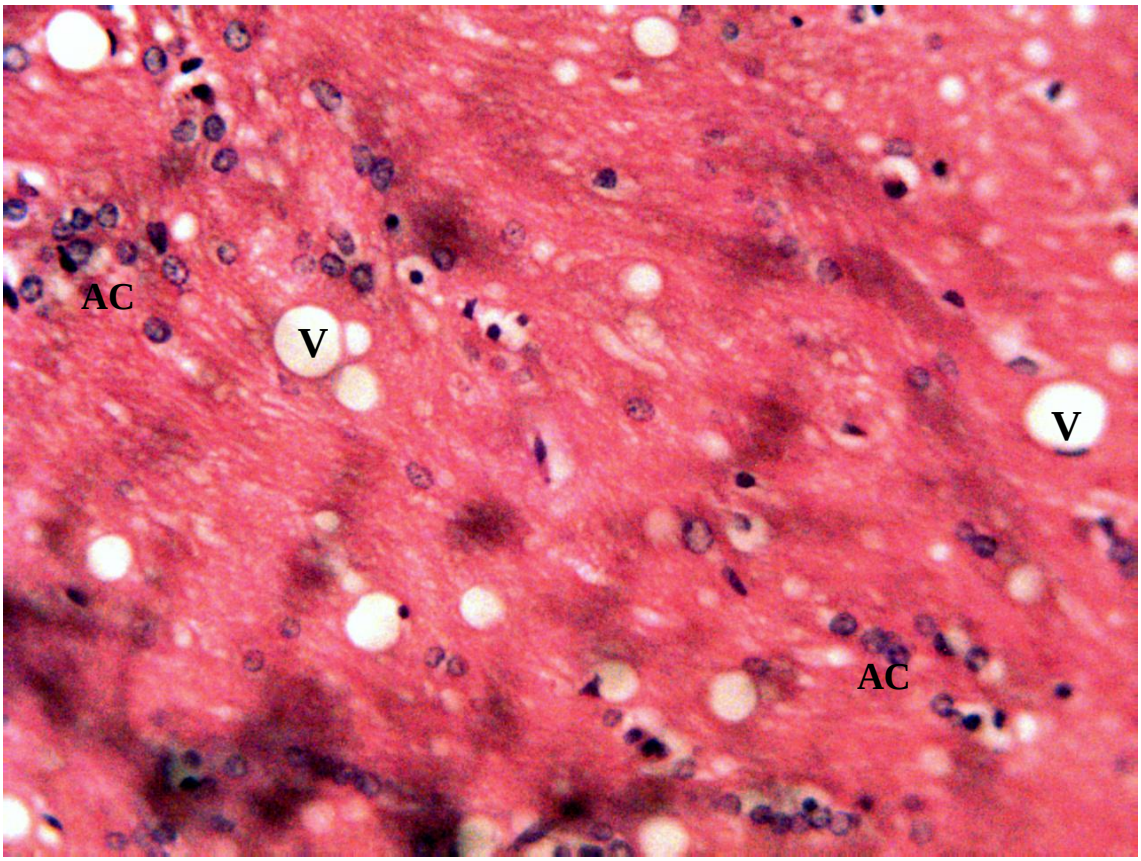


Plate IV: A crosssection of cerebral cortex of Wistar rat (group IV) exposed to mosquito coil smoke for 3 hours through 28 days showing some aggregation of cells (AC) and Vacoulations (V) (H and E X250).

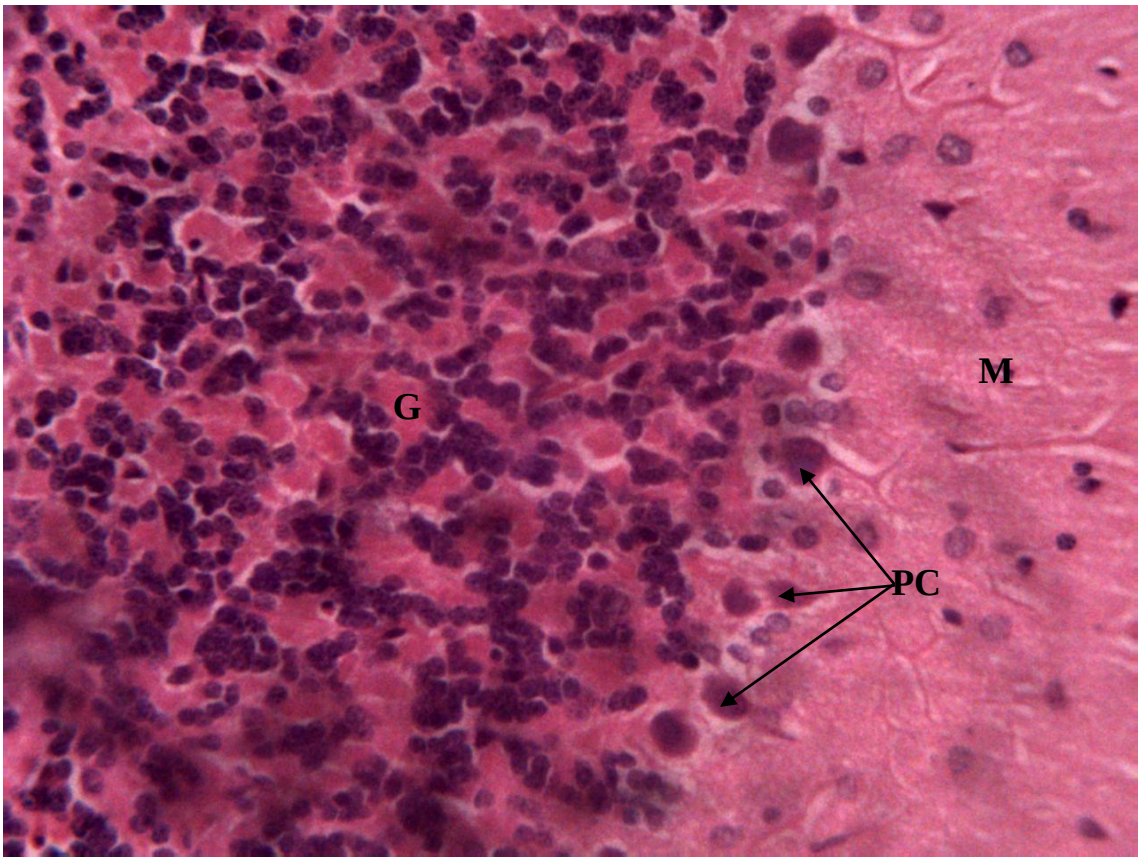


Plate V: A crosssection of cerebellum of Wistar rat for control (group I) exposed to fresh air showing normal architecture with Purkinje cells (PC), normal Purkinje cell layer, Molecular layer (M) and Granular layer (G) (H and E X250).

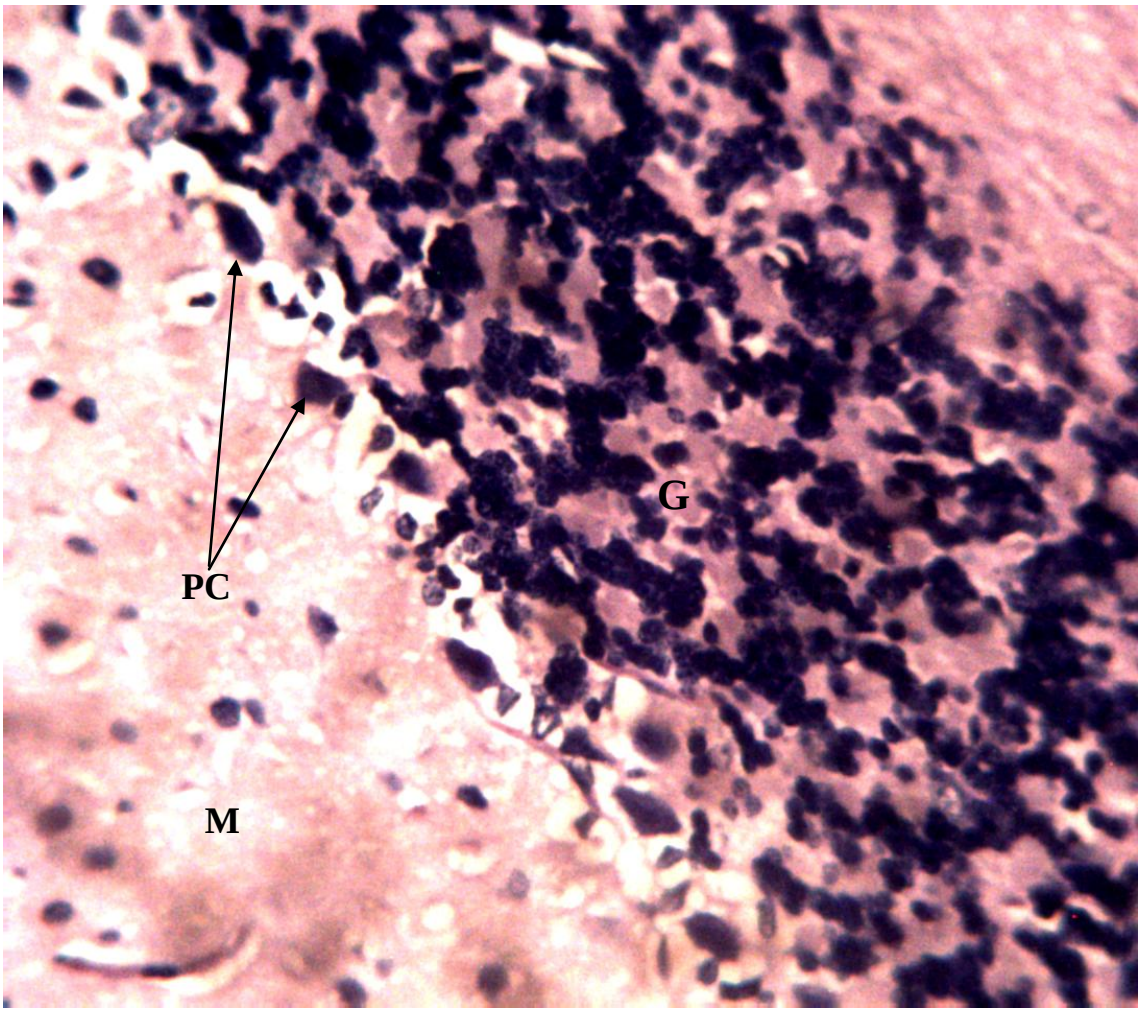


Plate VI: A crosssection of cerebellum of Wistar rat (group II) exposed to mosquito coil smoke for 1 hour through 28 days showing normal architecture with Purkinje cells (PC), normal Purkinje cell layer, Molecular layer (M) and Granular layer (G) (H and E X250).

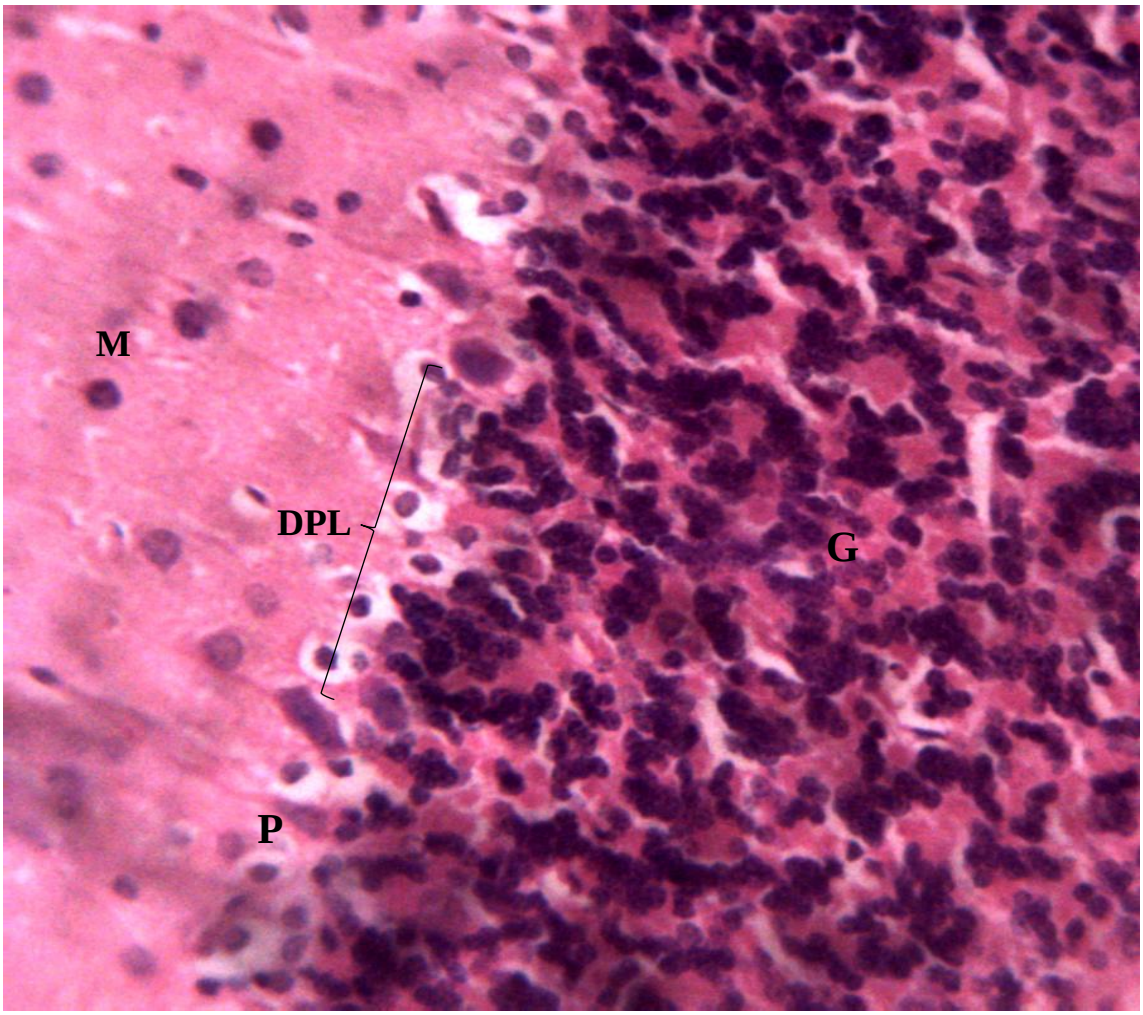


Plate VII: A crosssection of cerebellum of Wistar rat (group III) exposed to mosquito coil smoke for 2 hours through 28 days showing distorted Purkinje cell layer (DPL) (H and E X250).

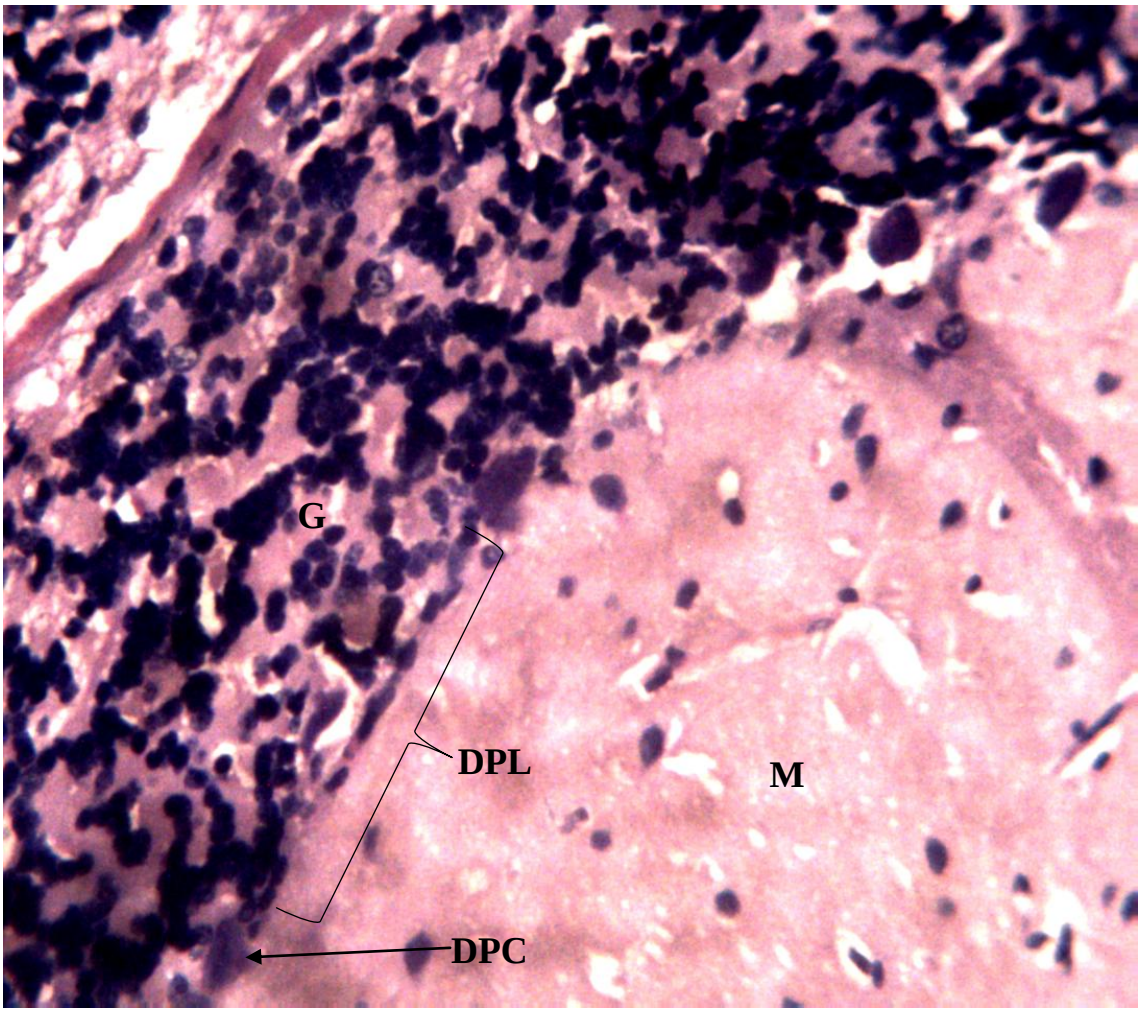


Plate VIII: A crosssection of cerebellum of Wistar rat (group IV) exposed to mosquito coil smoke for 3 hours through 28 days showing distorted Purkinje cell layer (DPL) and degenerating Purkinje cells (DPC) (H and E X250).

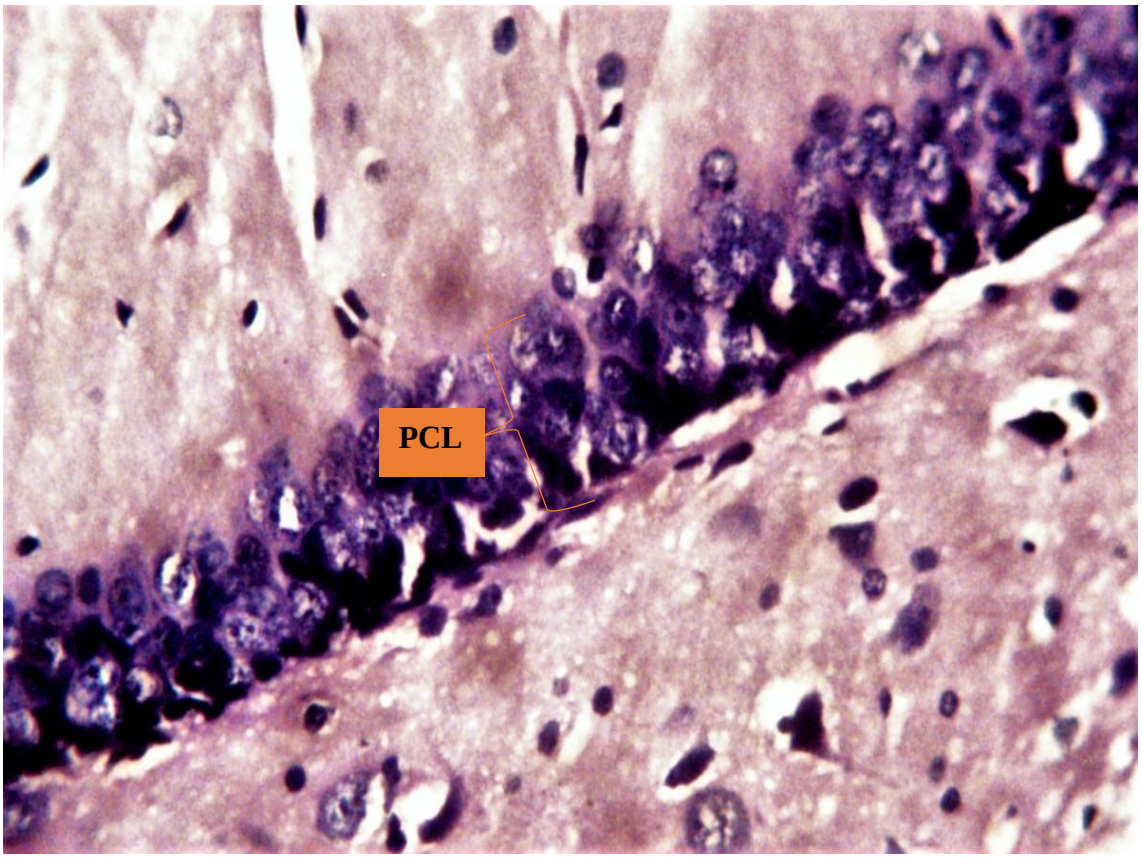


Plate IX: A crosssection of hippocampus of Wistar rat for control (group I) exposed to fresh air showing normal architecture with Pyramidal cells (PC) and Pyramidal cell layer (PCL) appearing normal (H and E X250).

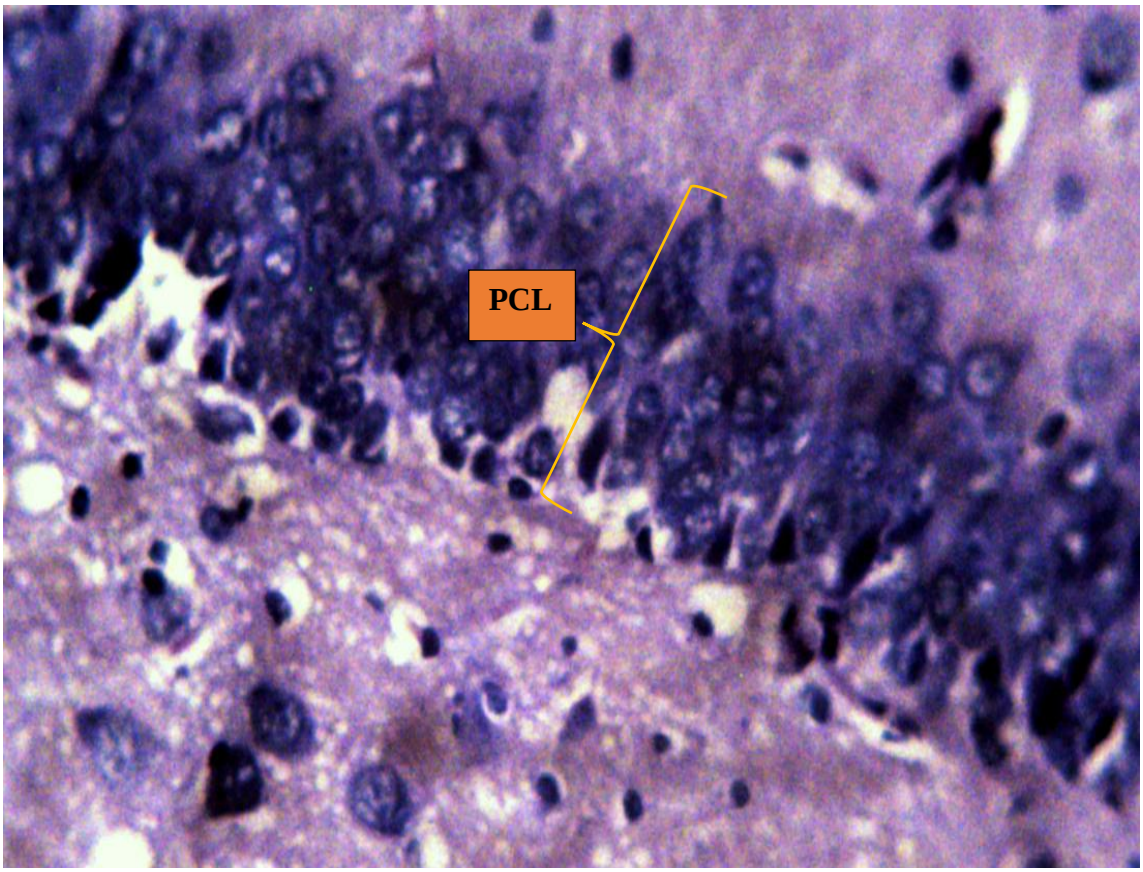


Plate X: A crosssection of hippocampus of Wistar rat (group II) exposed to mosquito coil smoke for 1 hour through 28 days also showing normal architecture with Pyramidal cells (PC) and Pyramidal cell layer (PCL) appearing normal (H and E X250).

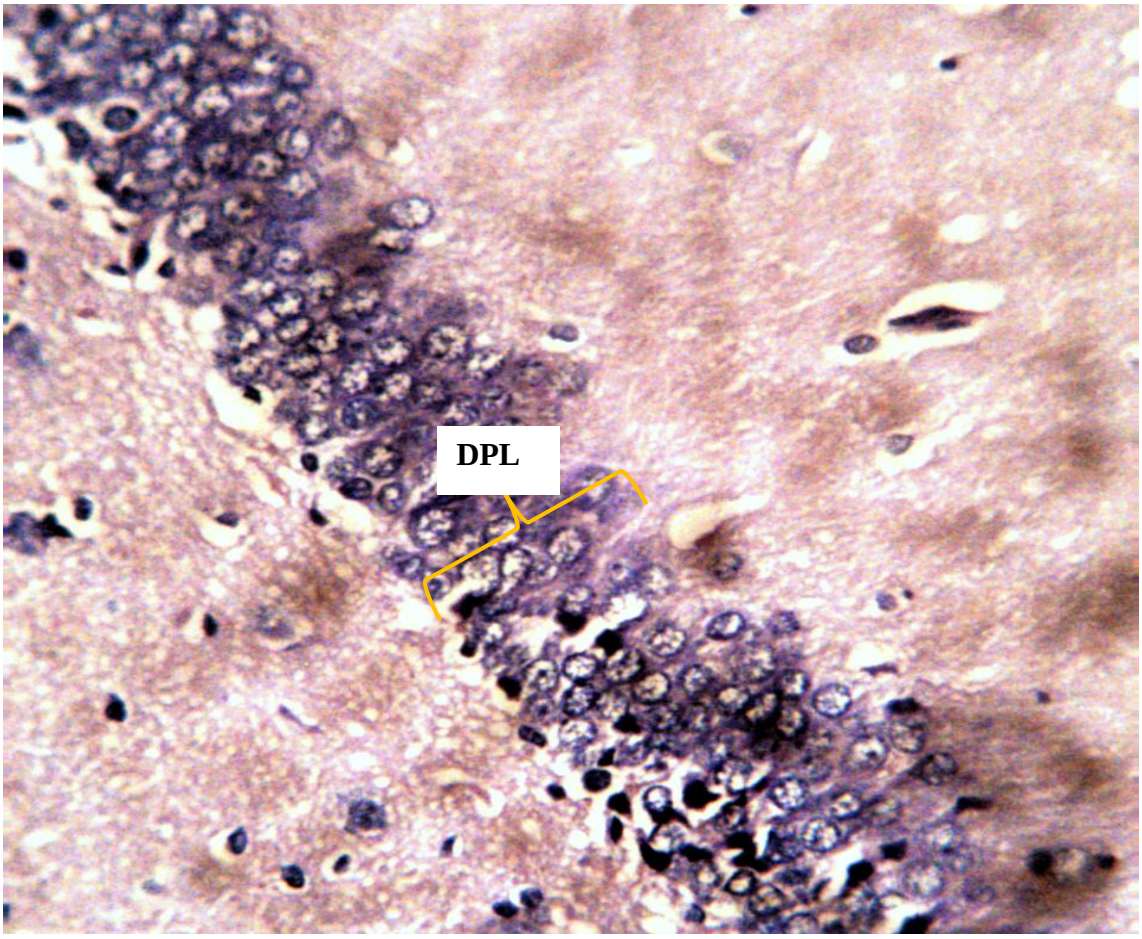


Plate XI: A crosssection of hippocampus of Wistar rat (group III) exposed to mosquito coil smoke for 2 hours through 28 days showing distorted Pyramidal cell layer (DPL) (H and E X250).

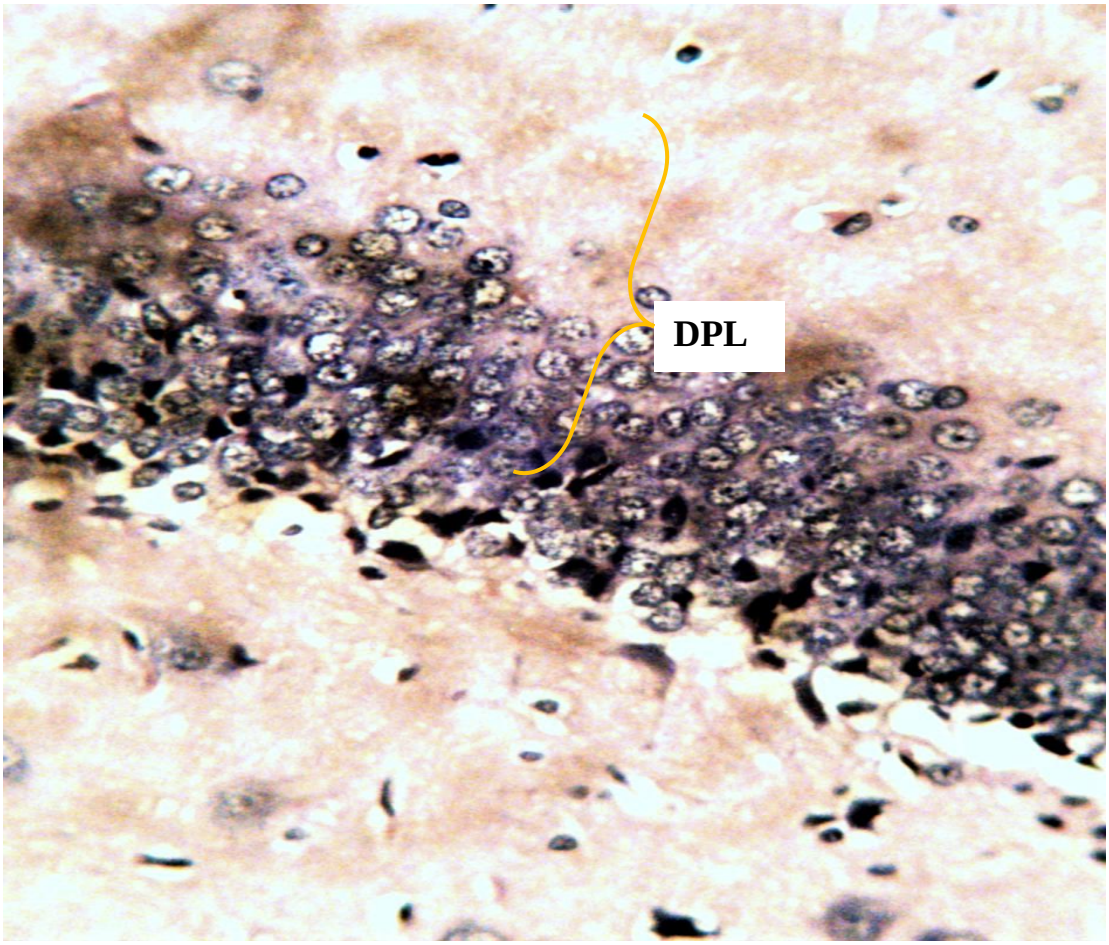


Plate XII: A crosssection of hippocampus of Wistar rat (group IV) exposed to mosquito coil smoke for 3 hours through 28 days showing distorted Pyramidal cell layer (DPL) (H and E X250).

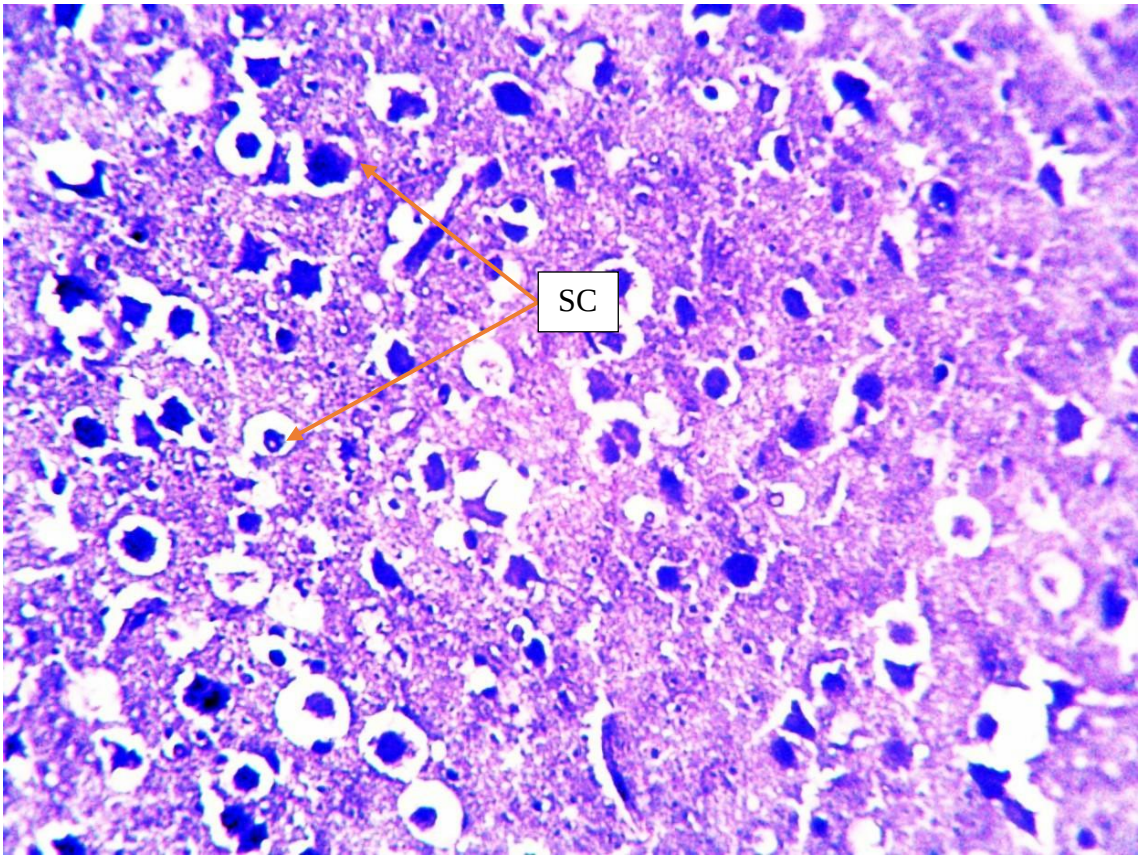


Plate XIII: A crosssection of cerebral cortex of Wistar rat from control (group I) exposed to fresh air showing normal architecture of Stellate cells (SC) with nissl substance(Cresyl FastViolet X250).

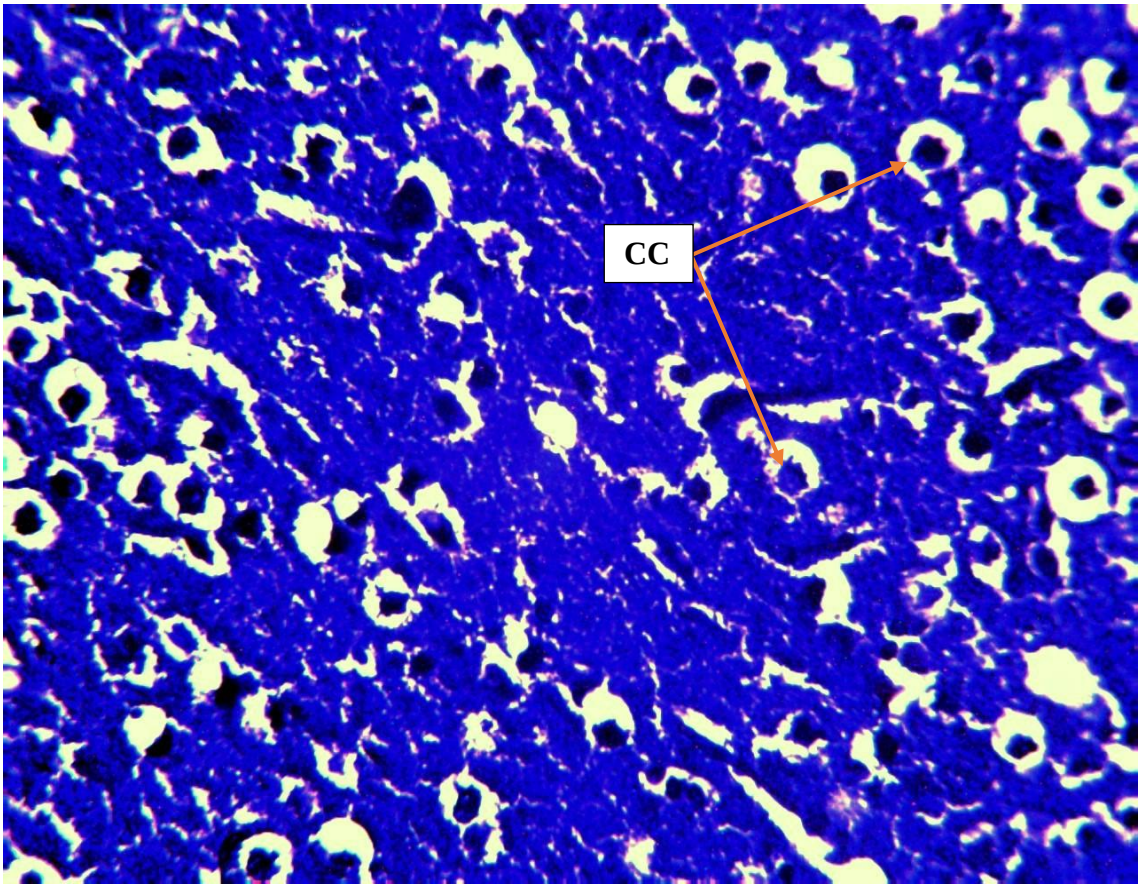


Plate XIV: A crosssection of cerebral cortex of Wistar rat (group II) exposed to mosquito coil smoke for 1 hour through 28 days showing somecondensationof cortical cells(CC) (Cresyl Fast Violet X250).

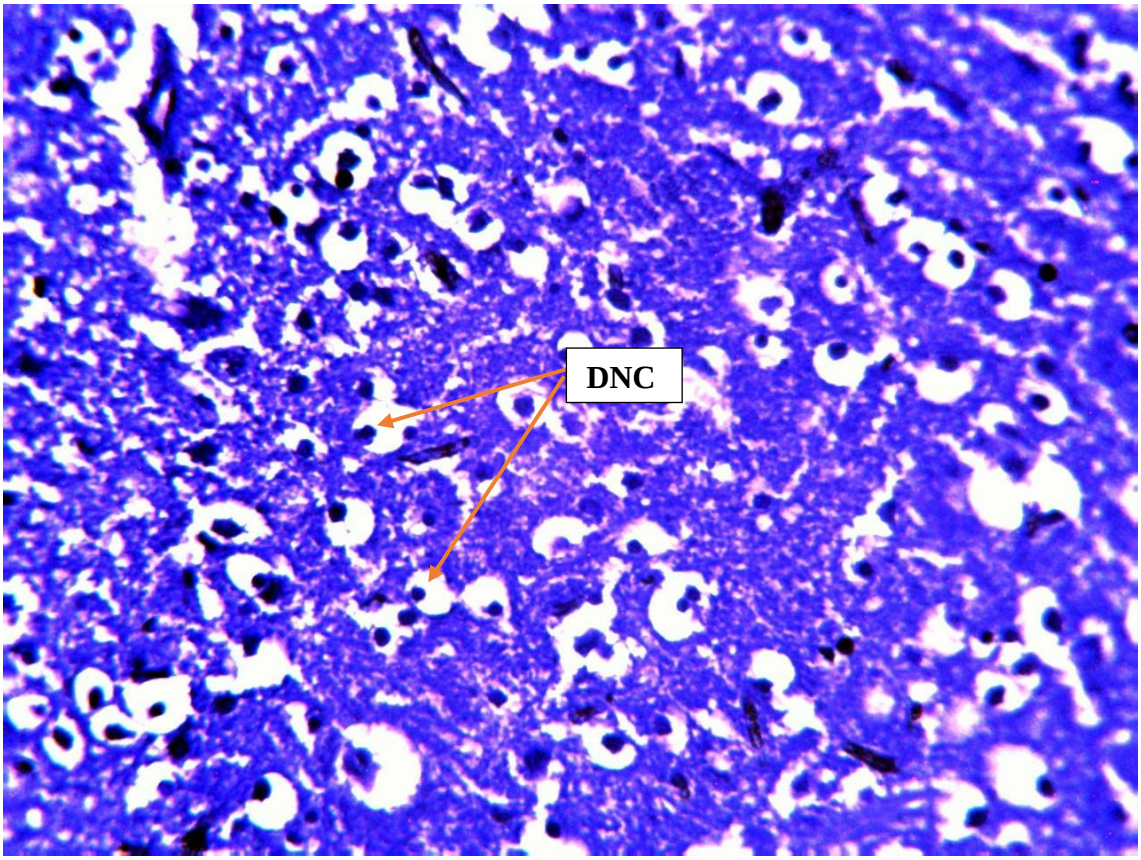


Plate XV: A crosssection of cerebral cortex of Wistar rat (group III) exposed to mosquito coil smoke for 2 hours through 28 days showing some degenerating neuronal cells (DNC) (Cresyl Fast Violet stain X250).

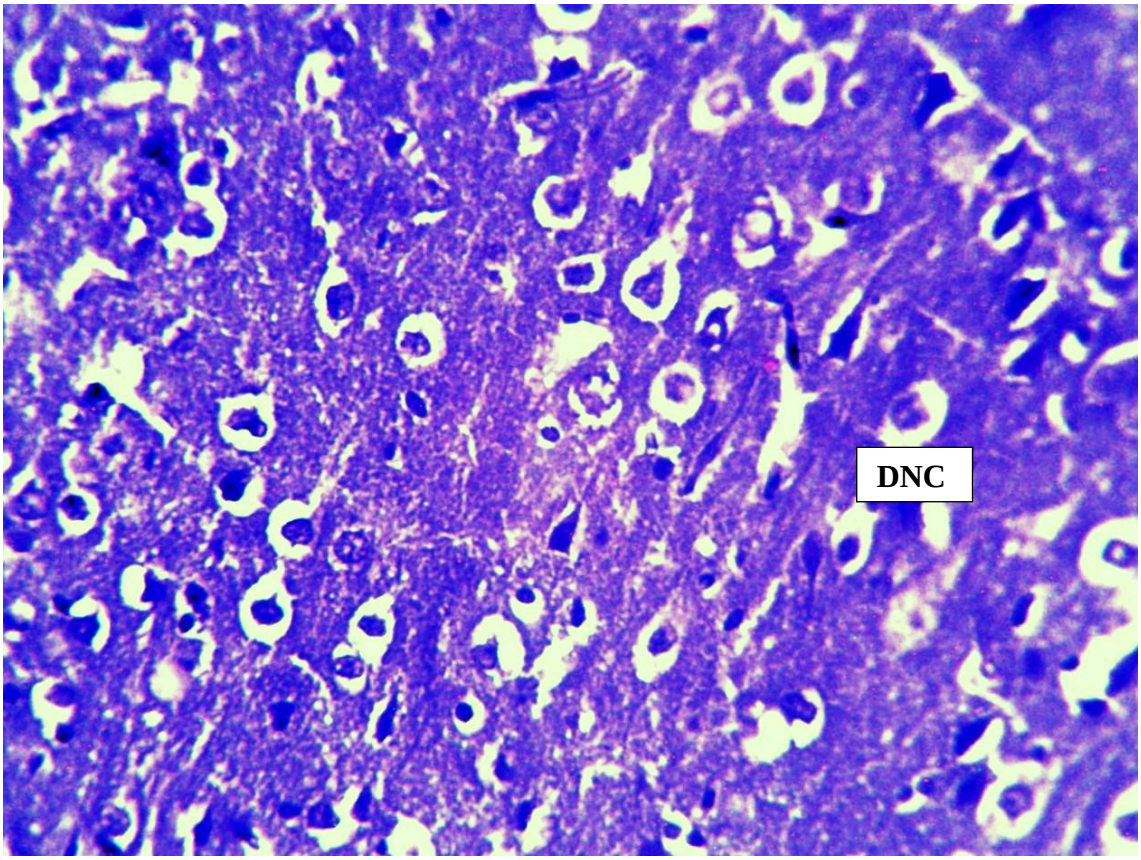


Plate XVI: Across section of cerebral cortex of Wistar rat (group IV) exposed to mosquito coil smoke for 3 hours through 28 days showing some faintly stained and degenerating neuronal cells (DNC) (Cresyl Fast Violet stain X250).

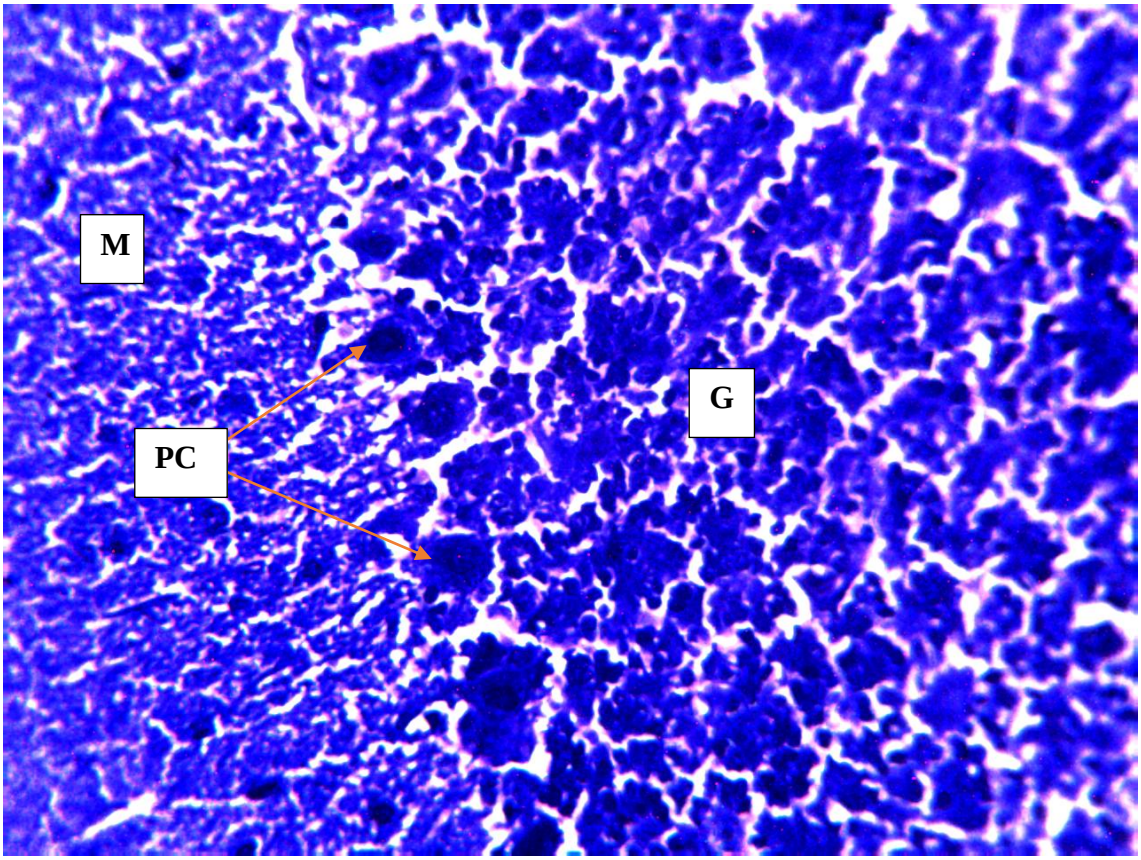


Plate XVII: A crosssection of cerebellum of Wistar rat from control (group I) exposed to fresh air showing normal architecture with Purkinje cells (PC), normal Purkinje cell layer, Molecular layer (M) and Granular layer (G) (Cresyl Fast Violet stain X250).

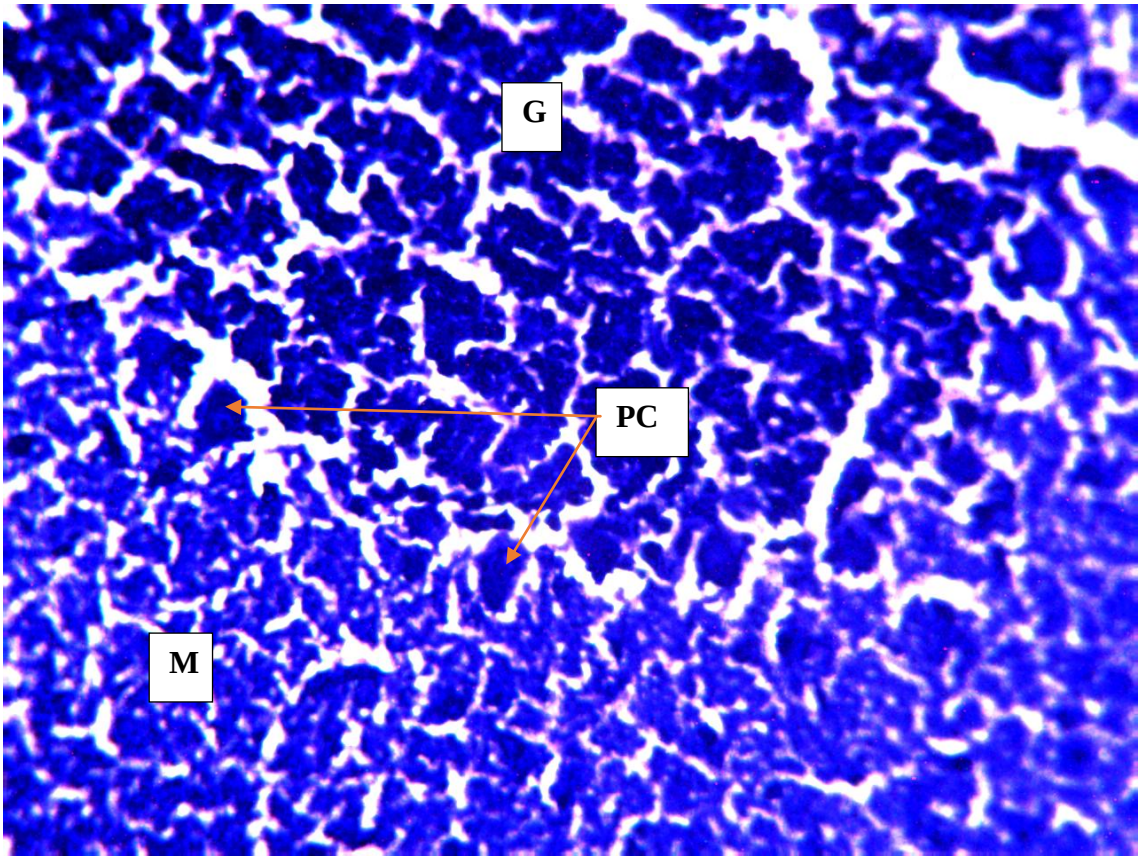


Plate XVIII: A crosssection of cerebellum of Wistar rat (group II) exposed to mosquito coil smoke for 1 hour through 28 days showing normal architecture with Purkinje cells (PC), Molecular layer (M) and Granular layer (G) (Cresyl Fast Violet stain X250).

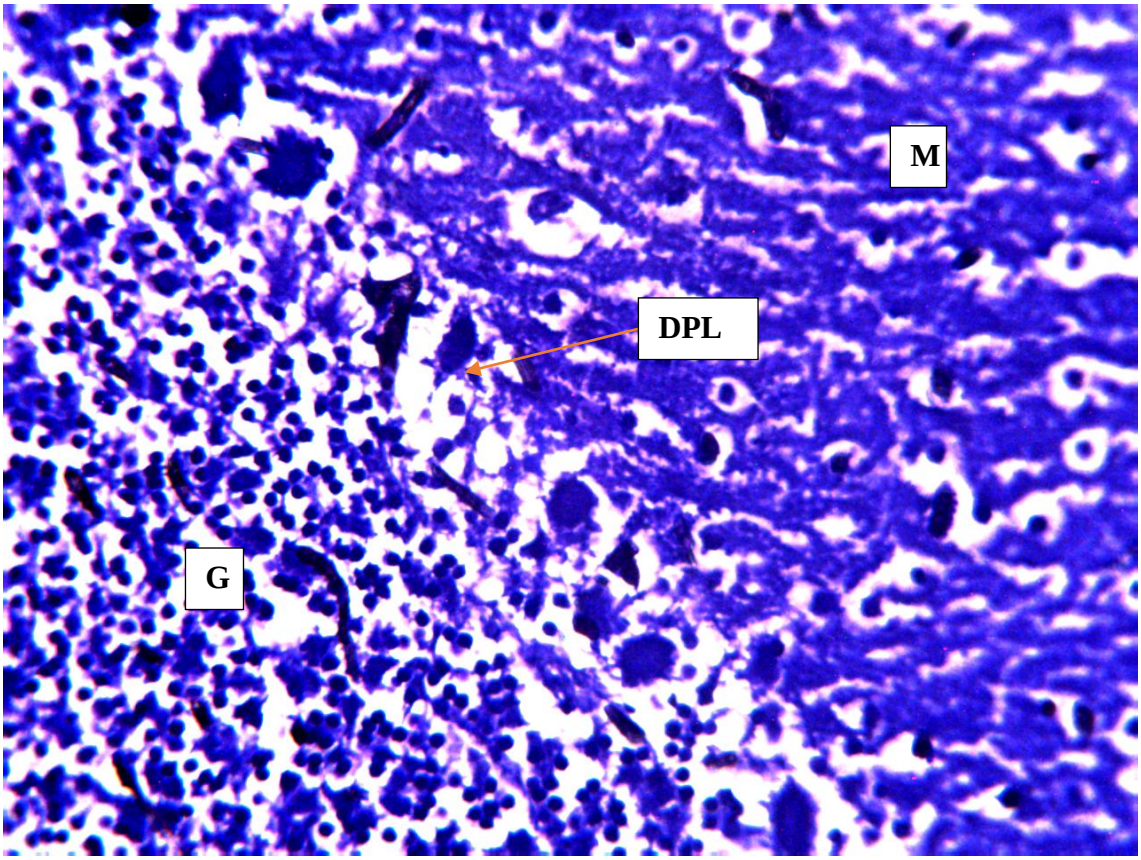


Plate XIX: A crosssection of cerebellum of Wistar rat (group III) exposed to mosquito coil smoke for 2 hours through 28 days showing distorted Purkinje cell layer (DPL) (Cresyl Fast Violet stain X250).

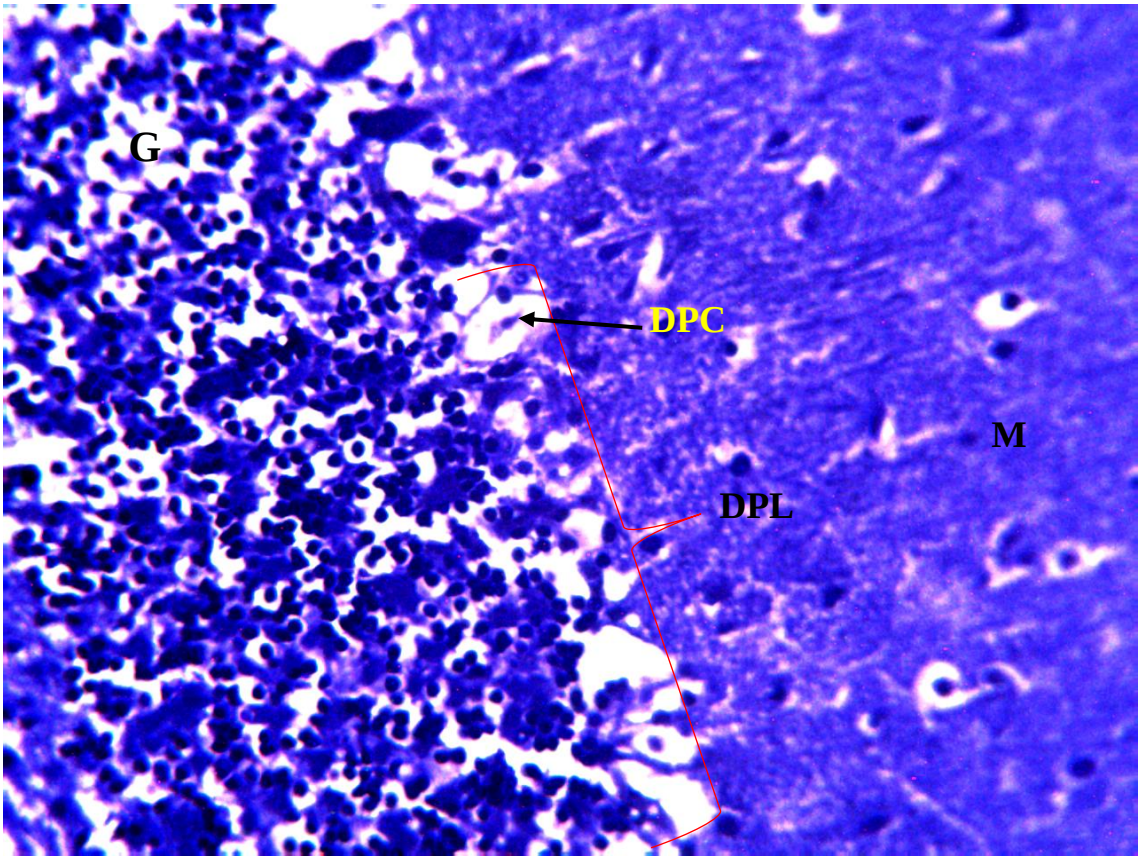


Plate XX: A crosssection of cerebellum of Wistar rat (group IV) exposed to mosquito coil smoke for 3 hours through 28 days showing distorted Purkinje cell layer (DPL) and degenerating Purkinje cells (DPC) (Cresyl Fast Violet stain X250).

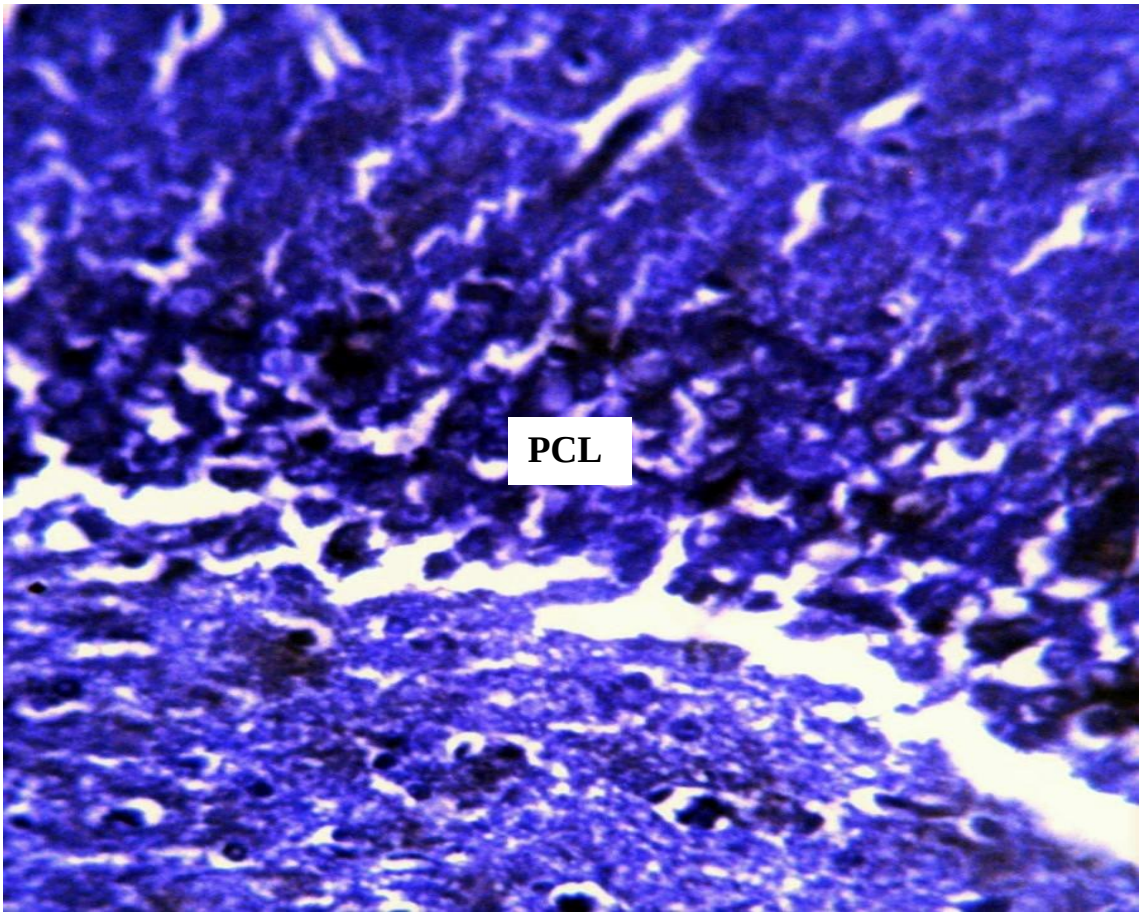


Plate XXI: A crosssection of hippocampus of Wistar rat for control (group I) exposed to fresh air showing normal architecture with Pyramidal cells (PC) and Pyramidal cell layer (PCL) appearing normal (Cresyl Fast Violet stain X250).

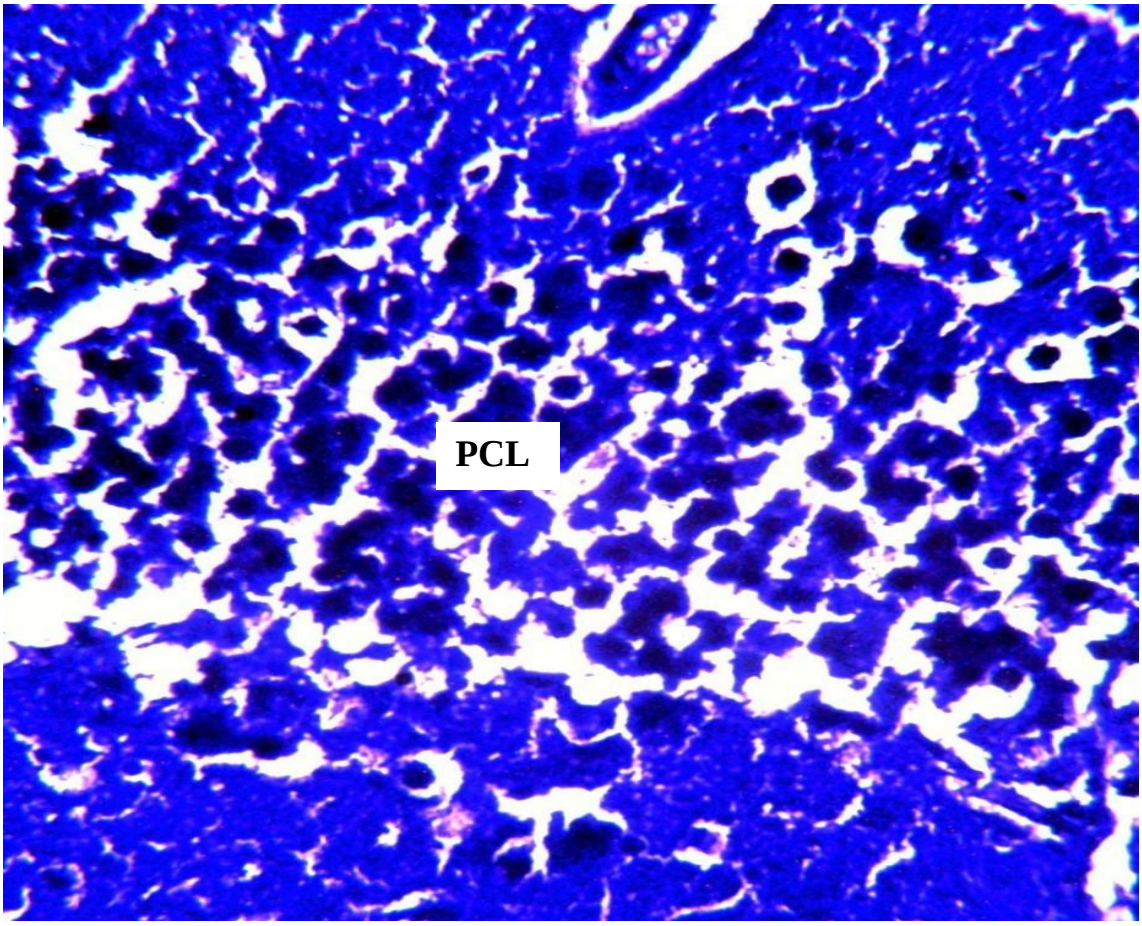


Plate XXII: A crosssection of hippocampus of Wistar rat (group II) exposed to mosquito coil smoke for 1 hour through 28 days also showing condensation of nissl substancein Pyramidal cell layer (PCL) appearing normal (Cresyl Fast Violet stain X250).

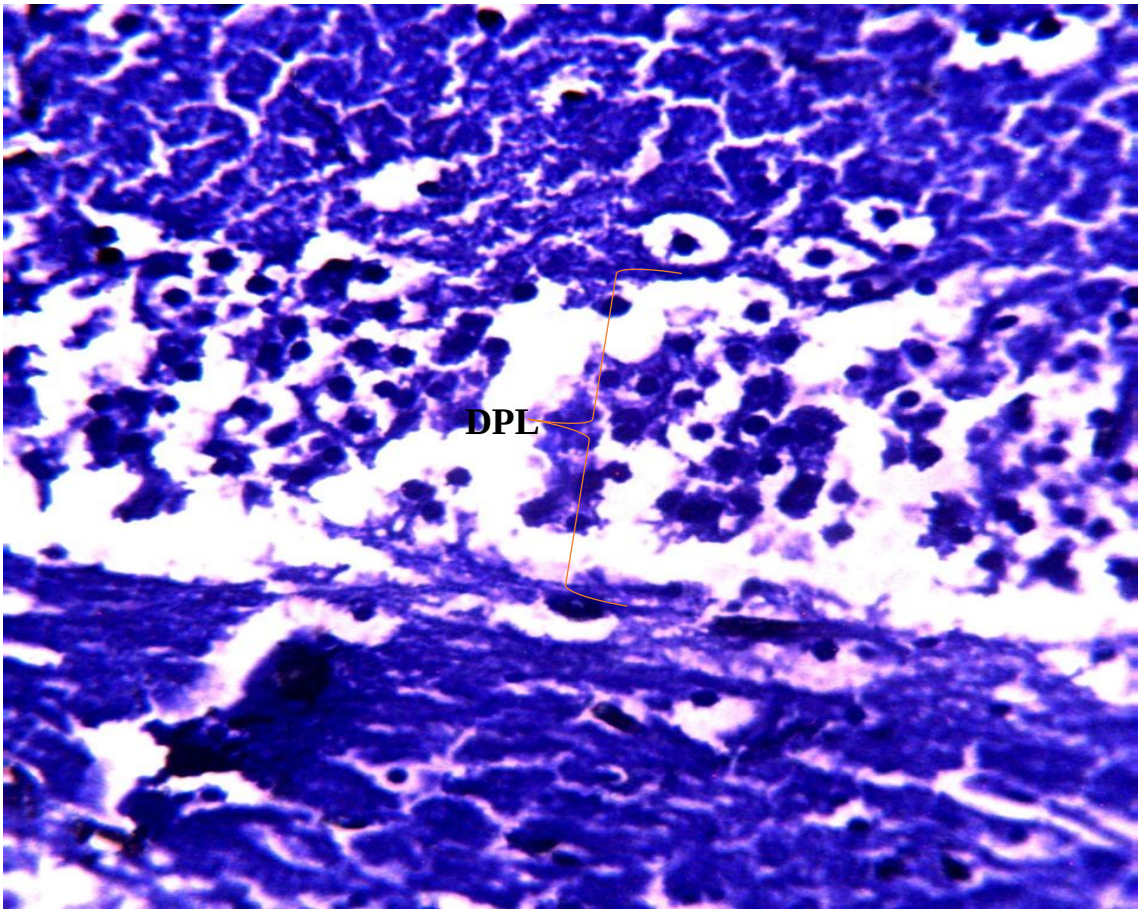


Plate XXIII: A crosssection of hippocampus of Wistar rat (group III) exposed to mosquito coil smoke for 2 hours through 28 days showing condensation of nissl substance in Pyramidal cell layer and distorted Pyramidal cell layer (DPL) (Cresyl Fast Violet stain X250).

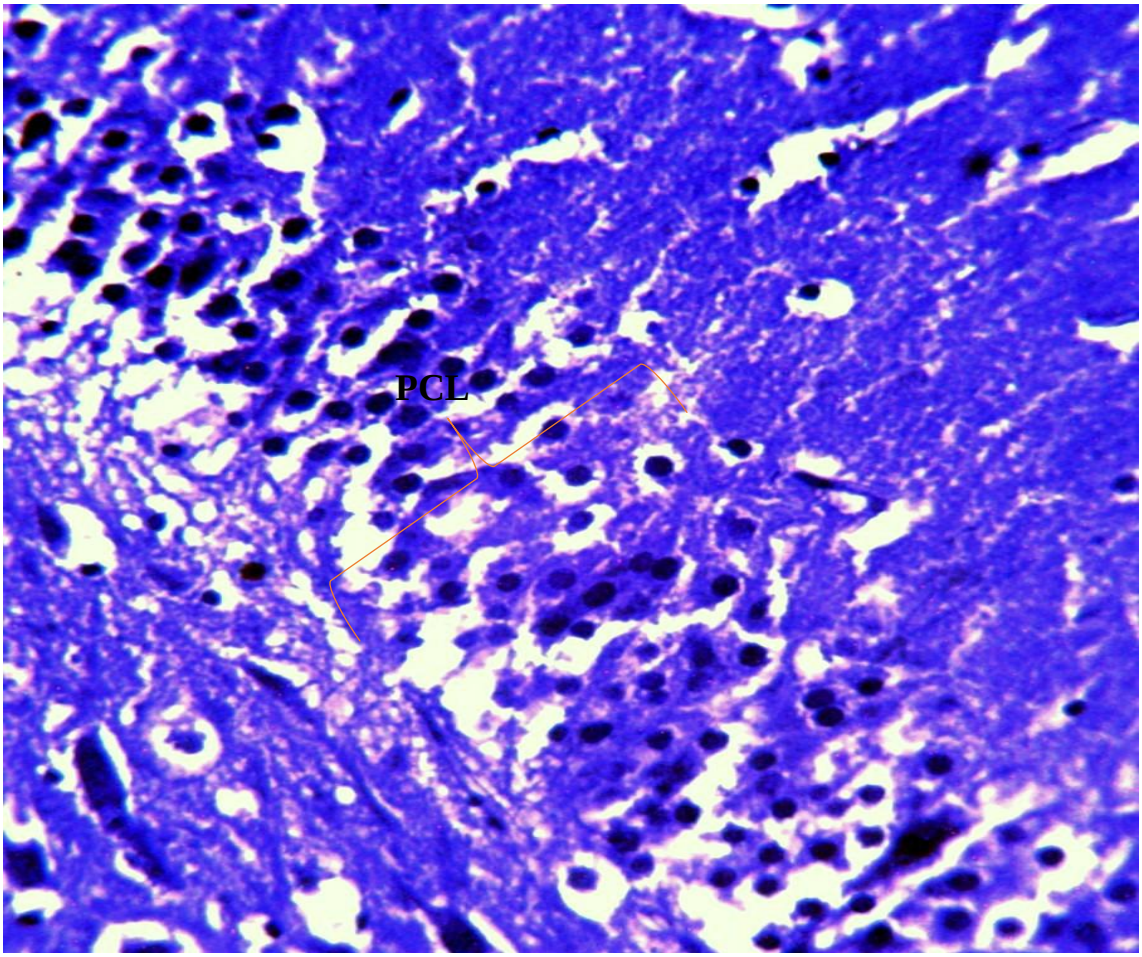


Plate XXIV: A crosssection of hippocampus of Wistar rat (group IV) exposed to mosquito coil smoke for 3 hour through 28 days showing more condensed nissl granules in the Pyramidal cell layer (PCL) (Cresyl Fast Violet stain X250).

CHAPTER FIVE

5.0 Discussion

The use of pesticides in field and for domestic pest control programs seems to have caused many biochemical and behavioural changes in man and other non-target organisms by influencing both the activities of many enzymes and other cellular processes. Brain is an important part of the biological system whose function helps to regulate other body parts. However, damage or any form of stress experienced in this organ may have serious impact on the entire organism.

Exposure to mosquito coil smoke did not show significant change in weight of the treated animals when compared with that of the control. However, non-significant increase and decrease were observed in the twenty-eight (28) days of exposure, this is in agreement with the work of Garba *et al.* 2007. When compared with the control, group III animals that were exposed to the smoke for 2 hours daily showed insignificant decrease in the mean weight while animals in group II and IV exposed to the smoke for 1 hour and 3 hours respectively showed an insignificant increase in the mean weight. This indicates that exposure to mosquito coil smoke could affect weight of animals positively or negatively but the 28 days duration may not be enough to show any significant difference.

After 28 days of exposure to mosquito coil smoke, there was gradual decrease in the short-term memory of the smoke exposed animals with significant difference recorded in group II in week 2, group III in weeks 2 and 3 and group IV in week 3 when compared with the control. The animals in the control group showed at least a minimum of 61.2% preference score for novel objects against the familial objects but in the smoke

exposed groups, there was less than 50% preference score for novel objects which indicate short-term memory impairment in the smoke exposed groups. This could partly be as a result of decrease in the Fe concentration (Mshelia *et al.,* 2013) as observed and partly due to decrease in acetylcholine (Tina *et. al.*, 2012). both of which play roles in cognition.

The present study showed no significant difference in spatial learning and memory between the control (group I) and all the smoke exposed (groups II, III and IV) animals after 28 days of exposure, this disagrees with the work of Mshelia *et al.,* 2013 who reported decrease in learning in mice.However, this could be as a result of specie differences or differences in genetic make-up.

The result from the motor activity test showed non-significant difference in the time taken for the animals to reach to the top of the staircase but showed a dose dependent insignificant decrease with the exception of weeks 3 and 4 noticed in animals exposed to the smoke for 3 hours daily (group IV) which maybe as a result of the excitability observed in the animals during the exposure period.

This study shows that exposure to mosquito coil smoke have caused an increase in the level of malondialdehyde (MDA) measured which is a product of lipid peroxidation (LPO) of polyunsaturated fatty acid, although this does not meet statistical significance. This is in line with the work of Olawale 2008, who reported that all pesticides must be toxic to be effective against pest and mechanism of pesticide action in animals is associated with the production of reactive oxygen species (ROS). The Malondialdehyde (MDA) level showed a dose dependent increase with group IV having the highest value,

since burning mosquito coil releases gases such as carbon monoxide (CO) and carbon dioxide (CO_2)and exposure to low levels of CO can lead to depression and confusion.This is in line with the work of Mshelia *et al.*, 2013.

On the other hand, statistically significant decrease in the activities of catalase (CAT) was recorded in all the smoke exposed groups when compared with the control. This could be as a result of enzyme overload which might have caused the observed increased in the malondialdehyde level.

Superoxide dismutase (SOD) activities showed a dose dependent decrease in the smoke exposed animals this conform to the work of Olawale *et al*. 2008, but only meet statistical significant decrease in group IV (3 hours exposed group), this could partly be as a result of the depletion of Cu, Zn and Mn which are important cofactors for SOD, indicating another possible mechanism of mosquito coil smoke induced neurotoxicity by leading to a classic case of oxidative stress. The GSH also shows a dose dependent decreased activity however, this does not meet statistical significance. This maybe because 28 days is not long enough to show statistical significance.

The analysis of trace elements namely; Copper (Cu), Iron (Fe), Zinc (Zn) and Manganese (Mn) in the cerebrum and cerebellum of adult male Wistar rats was conducted using Fast Sequential Atomic Absorption Spectrometry (FS-AAS). These trace elements play vital roles serving as co-factors for several enzymes and proteins to synthesis of numerous neurotransmitters. The vital roles played by these essential elements in the neural cells justifies their exploration in the current studies to reveal the possible effects of exposure to mosquito coil smoke on the concentration of these elements in the cerebrum and cerebellum of adult male Wistar rats.

The result of the present study showed statistically significant decrease in the amount of Cu, Fe, Zn and Mn in both the cerebrum and the cerebellum of the smoke exposed groups when compared with the amount found in the control group. Fe has the highest amount in both the cerebrum and cerebellum which indicates that the brain has the highest amount of Fe compared to other trace elements.

In the present study, Cu which is an essential co-factor for dopamine-β-hydroxylase, alpha amidating monoxygenase and Cu/Zn-superoxidedismutase and cytochrome C oxidasewas found to be depleted when compared with the control. This could have contributed to the decreased activity of Superoxide dismutase (SOD) observed in the smoke exposed groups which meet statistical significant decrease in group IV that were exposed to the smoke for 3 hours for 28 days. Since Cu plays a vital role in the normal activity of the enzyme Cytochrome C oxidase, it is likely that the depletion in the Cu concentration have played a part in the neuronal degeneration present in the histological tissues of the smoke exposed groups. Also, because Cu is a non-competitive antagonist of N-methyl-D-aspartate receptors(NMDARs) (Receptors that are critical for CNS development and memory among other functions), it's over expression causes excitotoxicity and subsequent neurodegeneration (Vishal and Stephen, 2008). This may not be unconnected with the neurodegeneration observed in the histological study.

Iron (Fe) deficiency in the brain leads to hypomyelination, abnormal cognition and changes in neurotransmitter metabolism (Tina, 2012). The observed Fe depletion might have played role in the short-term memory deficit recordedin the smoke exposed groups.

Gamma Aminobutyric Acid ($GABA_A$) a complex receptor requires Zn to bind neurotransmitter (GABA) in other to inhibit neuro-excitation at the synaps (Zerrouk, 2012). The current study showed a significant decrease in the concentration of Zn in the smoke exposed groups when compared with the control which suggest that there could be insufficient Zn to modulate such a complex receptor as GABA receptor and hence, the excitation noticed in the smoke exposed animals.

Also, statistically significant decrease in the level of Fe and Zn in rats has been associated with stress (Zerrouk, 2012). In the present study, the mosquito coil smoke exposed rats all showed significant depletion of Fe and Zn, this could further explain the recorded increase in the MDA measured in the smoke exposed groups and the decrease in their anti-oxidant enzymes activities. The depletion in the concentration of Manganese (Mn) in the smoke exposed groups as recorded in the present study could have contributed to the decreased activity of SOD because SOD2 can only function in the presence of Mn. Glutamine synthetase (GS), the most abundant manganoprotein is predominantly expressed in astrocytes where it converts glutamate to glutamine, because GS contain four Mn ions per octamer, Mn has been proposed to regulate GS activity and in fact, insufficient Mn has been proposed to increase glutamate trafficking, glutametergic signaling and excitotoxicity (Aaron, 2011).

In the present study, varying degrees of histopathological presentations were observed in the cerebrum, cerebellum and the hippocampus of adult male Wistar rats exposed to mosquito coil smoke for the period of 28 days. The observed histopathological manifestations include; cellular or neural degeneration, cellular aggregation, disruption of cellular layers and degeneration of cellular layers to vacuolations.

The cerebral cortex of the mosquito coil smoke exposed groups showed varying forms of histopathological manifestation depicting some neurodegenerative effects that could affect the normal functions of the cerebrum. The resulted effects include degeneration of pyramidal cells in the 1-hour smoke exposed group, degeneration of pyramidal cells and stellate cells in the 2 hours smoke exposed group to degeneration of pyramidal and stellate cells to vacuolation and aggregation of cells in the 3 hours smoke exposed group.

The variation in the degree of the observed damage could be as a result of the duration of exposure to the mosquito coil smoke. Mosquito coil smoke contain many gases and particles like CO, CO_2, Pyro(a)benzene and other hydrocarbon(Abubakar and Hassan, 2006). CO is known for its ability to displace oxygen from hemoglobin thereby, exposing the neural cells to hypoxic state (Abubakar and Hassan, 2006). This may result in arrest of cellular functions and leads to cellular death (Abubakar and Hassan, 2006). This could be some of the reasons for the neuronal degenerations observed in the present study.

In the cerebellar cortex of the smoke exposed animals, the histopathological manifestation noticed which include degeneration of Purkinje cells and disruption of the Purkinje cell layer could also affect normal neuronal functions. The Purkinje cells are the neuronal cells that communicate both to molecular and granular cell layers and their degeneration could mean breach of communication between the two cell layers (Ghez, 1985).

Mosquito coil smoke releases gasses and particles that could interfere with the normal cellular processes of energy generation (Liu, *et al*., 2003). The first result of energy depletion is failure of Na^+ and K^+ pumps, leading to depolarization of the neuronal membrane, then synaptic function and conductivity ceases (Dimitri, 2013). Depolarization causes neurons to release glutamate into the synaptic cleft which excites the neurons and when the glutamate is in excess, it becomes an excitotoxin. Since some glutamate receptors are non-selective cation-permeable ion channels, over activation of these channels causes a passive influx of Cl^- and Na^+ into cells causing cytotoxic oedema and rapid cell death. Additional structural damage develops hours or days as a result of Ca^+ influx into neurons (Dimitri, 2013).

Hippocampus has been shown to have high degree of susceptibility to hypoxia (Neal, 2012), state of hypoxia produces toxicity leading to neurodegeneration (Neal, 2012). In the hippocampus of the smoke exposed animals, degeneration of the pyramidal cells was observed which could explain the memory deficit noticed in the short-term memory as explored using novel object recognition test.

It can also be concluded from the present study that exposure to mosquito coil smoke could leads to some cellular processes that results in the condensation and shrinkage of nissl substances which could translate to less protein synthesis.

CHAPTER SIX

6.0 Conclusions and Recommendations

6.1 Conclusion

The present study shows that exposure to mosquito coil smoke for the period of 28 days has significanteffect on short-term memory. From the result of this study, we can see that the smoke exposed animals had less than 50% preference score for the novel object against the familial object which served as an indication of memory deficit. Normally, the animals were expected to have spent more time appreciating the novel object and not the familial object.

Exposure to mosquito coil smoke could be said to have minimal effects on motor activity. From the current study, no significant difference was recorded between the control and the smoke exposed animals which shows that other differences noticed was not as a result of some disability but as a result of the possible effects of the gasses and particles generated from the burning mosquito coil.

The varying degrees of the histopathology observed across the mosquito coil smoke exposed groups can be attributed to a pro-oxidative environment caused by the mosquito coil smoke polluted air.

Interestingly, the present study showed that exposure to mosquito coil smoke even for 28 days can leads to impairment of endogenous antioxidant system which favours accumulation of reactive oxygen species (ROS) either by overloading the anti-oxidant enzymeswith free radicals or by depleting some important co-factors such as Cu, Zn and Mn, thereby, leading toward a case of classical stress.

The decreased amount of trace elements assayed seems to play role in the aetiology of mosquito coil smoke induced neurotoxicity. The depletion in trace elements such as Cu, Zn and Mn may have contributed in the decreased activity of SOD recorded in the oxidative stress biomarker result as they play a vital role in the normal function of SOD. Catalase enzyme that scavenge hydrogen peroxide inions shows significant decrease, an indication of higher generation of hydrogen peroxide. This therefore stand to authenticate the fact that mosquito coil smoke exposure mediates its neurotoxicity through depletion of important trace elements which subsequently leads to oxidative stress.

6.2 Recommendations

Recommendation from this study are as follows;

1. Rambo mosquito coil users should be cautious of how they use the product as it could lead to short-term memory impirement.
2. That questionnaires be distributed among frequent users of mosquito coil in Nigeria to establish relationship between duration of mosquito coil and risk of neurotoxicity to ascertain demographic data.
3. Further work should be carried out in order to quantify the amounts of the various gasses and particles that could be generated from burning mosquito coil.
4. Further studies should be carried out on neurochemistry to quantify or ascertain the level of neurotransmitter depletion as a result of exposure to mosquito coil.
5. Molecular studies should also be carried out to ascertain if any, the extent of damage to DNA in the brain tissue as a result of mosquito coil smoke exposure.

6.3 Contribution to Knowledge

Exposure to mosquito coil smoke significantly affects short-term memory in GII week 2 (33.7±14.7), GIII week 2 and 3 (21.9±4.0 and 28.2±10.4) and GIV week 3 (36.1±11.6), at $p<0.05$.

Mosquito coil smoke exposure causes oxidative stress partly by decreasing the co-factors for some endogenous anti-oxidant enzymes in the cerebrum such as Cu (2.5±0.18), Zn (28.9±0.93) and Mn (8.3±0.81) $p<0.05$.

Mosquito coil smoke also causes its neurotoxicity in the hippocampus by increased generation of ROS, mainly hydrogen peroxide.

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