EFFECTS OF SUPPLEMENT, AQUEOUS EXTRACT AND DI-CHLOROMETHANE CAFFEINE FRACTION EXTRACT OF *Cola nitida* ON THE CEREBRAL CORTEX, HIPPO CAMPUS AND BIOCHEMICAL PARAMETERS OF ADULT LONG EVANS RATS

BY

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DECLARATION

I hereby declare that the research work reported in this dissertation entitled "**Effects of Supplement, Aqueous Extract and Di-chloromethane Caffeine Fraction of *Cola nitida* on the Cerebral cortex, Hippocampus and biochemical parameters of Adult Long Evans rats"** has been carried out by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria, under the supervision of Professor S. B. Oladele and Dr. A.A. Buraimoh. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree in any other institution.

_____ _____

Maimunatu Ahmad MUHAMMAD **Date**

CERTIFICATION

This dissertation entitled **"THE EFFECTS OF SUPPLEMENT, AQUEOUS EXTRACT AND DI-CHLOROMETHANE CAFFEINE FRACTION OF *Cola nitida* ON THE CEREBRAL CORTEX, HIPPOCAMPUS AND BIOCHEMICAL PARAMETERS OF ADULT LONG EVANS RATS"** by MAIMUNATU AHMAD MUHAMMAD meets the regulation governing the award of the degree of Master of Science (M.Sc.) in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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This research work is dedicated to Allah (S.W.A) for giving me the strength and health to carry out successfully the research reported in this dissertation and also to my parents Malam Ahmad Muhammad and Malama Maryam Muhammad.

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In the name of ALLAH, the most gracious and most high. My profound gratitude goes to the chairman of my supervisory committee Prof. S. B. Oladele for his continuous supervision and constructive criticism throughout the course of my research and to a father, a teacher and a member of supervisory committee Dr. A.A. Buraimoh for his contributions and encouragement towards the success of this study and my academic pursuit in general.

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ABSTRACT

Cola nut is a caffeine-containing nut of evergreen trees of the genus *Cola*, primarily of the species *Cola acuminata* and *Cola nitida*. It is one of the most common chewable nut in Nigeria. It is a central nervous system stimulant used in folk medicine as an aphrodisiac, an appetite suppressant, to treat migraine headache and indigestion. The aim of the present study was to investigate the possible effects of *Cola nitida* extracts on the cerebral cortex, hippocampus and some biochemical parameters. Forty (40) adult Long Evans rats of both sexes, with average weight of 210-230grams were randomly divided into four groups: A (control) = four rats (administered normal feed and distilled water);B= caffeine fraction group containing three sub groups of four rats each, B1, B2 and B3 were administered with di-chloromethane caffeine fractionof 19.2, 38.4 and 57.6mg/kg body weight, respectively; C= aqueous extract group containing three sub groups of four rats each, C1, C2 and C3 and were administered cola nut aqueous extract orally at the concentration of 300, 600 and 900 mg/kg body weight respectively; D= cola nut supplement group containing three sub groups of four rats each, D1, D2 and D3 were administered 10%, 20% and 30% w/w cola nut supplemented feed, respectively. The administration lasted for a period of 21 days (3 weeks). Behavioural test for spatial learning and memory was carried out using Morris water maze and anxiety-like behaviours using Elevated Plus Maze. Blood was collected via cardiac puncture for determination of haematological indices, Brain tissues used for determination of oxidative stress parameters were homogenised in cold saline thoseused for histological studies were fixed in Bouin's fluid processed and stained using Haematoxylin and Eosin (H and E) and toluidine blue stains. The Morris water maze test showed a non-significant decrease (P□ 0.05)in time to find the platform in all the treatment groups. A non- significant increase (P□ 0.05) in the meantime spent in the open arm, number of entriesinto the open arm, rearing and number of head dips in the elevated plus maze test

when compared with the control was observed. The result of the oxidative stress markers showedstatistical significant decrease insuperoxide dismutase (SOD) of the aqueous and caffeine fraction groups when compared with the control.No significant difference was observed with the other markers. The results of the haematological indices revealed a statistical significant increase(p□ 0.05) anddecrease (P□ 0.05) in lymphocytes and neutrophils counts respectively in the caffeine and aqueous extract groups. The histology results revealed neurodegenerative changes in the cerebral cortex and hippocampus of the treated groups, which range from neuronal degeneration, pyknosis and clumping of pyramidal cells in the hippocampus. It was concluded that ingestion of cola nut and its constituent by Long Evans rats caused no significant effect on learning and memory, decreased anxiety-like behaviours, anti-inflammatory effect by increasing lymphocytes and decreasing neutrophils level, non-antioxidant effect by significantly decreasing the level of superoxide dismutase (SOD) and neurodegenerative changes.

TABLE OF CONTENTS

CONTENTS

PAGE

TITLE PAGE ……...i

DECLARATION ii

CERTIFICATION.....iii

DEDICATION iv

ACKNOWLEDGEMENT.....v

ABSTRACT.....vi

TABLE OF CONTENTS viii

List of Figures.....xi

List of Tables xii

List of Plates xiii

List of Appendices.....xvii

List of Abbreviations xviii

CHAPTER ONE 1

1.0 Introduction.....1

1.1 Statement of Research Problem 2

1.2 Justification of the Study.....2

1.3 Significance of the Study 3

1.4.1 Aim of the Study 3

1.4.2 Objectives of the Study.....3

1.5 Research Hypothesis.....4

1.6 Scope of the Study.....4

1.7 Limitations of the Study 4

CHAPTER TWO 5

2.0 Literature Review 5

2.1 *Cola nitida*. 5

2.1.1 Plant Taxonomy.....6

2.1.2 Plant Description.....6

2.1.3 Cultivation 7

2.1.4 Distribution of Cola Nut 8

2.1.5 Reports on the effects of *Cola nitida.* 9
2.1.6 Phytochemical composition 11
2.1.7 Side effects of *Cola nitida* 11
2.2 Caffeine; the active component of *Cola nitida* **12**
2.2.1 Reports on the effects of caffeine 13
2.2.1 Mechanism of Action of Caffeine 14
2.3 Cerebrum **15**
2.3.1 The Gross anatomy of cerebrum 15
2.3.2 Histology of the cerebrum 18
2.3.3 Embryology of the Cerebrum 23
2.3.4 Functions of the Cerebrum 23
2.4 The Hippocampus **24**
2.4.1 Functions of the Hippocampus 27
2.4.2 Learning and Memory 27
CHAPTER THREE **29**
3.0 Materials and Methods **29**
3.1 Materials **29**
3.2 Plant Material **29**
3.3 Extraction of Plant Material **29**
3.4 Experimental Animals **30**
3.5 Acute toxicity study **30**
3.6 Experimental Design **30**
3.7 Neurobehavioral Studies **32**
3.7.1 Spatial memory and learning (Morris water maze). 32
3.7.2 Elevated Plus Maze 34
3.8 Animal Sacrifice **36**
3.9 Determination of Haematological Indices **36**
3.10 Tissue Preparation **37**
3.10.1 Fixation 37
3.10.2 Tissue processing 37
3.10.3 Sectioning 38
3.10.4 Staining 38
3.10.5 Photomicrograph 38
3.11 Estimation of Oxidative Stress Parameters **38**

3.11.1 Determination of Catalase activity (CAT) 38
3.11.2 Determination of Superoxide Dismutase (SOD) 39
3.11.3 Assessment of Lipid Peroxidation 40
3.11.4 Assay of Reduced glutathione (GSH) 40
3.12 Statistical Analysis 41
CHAPTER FOUR 42
4.0 Result 42
4.1 Acute Toxicity: 42
4.2 Physical Observation 42
4.3 Body Weight of the Animals 42
4.4 Morris water maze test 44
4.5 Elevated plus maze 46
4.6 Haematological indices 48
4.7 Oxidative stress 51
4.8 Histological Studies 54
CHAPTER FIVE 98
5.0 Discussion 98
CHAPTER SIX 104
6.0 Conclusion and Recommendations 104
6.1 Conclusion 104
6.2 Recommendations for Further Research 105
References 106
Appendices 115

List of Figures

Figure 2.3: Structural formula of caffeine and adenosine……13

Figure 2.4: Gross anatomy of the cerebrum showing different parts…….…....17

Figure 2.3: The Cellular layers of Cerebral cortex…….……22

List of Tables

Table 3.1: Grouping, dosages and duration of administration of *Cola nitida* extracts....31

Table 4.1: Weight of animals in grams before and after administration of *Cola nitida*...43

Table 4.1: Effect of *Cola nitida* on mean latencies for spatial memory using Morris water maze test……..45

Table 4.2: Effect of *Cola nitida* on anxiety-like behaviours using elevated plus maze…47

Table 4.3: Effect of *Cola nitida* on haematological indices……..…49

Table 4.4: Effect of *Cola nitida* on Oxidative Stress Parameters…….....51

List of Plates

Plate I: A and B Showing the pictures of Cola nut plant and seeds……..……..……..**7**

Plate II: Showing the picture of Cola nut seeds….....……....**7**

Plate III: The Structure of rat hippocampus…..……...…..……**26**

Plate IV: Morris Water Maze pool……..….……...…….**33**

Plate V: Elevated plus maze…….……...**35**

Plate VI: Section of cerebral cortex of rat showing the cell layers from Molecular (I), Outer granular (II), Outer pyramidal (III), Internal granular (IV), Internal pyramidal (V) and Multiform (VI). (H and E × 40) …….……...**54**

Plate VII: Section of cerebral cortex (layer II and III)of rat from control group (H and E × 250)……...**55**

Plate VIII: Section of cerebral cortex (layer II and III) of rat treated with 300mg/kg aqueous extract of *Cola nitida* (H and E × 250) ……..……..……..…**56**

Plate IX: Section of cerebral cortex(layer II and III) of rat treated with 600mg/kg aqueous extract of *Cola* (H and E × 250)…..…….…….…**.....57**

Plate X: Section of cerebral cortex of rat treated with 900mg/kg aqueous extract of *Cola nitida* (H and E × 250)……..……..…**58**

Plate XI: Section of cerebral cortex of rat supplemented with 10% *Cola nitida* (H and E × 250)……..……...…**59**

Plate XII: Section of cerebral cortex of rat supplemented 20% *Cola nitida*(H and E × 250)……..……...……...…**60**

Plate XIII: Section of cerebral cortex of rat) feed supplemented with 30% *Cola nitida*(H and E × 250)……...…….**61**

Plate XIV: Section of cerebral cortex of rat administered 19.2mg/kg caffeine extract(H and E × 250) ……...……..…..…**62**

Plate XV: Section of cerebral cortex of rat administered38.4mg/kg caffeine extract(H and E × 250)……...…..…**63**

Plate XVI: Section of cerebral cortex of rat administered57.6mg/kg caffeine extract(H and E X 250)……..…..……**64**

Plate XVII: Section of hippocampus of rat showing CA2, CA3, CA4 regions and the dentate gyrus. (H and E × 40)……..**65**

Plate XVIII: Section of hippocampus of rat from control group(H and E × 250)……..…… …**66**

Plate XIX: Section of hippocampus of rat treated with 300mg/kg aqueous extract of *Cola nitida* (H and E × 250)…….……..…….…….…**67**

Plate XX: Section of hippocampus of rat treated with 600mg/kg aqueous extract of *Cola nitida* (H and E × 250)……..……...…..……..……..……**68**

Plate XXI: Section of hippocampus of rat treated with 900mg/kg aqueous extract of *Cola nitida*(H and E × 250)…….……...…..……..……..**69**

Plate XXII Section of hippocampus of rat 10% supplemented with *Cola nitida*(H and E × 250)……...……..…….…….**70**

Plate XXIII: Section of hippocampus of rat supplemented with 20% *Cola nitida*(H and E × 250)……..……..……**71**

Plate XIV: Section of hippocampus of rat supplemented with 30% *Cola nitida* (H and E × 250)…….……...……...……**72**

Plate XXV: Section of hippocampus of rat administered 19.2mg/kg caffeine extract(H and E × 250)…….…….……..**73**

Plate XXVI: Section of hippocampus of rat administered 38.4mg/kg caffeine extract (H and E × 250)……..…….….**74**

Plate XXVII: Section of hippocampus of rat administered 57.6mg/kg caffeine extract(H and E × 250)…….……...……...…….**75**

Plate XXVIII: Section of cerebral cortex of rat from control group (H and E × 250).....…….……...……**76**

Plate XXIX: Section of cerebral cortex of rat treated with 300mg/kg aqueous extract of *Cola nitida* (Toluidine blue stain × 250)…….……**77**

Plate XXX: Section of cerebral cortex of rat treated with600mg/kg aqueous extract of *Cola nitida*(Toluidine blue stain × 250)……..…**78**

Plate XXXI: Section of cerebral cortex of rat treated with900mg/kg aqueous extract of *Cola nitida* (Toluidine blue stain × 250)…….……..……**79**

Plate XXXII: Section of cerebral cortex of rat supplemented with 10% *Cola nitida* (Toluidine blue stain × 250)……...……..……**80**

Plate XXXIII: Section of cerebral cortex of rat supplemented with 20% *Cola nitida* (Toluidine blue stain × 250)…….……**81**

Plate XXIV: Section of cerebral cortex of rat supplemented with 30%*Cola nitida*(Toluidine blue stain × 250)…….……**82**

Plate XXXV: Section of cerebral cortex of rat administered with 19.2mg/kg caffeine extract (Toluidine blue stain × 250)……**83**

Plate XXXVI: Section of cerebral cortex of rat administered 38.4mg/kg caffeine extract(Toluidine blue stain × 250)…….……....……**84**

Plate XXXVII: Section of cerebral cortex of rat administered 57.6mg/kg caffeine extract (Toluidine blue stain × 250)……...……..……**85**

Plate XXXVIII: Section hippocampus from control group(Toluidine blue stain × 250)……..……...……**86**

Plate XXXIX: Section of hippocampus of rat treated with 300mg/kg aqueous extract of *Cola nitida*(Toluidine blue stain × 250)…….…….……**87**

Plate XL: Section of hippocampus of rat treated with600mg/kg aqueous extract of *Cola nitida*(Toluidine blue stain × 250)……..…….……**88**

Plate XLI: Section of hippocampus of rat treated with 900mg/kg aqueous extract of *Cola nitida*(Toluidine blue stain × 250)……..…….……...**89**

Plate XLII: Section of hippocampus of rat supplemented with 10% *Cola nitida*(Toluidine blue stain × 250)……..……...**90**

Plate XLIII: Section of hippocampus supplemented with 20% *Cola nitida*(Toluidine blue stain × 250)…….……...……..….**91**

Plate XLIV: Section of hippocampus of rat supplemented with 30% *Cola nitida*(Toluidine blue stain × 250)…….**92**

Plate XLV: Section of hippocampus of rat administered 19.2mg/kg caffeine extract (Toluidine blue stain × 250)…….**93**

Plate XLVI: Section of hippocampus of rat administered 38.4mg/kg caffeine extract (Toluidine blue stain × 250)…...……..**94**

Plate XLVII: Section of hippocampus of rat administered 57.6mg/kg caffeine extract(Toluidine blue stain × 250)…….……**95**

List of Appendices

Appendix I: Calculation of the extracts doses……...112

List of Abbreviations

CAT	Catalase
CC	Central chromatolysis
CDPC	Clumped Degenerating Pyramidal Cells
DN	Degenerating neuron
DPC	Degenerating Pyramidal cells
ECA	Entry in to close arm
EOA	Entry in to open arm
EOS	Eosinophils
GSH	Glutathione concentration
Hb	Haemoglobin
HD	Head dip
IU	International unit
LCDPC	Layer of Clumped Degenerating Pyramidal Cells
LYM	Lymphocytes
MDA	Malondialdehyde
NEU	Neutrophils
NPC	Normal pyramidal cells
PCV	Packed Cell Volume
PK	Pyknosis
PC	Pyramidal cells
S	Seconds
SEM	Standard Error of Mean
SC	Stellate cells
SOD	Superoxide Dismutase
TBARs	Thiobabituric acid reaction

TOA	Time spent in open arm
TCA	Time spent in close arm
V	Vacuolation
WAA	Weight after administration
WBA	Weight before administration
WBC	White blood cells
WHO	World Health Organization
%WC	Percentage weight change

CHAPTER ONE

1.0 Introduction

Plants have been an important source of medicine for thousands of years. The world health organization (WHO) estimated that 80% of people still rely on traditional remedies such as herbs for their medicines (WHO, 2000; Tripathi and Tripathi, 2003). Plants are also a source of modern medicines. It is estimated that approximately a quarter of processed drugs contain plant extracts or active ingredients obtained from or modelled on plant substances (Tripathi and Tripathi, 2003). Plant-derived substances have recently become of great interest due to their versatile applications. Medicinal plants are the richest resource of drugs of traditional system of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*,2008).

Medicinal plants were used by people from different cultures without the knowledge of their active ingredients. This is common practice which is laden with hazards as the extract may contain some toxic constituents (Lown, 1993).

Cola nitida commonly called cola nut in English, "Goro" in Hausa, "Obi" in Yoruba, "Oji" in Igbo, "Irevu" in Ebira and "Ebi" in Nupe is a genus of 125 species of trees native to tropical rainforest of Africa. Cola is one of the major tree crop grown in Nigeria today, and the tree on which it grows is believed among some Nigerian tribes to be the first tree on the earth(Lowor, 2010). It is a smooth fruit consisting two cotyledons of the family Sterculoideae. It is cultivated in the tropical West Africa. Some species are also cultivated for their nuts in Madagascar, Brazil, Jamaica and other humid tropics. The seed contains caffeine and is bitter. The first taste of *Cola nitida* is bitter, but it

sweetens upon chewing (Lovejoy *et al*., 1980). It isan important part of the traditional spiritual practice of culture and religion in West Africa, particularly in Nigeria.Cola nut has been reportedly used in folk medicine as an aphrodisiac, an appetite suppressant, to treat migraine headache and indigestion (Esimone *et al.*, 2007).

1.1 Statement of Research Problem

Cola nitida has neurodegenerative effects on the cerebellum of Wistar rats(Buraimoh *et al.*, 2014).

Memory and cognition enhancement procedure is not well understood in the field of neuroscience and due to high consumption of *Cola nitida* by Nigerians, especially students to increase alertness and probably memory, there is need to investigate and compile empirical evidence to either proof or disprove this belief.

Furthermore, considering the use of the fruit by people in West Africa, especially Nigeria, who believe that it has a divine power of curing some diseases, there is need to investigate the effects of *Cola nitida* on neurobehavioral activities and vital tissues of the body such as cerebrum and hippocampus at microscopic level.

1.2 Justification of the Study

Despite reported evidence of neurodegeneration that *Cola nitida* has on the cerebellum (Buraimoh *et al*., 2014), traditional, cultural and social use in our environment is still on the rise. Therefore, there is need to investigate further; the effect of *Cola nitida* on other parts of the nervous system, such as the cerebral cortex and hippocampus.

1.3 Significance of the Study

The study could be of great importance inidentifying and evaluating the regions of the brain that may be affected by *Cola nitida*, give insight to people, especially students on the effect of the plant on memory, cognition, anxiety-like behaviours and its relationship with biochemical parameters.

1.4 Aim and Objectives of the study

1.4.1 Aim of the Study

The aim of the study was to investigate the effects of supplement, aqueousextract and di-chloromethane caffeine fraction extract administration of *Cola nitida* on the cerebrum and hippocampus of adultLong Evans rats.

1.4.2 Objectives of the Study

The objectives of this study were to:

I. Investigate the effect of *Cola nitida* on spatiallearningand memory, andanxiety-like behaviour using Morris water maze and elevated plus maze, respectively.
II. Investigate the effect of *Cola nitida* on haematological indices (PCV, WBC, Hb, and differential cell counts) of Long Evans rats.
III. Evaluate the comparative effect of*Cola nitida* on the oxidative stress markers(GSH, MDA, CAT and SOD) of the cerebrum of Long Evans rats.
IV. Examine the effect of *Cola nitida* on the histology of cerebral cortex and hippocampus of Long Evans rats.

1.5 Research Hypothesis

- *Cola nitida* has no effect on the histology of the cerebrum and hippocampus of adult Long Evans rats.
- *Cola nitida* has effect on neurobehavioursof adult Long Evans rats.

1.6 Scope of the Study

This work would be limited to the effects of *Cola nitida* and its active ingredient caffeine on the histology of cerebral cortex and hippocampus, neurobehaviours, haematological indices and some biochemical parameters.

1.7 Limitations of the Study

- Electron microscopy was not done to show the detailed structure of the cerebral cortex and hippocampus due to lack of electron microscope.
- Neurotransmitters were not assayed due to lack of fund.

CHAPTER TWO

2.0 Literature Review

2.1*Cola nitida*.

The Cola nut is a caffeine-containing nut of evergreen trees of the genus *Cola*, primarily of the species *Cola acuminata* and *Cola nitida* (Erbe and Lawrence, 2014). It is one of the most common chewable nut in Nigeria (Umoren *et al.,* 2009). Cola nuts are an important part of the traditional spiritual practice of culture and religion in West Africa, particularly in Nigeria (Adewale-Somadhi, 2004). It is used as a religious object and sacred offering during prayer, ancestor veneration and significant life events, such as wedding, naming ceremonies and funerals. It has many pharmacological properties and contains some active principles. It prevents sleep, thirst, hunger and act as an antidepressant(Adewale-Somadhi, 2004). The Cola nuts are a source of antioxidants and contain a wide range of complex secondary plant metabolites such as theobromine, d-catechin, L-epicatechin and kolatin (Lowor*et al*., 2010). The use of the plant in the treatment of certain diseases has been reported by several authors (Ayebe *et al.*, 2012). It is also used in traditional divination, a system called "Obi divination" in Yoruba traditional religion. For this use, only cola nuts divided into four lobes are suitable. They are cast upon a special wooden board and the resulting pattern are read by a trained diviner (Epega, 2003). In Nigeria, it is generally acknowledged that the Yorubas' grow the cola nuts; the Hausas' eat it while the Igbos' celebrate it. There is a proverb in the South East which says "He who brings cola brings life and let us who eat in good health condition". But whoever refuses his brother cola "may he get doom of our fore fathers". If a southerner gives you cola when you go to his house, this is a clear indication that you are welcome (cola nut tradition in Igbo land).

2.1.1 Plant Taxonomy

KINGDOM	Plantae
DIVISION	Magnoliophyta
CLASS	Magnoliopsida
ORDER	Malvales
FAMILY	Malvaceae
SUBFAMILY	Sterculiadeae
GENUS	*Cola*
SPECIES	*nitida*
BINOMIAL NAME	*Cola nitida (*Burdock *et al*., 2009)

2.1.2 Plant Description

Cola nitida is an evergreen tree growing to a height of 12 to 20 meters (39 to 66 ft). The trunk is up to 1.5 m (4 ft 11.1 inches) in diameter and older trees develop buttresses. The bark is thick and fibrous, with deep longitudinal fissures. It is grey or brownish-grey, with pinkish-red wood becoming visible when the bark is damaged. The leaves have stalks and are alternate, oblong, glabrous, leathery and tough, with untoothed wavy margins and up to 33 cm (13 inches). The flowers have parts in fives. They grow in panicles from the leaf axils and have no petals. Male flowers have a deeply lobed, cup-shaped calyx about 2 cm (0.8 in) in diameter with two whorls of stamens. Female flowers are larger at 5 cm (2.0 in) diameter, with five carpels. The calyx is yellow with red nectar guides, and are followed by fleshy fruits up to 13 cm (5.1 in) long and 7 cm (2.8 in) wide. When ripe, the pods split open to reveal the seeds which may be mottled white, reddish-grey or brown. The seeds are bitter when fresh but become more aromatic as they age (Kuame, 2006).



(A) *Cola nitida* plant (B) Matured Cola fruit and seeds.

PlateI. A and B Showing the pictures of cola nut plant and seeds(www. Britannica.com)



PlateII: The picture of cola nut seeds (Personal photograph) July,2015.

2.1.3 Cultivation

Originally a tree of tropical rainforest, it needs a hot humid climate, but can withstand a dry season on sites with a high ground water level. It may be cultivated in drier areas where ground water is available. *Cola nitida* is a shade bearer, but develops a better

spreading crown which yields more fruits in open places. Though. it is a lowland forest tree, it has been found at altitudes over 300 m on deep, rich soils under heavy and evenly distributed rainfall(Kuame, 2006).

Regular weeding is a must and can either be done manually or by using herbicides. Some irrigation can be provided to the plants, but it is important to remove the water through an effective drainage system, as excess water may prove to be detrimental for the growth of the plant. When not grown in adequate shade, the cola nut plant responds well to fertilizers. Usually, the plants need to be provided with windbreaks to protect them from strong gales(Kuame, 2006).

Cola nuts can be harvested mechanically or by hand, by plucking them at the tree branch. When kept in a cool, dry place, cola nuts can be stored for a long time (Erbe and Lawrence, 2014).

2.1.4 Distribution of Cola Nut

Cola nitida is native to Sierra Leone, Liberia, Ivory Coast, Ghana andNigeria. It is a lowland tree but can be found up to altitudes of about 300 meters (980 ft.) in areas with deep, rich soils and evenly distributed rainfall. It has been cultivated in other parts of tropical Africa as well as India, Jamaica, Brazil, and Hawaii (World Agroforestry Centre, 2015). It can grow where there is around 1,200 millimeters of rain but does better where the annual rainfall exceeds 1,700 millimeters (67 in), spread across eight months or more. Its temperature range is 23 to 28 °C (73 to 82 °F) and it grows on both light and heavy soils as long as they are deep (Lim, 2012).

2.1.5 Reports on the effects of *Cola nitida.*

Cola nitida contains caffeine which is a central nervous system stimulant, when chewed, the caffeine will dissolve in the saliva and be absorbed in the system. This stimulates a person and keeps him or her awake for a long period of time. This is practiced by students and drivers on a long journey who need to remain awake for unusually long period (Jayeola, 2001). In humans, it was reported to enhance alertness and physical energy, to elevate mood, to increase tactile sensitivity and to suppress the appetite (Umoren *et al*., 2009). Autonomic changes such as increased body temperature, increased blood pressure and increased respiratory rate were reported. Consumption of a diet comprising cola nuts for 7 days elevated the mean arterial pressure (MAP) of normal rats (Osim and Udia, 1993).

The plant was reported to have some antibacterial effects; The work of Adeniyi *et al*. (2004), reported that the methanol extract of root bark for both *Cola nitida* and *Cola milleni* were found to be potent against both *Mycobacterium bovis* and strains of *Mycobacterium vaccae*. According to Muhammad and Fatima (2014), aqueous and methanol extracts of red and white variety of colanut showed bacteriocidal activity against *Streptococcus anginosus,* a gram positive bacteria.

An important research on ocular activity done by Igwe *et al.*(2007) states that *Cola nitida* improved the near point of convergence by 43% and increased the amplitude of accommodation by 11%, while existing heterophorias are ameliorated. He suggested that somnolence and ocular muscle imbalance common features of the elderly could be ameliorated or relieved.

It is chewed in many West African cultures, individually or in a social setting to restore vitality and ease hunger pangs. *Cola nitida* has been reportedly used in folk medicine as an aphrodisiac, an appetite suppressant, to treat migraine headache and indigestion and also being used to clean the teeth and gums (Esimone *et al*., 2007). *Cola nitida* has been used to control vomiting in pregnant women (Haustein, 1971; Chukwu *et al*., 2006). It has also been applied directly to the skin to treat wounds and inflammation (Newall *et al*., 1996).

In Sierra Leone, an infusion of macerated leaves and salt is used to manage diarrhea and asthma (Lebbie and Guries, 1995). In Mali, macerated powdered nut or bark of *Cola nitida* is used for amenorrhea (Togola *et al.,* 2008). It has also been applied directly to the skin to treat wounds and inflammation (Newall *et al.*, 1996). In some developed countries, however, *Cola nitida* extract is used industrially for the manufacturing of many cola-type soft drink flavors (Beattie, 1970). It has also been reported to be used in the manufacture of non-alcoholic beverages, soft drink, chocolates and sweets (Ogutuga, 1957; Beattie, 1970; FAO, 1982; Asogwa *et al*., 2006). It's uses have inevitably created a high demand in excess of its production in Nigeria (Oladokun, 1985).

Other uses of cola nut include; dyeing and water purification, the timber has a use in building work, boat construction, carpentry, musical instruments, utensils and carving. It is also useful as firewood. The pods have been used to make fertilizer and soap, and they can be used as a substitute for up to 60% of the maize in poultry feed (Lim, 2012).

2.1.6 Phytochemical composition

The major constituent of *Cola nitida* has been shown to be alkaloid of which caffeine is the most common (Evans, 1996). The chemical composition of cola nut includes: caffeine (2% - 3.5%), theobromine (1.0% - 2.5%), theophylline, phenolics (phlobaphens, epicatechin, D-catechin, tannic acid), sugar (cellulose) and water (Muhammad andFatima 2014). Another report by Odebunmi *et al*. (2009) states the nutrient composition of *Cola nitida* as: moisture 66.4%, dry matter 33.60%, crude fat 5.71%, crude protein 2.63%, ash 1.50%, crude fibre 7.13% total carbohydrate 28.56%, and the minerals (mg/kg dry matter) – K 3,484 mg, Ca 124.4 mg, Mg 392 mg, Fe 16.43 mg, Zn 5.24 mg and P 411.43 mg. Sugar has also been shown to be slightly abundant in the seed (Oladukun, 1989; Obika *et al.*, 1996). It is commonly used by people to discharge hungerdue to its high sugar content (Obika *et al.*, 1996).

2.1.7 Side effects of *Cola nitida*

The seed has been reported to have some side effects, due to its high amount of N-nitroso compounds which are carcinogenic. In Nigeria, where the chewing of cola nut is a common practice, there is high incidence of oral and gastro intestinal cancer which may be related to that habit (Atawodi, 1995). *Cola nitida* is not advised for individuals with stomach ulcers due to its caffeine and tannin contents (Ibu *et al.*, 1986; Newall *et al*., 1996).

Cola nuts, when marketed or used as herbal medicinal remedy, have been the subject of a number of precautionary warnings to consumers, primarily related to the caffeine content of the nuts. For example, the American Herbal Products Association includes *Cola acuminata* and *Cola nitida* on its lists of herbs that may cause irritation to the

gastro intestinal tract, that may induce nervous system stimulation, and that should not be used in pregnancy unless otherwise directed by a qualified expert (McGuffin *et al*., 1997). The potential for kola nuts and their extracts to interact pharmacologically with a number of drugs included, but not limited to ephedrine, phenelzine, monoamine oxidase inhibitors, adenosine, clozapine, benzodiazepines, propranolol and phenylpropanolamine and quinolone antibiotics has been noted (Brinker, 2001).Animal experiment showed that chronic consumption of cola nut and caffeine diets caused decrease in food intake and body weight (Umoren *et al.*, 2009). It has also been reported that it has a mimicked malaria-like symptoms when chewed in high concentration (Alaribe *et al.,* 2003). Buraimoh *et al.* (2014). Reported that *Cola nitida* has neurodegenerative effects on the cerebellum of Wistar rats and causes an increased physical activity as well as alertness.

2.2 Caffeine; the active component of *Cola nitida*

Caffeine is a bitter, white crystalline xanthine alkaloid that acts as a stimulant drug and an acetyl cholinesterase inhibitor (Cardoso-Lopes *et al.*, 2009). Caffeine ($C_8H_{10}N_4O_2$) is the most widely consumed psychoactive substance in the world. It is found in varying quantities in the seeds, leaves, and fruits of some plants, where it acts as natural pesticides that paralysis and kills certain insects feeding on the plants. The caffeine molecule is structurally similar to adenosine, and is capable of binding to adenosine receptors on the surface of cells without activating them, thereby acting as a competitive inhibitor (Fisone, 2004).

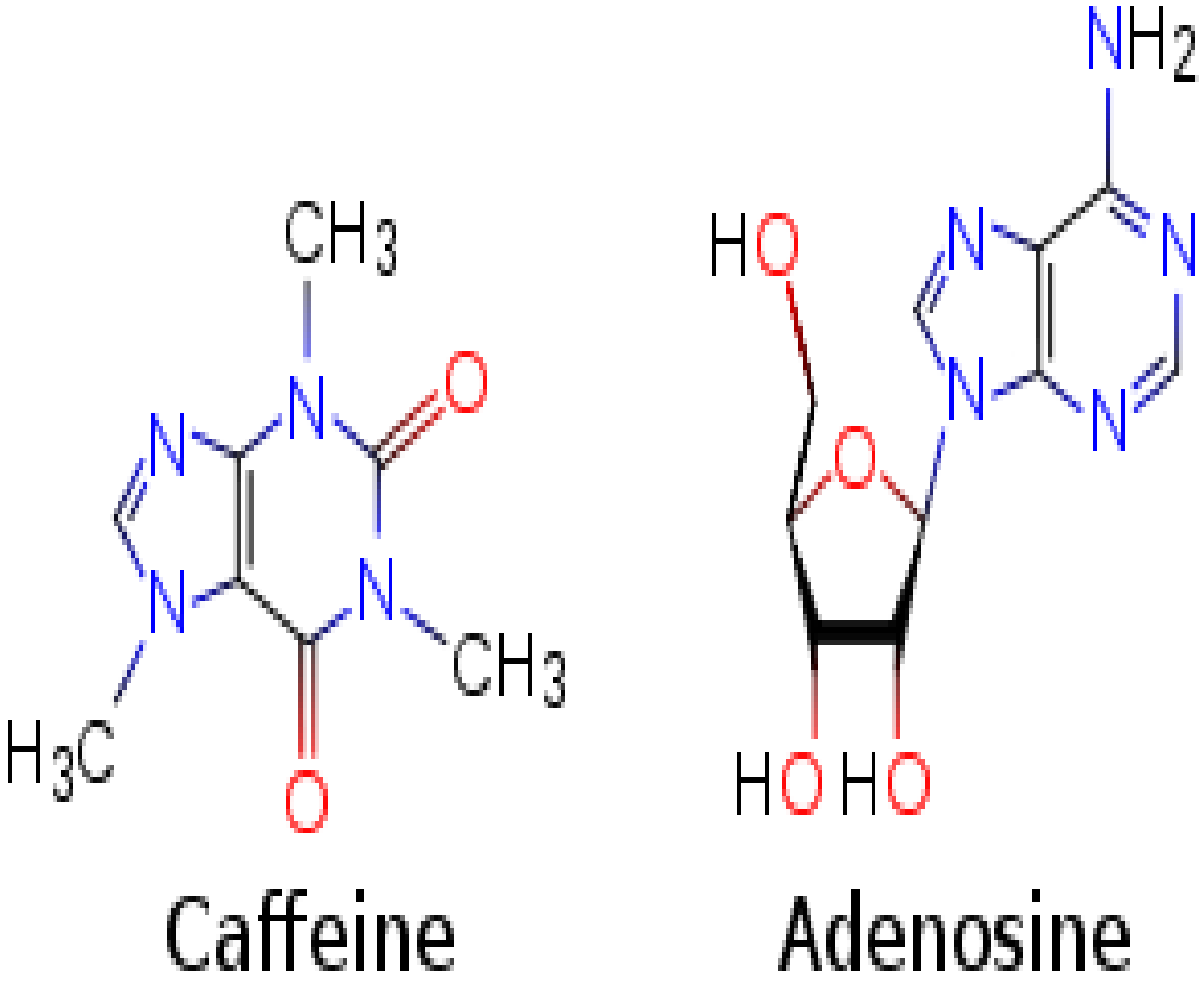


Figure 2.3: Showing the structural formula of caffeine and adenosine (www.chemistryproject 2011.blogspot.com).

2.2.1 Reports on the effects of caffeine

Caffeine stimulates the central nervous system first at the higher levels, resulting in increased alertness and wakefulness, faster and clearer flow of thought, increased focus,

and better general body coordination, and later at the spinal cord level at higher doses (Karau *et al.,* 2010). This stimulatory activity is achieved through several mechanisms including increased ATP production through cyclic adenosine monophosphate (cAMP) accumulation and inhibition of dopamine re-uptake (Huang *et al*., 2005). Urzúa *et al.* (2012) observed a weight loss in all the groups during the first 15 days of their study and over the 60 days after diabetes induction and administration of various dosages of caffeine. Lopez-Garcia *et al.*(2006) on the other hand, reported that increases in caffeine intake may lead to a small reduction in long-term weight gain. It has been suggested that caffeine could contribute to the protection of β pancreatic cells from free radicals produced by oxidative stress, prevent membrane damage and preserve the β pancreatic cell due to its' antioxidant effect (Urzua *et al.,* 2012). The effect of caffeine on pregnancy was reported by Russell (2007), he observed that consuming more than 300 milligrams of caffeine a day will increase one's chances of a miscarriage, and based on studies on animals, high levels of caffeine may also cause birth defects, preterm delivery, reduced fertility and low birth weight.

2.2.1 Mechanism of Action of Caffeine

Caffeine acts through various mechanisms of action. According to Davis (2003), caffeine manages to affect the body by stimulating the central nervous system (CNS). Its lipophilic properties grant caffeine entry through the blood brain barrier (BBB), where it interferes with adenosine receptors. There is a similarity between the molecular structures of caffeine and adenosine, a naturally occurring substance in the brain(Fisone, 2004).

This similarity allows caffeine to bind to the same receptor sites as adenosine. However, the binding of caffeine to an adenosine receptor site does not produce the same effect as binding of adenosine to the receptor. Thus, caffeine is classified as an adenosine antagonist; a drug that attaches to a receptor and blocks the action of any other molecule trying to bind to the receptor site (Julien, 2005). The other mechanisms by which caffeine acts include mobilization of intracellular calcium and inhibition of specific phosphodiesterase both of which only occur at high non-physiological concentrations of caffeine.

2.3 Cerebrum

2.3.1 The Gross anatomy of cerebrum

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

entire supratentorial cranial cavity. The frontal lobes occupy the anterior cranial fossae, the temporal lobes occupy the lateral parts of the middle cranial fossae, and the occipital lobes extend posteriorly over the tentorium cerebelli (Moore, 2010). 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).

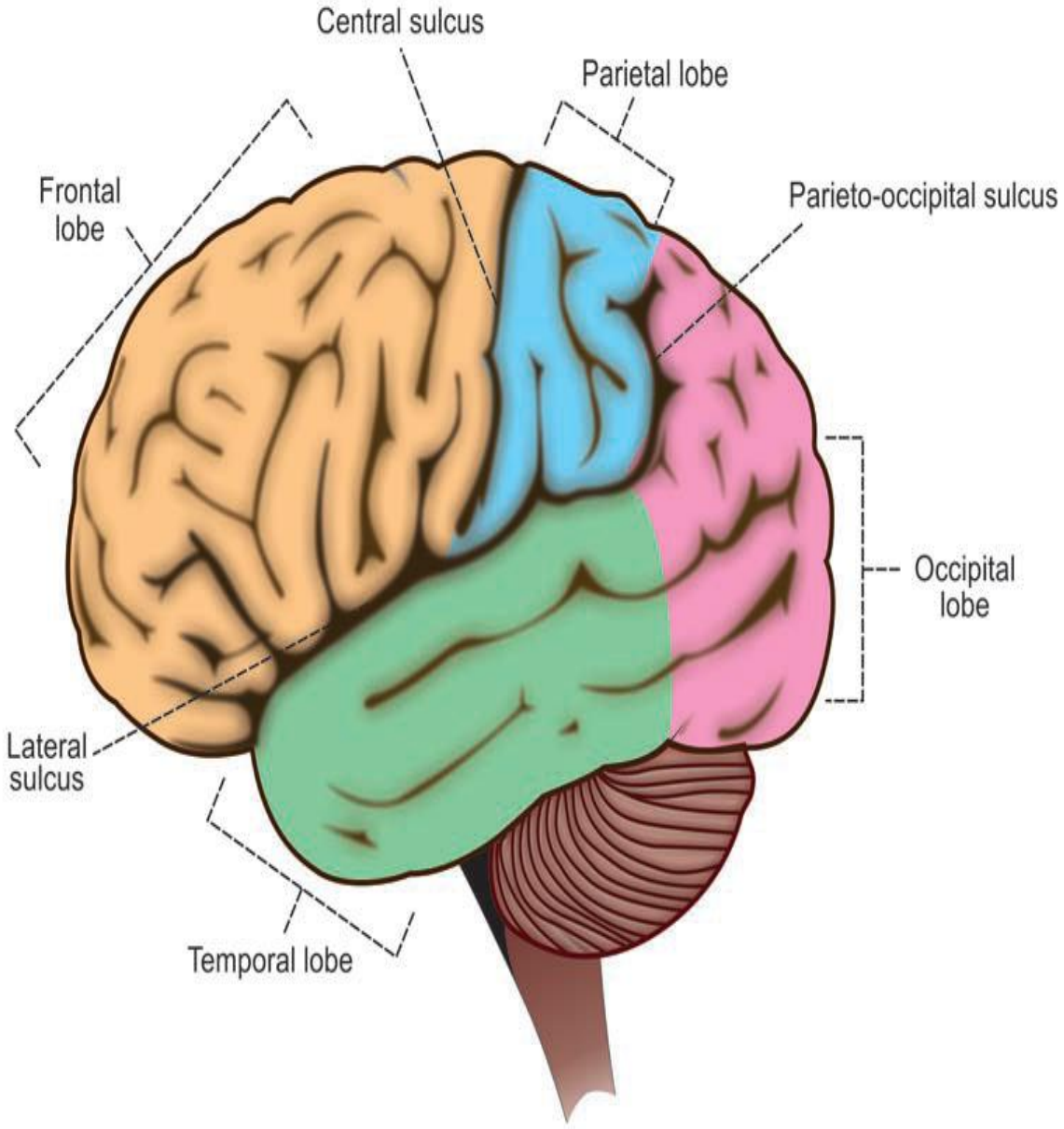


Figure 2.4: Gross anatomy of the cerebrum showing different parts (New healthguide.org).

2.3.2 Histology of the cerebrum

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

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 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 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 2.3, according to Kandel *et al*. (2000); Shipp (2007) and Saladin (2010).

I. Molecular or plexiform layer

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.

II. External granular layer

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

III. Outer pyramidal layer

Outer or 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).

VI. Internal granular layer

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).

V. Internal pyramidal layer

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).

IV. Polymorphic or Multiform layer

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 lemnsical inputs (Kandel *et al.*, 2000).



Figure 2.5: The Cellular layers of Cerebral cortex (Kandel *et al.*, 2000).

2.3.3 Embryology of the Cerebrum

Cerebral hemispheres arise as outgrowths from the lateral wall of prosencephalon during 5-6 weeks. These gradually enlarge to cover thalamus, midbrain and pons. Further growth results in formation of lobes and poles. Increased growth in a limited area result in formation of sulci and gyri. The basal part of the hemisphere increases in size to form two big nuclei connected together by fibres. These nuclei are the caudate and lentiform nuclei. Between these two nuclei pass fibres both ascending and descending. These form internal capsule (projection fibres). The commissural fibres develop in the lamina terminalis (Chaurasia, 2004).

2.3.4 Functions of the 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 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 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).

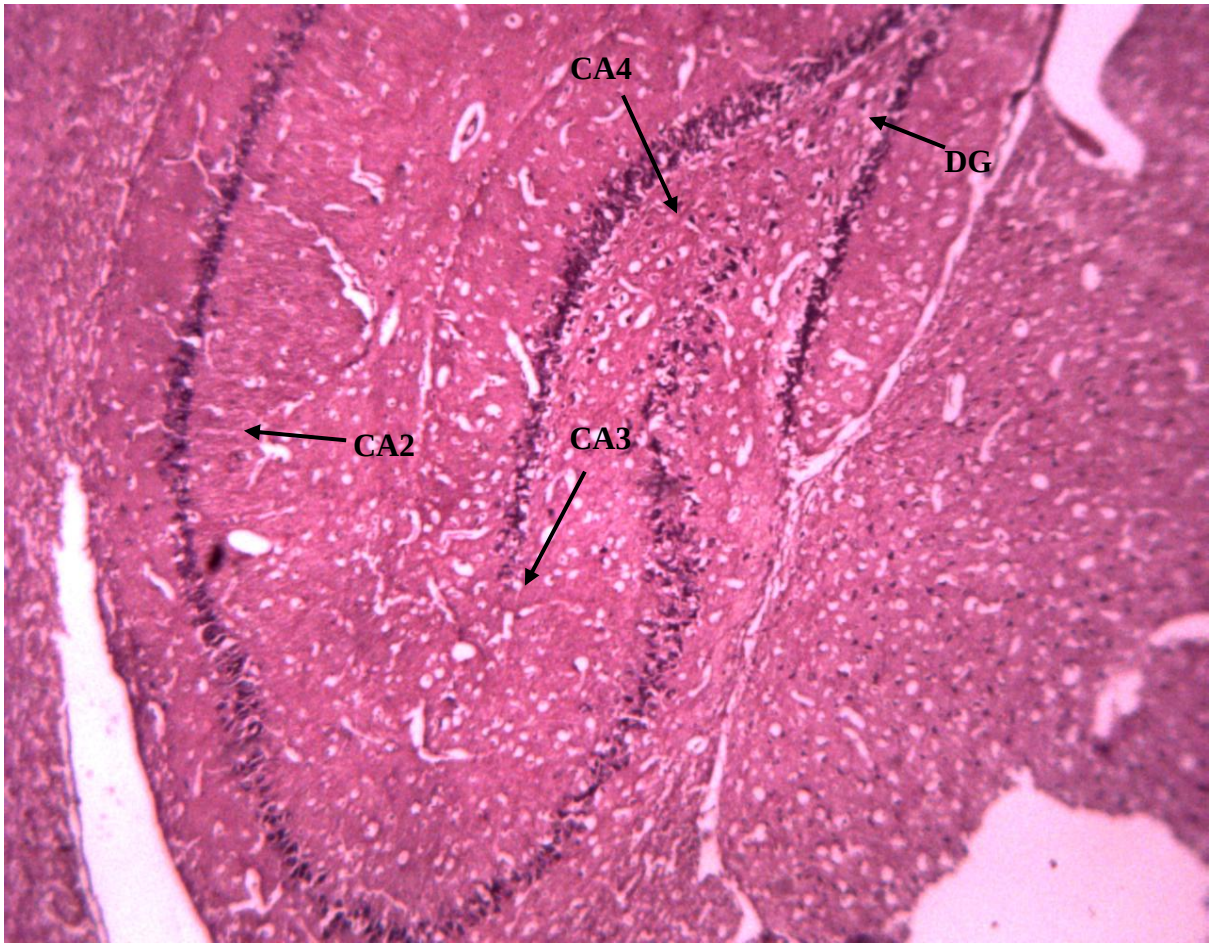
Olfaction: The olfactory bulb in most vertebrates is the most anterior portion of the cerebrum, and makes up a relatively large proportion of the telencephalon. However, in humans, this part of the brain is much smaller, and lies underneath the frontal lobe. The olfactory sensory system is unique in 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).

2.4 The Hippocampus

The hippocampusis a major component of the brains of humans and other mammals. It belongs to the limbic system and plays important role in the consolidation of information from short-term memory to long-term memory and spatial navigation. Like

the cerebral cortex, with which itis closely associated, it is a paired structure, with mirror-image halves in the left and right sides of the brain. The hippocampus is located under the cerebral cortex; and in primates it is located in the medial temporal lobe, in rodents, the hippocampus has been studied extensively as part of a brain system responsible for spatial memory and navigation (Conrad, 2008).

It has the shape of a curved tube, which has been variously compared to a seahorse, a ram's horn (*Cornu Ammonis*, hence the subdivisions CA1 through CA4), or a banana. It can be distinguished as a zone where the cortex narrows into a single layer of densely packed pyramidal neurons 3 to 6 cells deep in rats, which curl into a tight U shape; one edge of the "U," field CA4, is embedded into a backward-facing, strongly flexed, V-shaped cortex, the dentate gyrus(Amaral *et al.*, 2006).



PlateIII: The Structure of rat hippocampus (Personal photograph) September, 2015.

2.4.1 Functions of the Hippocampus

The hippocampus is an additional channel through which incoming sensory signals can initiate behavioural reactions for different purposes. It has been suggested that the hippocampus provides the drive that causes translation of short-term memory into long term memory, that is, the hippocampus transmits some signal or signals that seem to make the mind rehearse overand overthe new information until permanent storage takes place (Guyton and Hall, 2006). The hippocampus also plays an important role in anxiety (Gray *et al.*, 2014).

2.4.2 Learning and Memory

Learning is the process by which we acquire knowledge about the world, while memory involves encoding, storage and retrieval of such information for future use (Sharma *et al*., 2010).

Memory can be classified according to its duration, into short term memory and long term memory. Short term memory is a type of memory that last for seconds or at most minutes, it involves processing of information in the hippocampus and the medial temporal lobe, to create memory traces that later can be converted to the long term memory. Long term memory can store larger quantities of memory that last for years and sometimes for a lifetime (Sharma *et al.*, 2010). Memory is broadly divided into three viz: sensory, short-term and long-term memories. Sensory memory holds sensory information for a few seconds or less after an item is perceived. The ability to look at an item, and remember what it looked like with just a second of observation, or memorization, is an example of sensory memory. The brain stores sensory information

for very short periods of time in a working memory, to be able to use it later (Arash *et al.,* 2014). Brain areas involved in the neuroanatomy of memory include the hippocampus, the amygdala, the striatum, or the mammillary bodies which are thought to be involved in specific types of memory. For example, the hippocampus is believed to be involved in spatial learning and declarative learning, while the amygdala is thought to be involved in emotional memory (Labark and Cabeza, 2006).

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

Distilled water, Cages, Feed, Syringes, weighing balance, dissecting kit, dissecting tray, formalin, Chloroform, Cotton wool, Morris water maze and elevated plus maze testing models, H&E stain, Toluidine blue stain.

3.2 Plant Material

Fresh*Cola nitida*was purchased from Yangoro market Kwangila, Zaria. Identification and authentication of the seeds was done in the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria and was assigned specimen voucher no. 1526.

3.3 Extraction of Plant Material

The fresh seeds of *Cola nitida* were grated into pieces and air dried. The dried pieces were grinded into powder and weighed, 1000g of powdered cola nut was used for the aqueous extraction using cold maceration. Sixty-three (63) grams of aqueous extract was yielded.

1200 g of another portion of the dried powdered cola nut was used for fractionation of the caffeine content, it was soakedin 50% methanol for 75hours, the mixture was filtered and concentrated to $1/10^{th}$ and was allowed to cool and made alkaline using ammonium hydroxide solution. It was partitioned with di-chloromethane, chloroform fraction containing the caffeine was evaporated in vacuum to dryness. 4 grams of

caffeine crystal was obtained. The extractions were carried out at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

3.4 Experimental Animals

Fourty (40) adult Long Evans rats were obtained from the Animal House of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria. The animals were kept and maintained in the laboratory for two weeks to acclimatize prior to the study.

3.5 Acute toxicity study

The LD_{50} was determined using the up and down method (Bruce, 1985). Six rats were dosed up and down. The first two rats were dosed below an already established LD_{50}>2000 mg/kg (Ayebe *et al*., 2012) and were observed for 48 hours, the physical examination showed no any change in the rats Two animals were dosed up by a factor of 1.5 multiplied by the already established $LD_{50.}$ The LD_{50} was found to be greater than 3000mg/kg.

3.6 Experimental Design

Fourty (40) adult Long Evans rats of both sexes, weighing 210-230grams were randomly divided into four groups:

A= control and was administered normal feed (without mix) and 2 ml of distilled water.

B= caffeine fraction group containing three sub groups of four rats each. B1, B2 and B3 were administered with caffeine fraction extract19.2, 38.4 and 57.6mg/kg body weight respectively. The LD_{50} of caffeine was 192mg/kg body weight of rats' oral administration (Erowid.org, 2015).

C= aqueous extract group containing three sub groups of four rats each. C1, C2 and C3 and were administered cola nut aqueous extract orally at the concentrations of 300, 600 and 900 mg/kg body weight respectively

D= cola nut supplement group containing three sub groups of four rats each. D1, D2 and D3 were administered 10%, 20% and 30% w/w cola nut supplemented feed respectively using the method of Utu-Baku *et al.,* (2009). The experiment lasted for twenty-one days.

Table 3.1: Groups, treatment and duration of administration of *Cola nitida* Extracts

GROUPS	N	TREATMENT	DURATION
A	4	Normal feed and distilled water	21 days
B1	4	19.2mg/kg caffeine extract from *Cola nitida*	21 days
B2		38.4mg/kg caffeine extract from *Cola nitida*	21 days
B3	4	57.6mg/kg caffeine extract from *Cola nitida*	21 days
C1		300mg/kg aqueous extract of *Cola nitida*	21 days
C2	4	600mg/kgaqueous extract of C*ola nitida*	21 days
C3	4	900mg/kg aqueous extract of *Cola nitida*	21 days
D1		10% *Cola nitida* supplemented feed w/w	21 days
D2	4	20% *Cola nitida* supplemented feed w/w	21 days
D3	4	30% *Cola nitida* supplemented feed w/w	21 days

N = number of rats per group,

Route of administration = oral

3.7 Neurobehavioral Studies

There are a wide variety of neurobehavioral tests available for laboratory rodents from tests of basic locomotor and sensory function, to analyses of more complex behaviour related to cognition and emotionality.

3.7.1 Spatial memory and learning (Morris water maze).

Morris water maze is a neurobehavioral model used to study spatial memory and learning.

Morris water maze was done according to the method of Liu *et al.* (2011). The rats were placed in a small pool of water which contained an escape platform hidden a few millimetres away and below the water surface. The rats were released and allowed to swim around the pool in search of an exit and subsequent trials were performed to know if the rat will be able to locate the platform in a shorter time. As the rat was learning to locate the hidden platform, the time was measured as latencyand recorded, the procedure was conducted for five days as training. After the training, the rats were administered with *Cola nitida* extracts once a day over a period of three (3) weeks and the above procedure was repeated after every one week. The memory of the rats was evaluated and compared with the time taken in locating the platform before and during administration.



Plate IV: Morris Water Maze. (Personal photograph) July, 2015.

3.7.2 Elevated Plus Maze

The plus maze was made of plywood and consisted of two open arms and two closed arms which extended from a central platform. It was elevated 38cm above the floor.

3.7.2.1. *Elevated plus-maze for anxiety-like behaviour*.

The testing session consists of putting the animal in the apparatus and recording the following behaviours: total time spent in the open arm, total time spent in the closed arm, open arm entries, closed arm entries, stretch attend postures (rearing) and head dip. An increase in open arm activity (duration and/or entries) reflects anti-anxiety behaviour. Anxiety-related behaviour is measured as preference for the closed arms (Rodgers *et al.*, 1995). The maze is cleaned with 70% ethanol after every trial and with 10% bleach at the end of every day. This test cannot be repeated in the same animals with the same results.



Plate V: Elevated plus maze. A. M. Muhammad (Personal photgraph) July, 2015.

3.8 Animal Sacrifice

All animals were humanely sacrificed on the 22ndday of the experiment by using 5mg/kg intravenous thiopental sodium anaesthesia. The range for induction of anaesthesia using thiopental sodium is 3-7mg/kg (Kataria *et al*., 2012). The brain was dissected out and collected, fixed in Bouin's fluid for histological processing using H & E and Toluidine blue special stain.

3.9 Determination of Haematological Indices

Blood sample was collected after dissection by inserting a needle into the heart (cardiac puncture) which was still pumping. It was preserved in EDTA bottlesat 4^{o}c before the analysis. Packed Cell Volume (PCV), total haemoglobin (Hb), Total White Blood Cell (WBC) Count and Differential White Blood Cell Count were determined in the Department of Human Anatomy, Ahmadu Bello University, Zaria, based on the method of Schalm *et al.* (1975).

3.9.1 Packed Cell Volume (PCV) Determination

A capillary tube was filled with blood sample provided in EDTA bottle and then sealed with plastine at one end. It was then centrifuged at 12,000 rmp for five minute using microhematocrit centrifuge. The value read hematocrit reader was expressed in percentage.

3.9.2 Haemoglobin (Hb) Determination

A spencer hematoglobinometre chamber was charge with a drop of blood and the color was matched against the standard color of hematoglobinometre. The corresponding value on the scale in g/dl was read.

3.9.3 White Blood Cell (WBC) Count

The blood sample was pipetted to 0.5 mark inWBC pipette and was mixed with 20µl of diluting factor in a test tube to give 1:20 dilution. WBC were counted manually under microscope using the improved Neuber's hemocytometer and calculated using the formula:

3.9.4 Differential White Blood Cell Count

A thin film of blood was made by gently spreading of a drop of blood on a clean grease free slide and allow to dry. The film was then stained with Leishmann's stain for about 2 minutes buffered; water was added and then left for 8-10 minutes to dry. The slides were rinsed in water and then viewed under (low power magnification) microscope. The result was expressed in percentage.

3.10 Tissue Preparation

The tissue preparation method for the histological analysis was a technique outlined by Culling (1963)andBradbury (1977). The stage of the technique includes; fixation, tissue processing, sectioning, staining, and photomicrography.

3.10.1 Fixation

The cerebral cortex was carefully removed from the skull and fixed in Bouins fluid.

3.10.2 Tissue processing

The trimmed cerebrum was processed with the aid of automatic tissue processor at the Department of Human Anatomy, Ahmadu Bello University, Zaria.

3.10.3 Sectioning

Sections of the processed tissues were cut using rotatory microtome at 8µ.

3.10.4 Staining

Two types of staining techniques were used, which includes H and E (Haematoxylin and Eosin) for general tissue structure and a nuclear stain (toluidine blue).

3.10.5 Photomicrograph

The photomicrographsof the tissue sections were obtained at magnification × 250 using MD900 AmScope digital camera and a light microscope.

3.11 Estimation of Oxidative Stress Parameters

The rats were decapitated under 5 mg/kg mild thiopental sodium anaesthesia according to the method of Kataria *et al,* (2012) and the brain was rapidly excised, weighed and a portion was subsequently homogenized in phosphate buffer4ml/g with an up-and -down strokes in a mortar and pestle. The homogenate was centrifuged at 1500 revolutions for 5 min and the supernatant was collected and used for the test. The test was carried out in the Department of Biochemistry, Ahmadu Bello University, Zaria.

3.11.1 Determination of Catalase activity (CAT)

Catalase activity was determined spectrophotometer at 570 nm using the method described by Sinha (1972). 5 % Potassium heptaoxochromate (VI), $K_2Cr_2O_7$: 5 g of $K_2Cr_2O_7$ was dissolves in little quantity of distilled water and made up to 100 ml. 0.2 M H_20_2: 0.6 ml of H_20_2 was dissolved in little quantity of distilledwater and made up to

100 ml. it was stored at 4°C. 0.0lM phosphate buffer: 1.2g of NaH_2P0_4 and 1.4lg of $Na_2HPO_4$was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 10.2. Diebroinate/Acetic acid solution 5 % Potassium heptaoxochromate (VI) $K_2Cr_2O_7$ was mixed with glacial acetic acid in the ratio 1:3, and was stored in brown bottle at room temperature.0.9 ml or distilled water was added to 0.1 ml of microsome and mixed thoroughly. 2.5 ml of phosphate buffer was put in a small conical flask; 0.5 ml of microsome was added; and 2.0 ml of H_20_2 added, starting the stop watch. The reaction mixture will be thoroughly mixed and the reaction will be stopped after every 60 seconds for 3 minutes with Dichroniate/Acetic acid solution. It was heated in a water bath for 10 minutes at 80°C. Absorbance was read at 570 nm.

3.11.2 Determination of Superoxide Dismutase (SOD)

Superoxide Dismutase (SOD) activity was determined spectrophotometer by a method described by Fridovich (1989). The ability of superoxide dismutase (SOD) to inhibit auto oxidation of adrenaline at pH 10.2 formed the basses of this assay. 0.05 M phosphate buffer: 6.97 g of diphosphate K_2HPO_4 and 1.36 g of KH_2PO_4 was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 7.8. 0.05 Carbonate buffer: 14.3g of Na_2CO_3 and 4.2g of $NaHC0_3$ was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 10.2. 0.3 mM Adrenaline: 0.01 g of adrenaline was dissolved in 17 ml of distilled water. The solution was prepared fresh. 0.1 ml of microsome was diluted in 0.9 ml of distilled water to make 1:10 dilution of microsome. An aliquant mixture of 0.20 ml of the diluted microsome was added to 2.5 ml of 0.05 M Carbonate buffer. The reaction started with the addition of 0.3m1 of 0.3 mM adrenaline. The

reference mixture contains 2.5 ml of 0.05 M Carbonate buffer. 0.3rnl of 0.3 mM adrenaline and 0.20 ml of distilled water. Absorbance was measured ever 30 up to 150 s at 48Onm. 1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

3.11.3 Assessment of Lipid Peroxidation

Lipid peroxidation as evidenced by the formation of TBARS was measured by the modified method of Niehaus and Samuelson (1968). 150µl of serum, (O.25M sucrose solution) were treated with 2m1 of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.37%, 0.25N HCL and 15% TBA) and place in water bath for l hour at 90°C. The mixture was cooled and centrifuged at 3000rpm for 5mm at 4°C. The absorbance of the pink supernatant 2.0ml was measured against a reference blank using spectrophotometer at 535nm.

3.11.4 Assay of Reduced glutathione (GSH)

Reduced glutathione (GSH) concentration measurements were done according to ElIman (1959) as described by Rajagopalan *et al*. (2004). 0.2M phosphate buffer: 8.40g of NaH2PO4 and 9.94 of Na2HPO4 was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 8. To 150µ1 of serum or tissue homogenate (in phosphate -saline PH 7.4), 1.5m1 of 10% TCA was added and centrifuged at 1 500g for 5mm. 1 ml of supernatant was treated with 0.5 ml of ElIman's reagent (19.8 mg of 5, 5'-clithiobis (nitro benzoic acid) (DNTB) in 100 ml of 0.1% sodium nitrate) and 3 ml of phosphate buffer (0.2 M, p1-I 8). The absorbance was read at 412 nm.

3.12 Statistical Analysis

All results obtained were analysed using Statistical Package for Social Scientist (SPSS version 20.0) and the results were expressed as mean ± SEM. Differences among means of the groups were determined using One-way analysis of variance (ANOVA) with Turkey Post Hoc test. Values were considered significant at p 0.05. Tables were produced using Microsoftexcel 2013.

CHAPTER FOUR

4.0 Result

4.1 Acute Toxicity:

The oral administration of *Cola nitida* at the dose of 3000 mg/kg did not exhibit death and any signs of toxicity up to 48hours. The acute toxicity test (LD_{50}) demonstrated that *Cola nitida* extract was not lethal up to a dose of 3000 mg/kg. Thus, the LD_{50} is 3000 mg/kg.

4.2 Physical Observation

Observation of the animals showed increased physical activities such as stretch attend and increased movement in the caffeine extract groups, the aqueous extract groupalso had increased activity but the animals were not as active as those in the caffeine treated group. No obvious physical changes were noticed in the control and the crude cola nut supplement groups.

4.3 Body Weight of the Animals

The results of body weight showed no significant increase ($p \square 0.05$) in weight of animals in the aqueous extract and caffeine fraction groups (B and C groups) when compared with control group, there was no significant decrease ($p \square 0.05$) in weight of the crude administered group (D) as shown in Table 4.1.

Table 4.1: Weight of animals in grams before and after administration of *Cola nitida*.

Groups	**Weight before administration Mean ± SEM**	**Weight after administration Mean ± SEM**
A	221.25 ± 21.69	239.25 ± 20.27
B1	221.75 ± 35.26	231.50 ± 35.80
B2	222.50 ± 22.37	230.75 ± 22.61
B3	222.25 ± 19.93	240.50 ± 20.67
C1	222.25 ± 28.22	230.00 ± 28.60
C2	221.50 ± 22.70	222.25 ± 22.74
C3	221.50 ± 22.69	223.00 ± 21.88
D1	221.25 ± 21.43	203.75 ± 8.28
D2	221.75 ± 18.95	197.00 ± 23.72
D3	222.25 ± 19.06	200.75 ± 20.67

A= Control, B (B1-B3)= Caffeine fraction group, C (C1-C3)= Aqueous extract group, D (D1-D3)= Cola nut supplement group, n= 4.

4.4 Morris water maze test

The results of spatial learning and memory using Morris water maze test showed that there was a decrease in the meantime taken for the animals to complete Morris water maze task in all the groups, the result was only significant ($p < 0.05$) in the group administered 600mg aqueous extract group at week two (2) when compared with the control as shown in Table 4.2. The results showed that animals in control group had an increased latency time (A ,3.23 ± 0.70) in Morris water maze activity at week 3 and other groups had non- significant decreased time($p > 0.05$)(B1 1.82 ± 0.19, B2 1.72 ± 0.49, B3 2.11 ± 0.21, C1 1.74 ± 0.31, C2 1.87 ± 0.24, C3 1.98 ± 0.40, D1 1.82 ± 0.33, D2 1.94 ± 0.26, D3 1.62 ± 0.15).

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Table 4.2:Mean latencies of rats administered*Cola nitida*in Morris water maze test.

Groups	Treatment	Training	Week 1	Week 2	Week 3
		Mean ±SEM(s)	**Mean ±SEM(s)**	**Mean ±SEM(s)**	**Mean ±SEM(s)**
A	2 ml distilled water	1.94 ± 0.08	2.87 ± 0.71	1.59 ± 1.13	3.23 ± 0.70
B1	19.2 mg/kg caffeine extract from *Cola nitida*.	3.51 ± 0.66	3.15 ± 0.76	2.01 ± 1.36	1.82 ± 0.19
B2	38.4mg/kg caffeine extract from *Cola nitida*.	2.77 ± 1.21	1.41 ± 0.05	1.65 ± 0.98	1.72 ± 0.49
B3	57.6mg/kg caffeine extract from *Cola nitida*.	3.07 ± 0.83	2.65 ± 0.36	2.15 ± 1.36	2.11 ± 0.21
C1	300mg/kg aqueous extract of *Cola nitida* .	5.84 ± 1.83	1.73 ± 0.26	2.23 ± 1.52	1.74 ± 0.31
C2	600mg/kg aqueous extract of *Cola nitida.*	3.45 ± 0.86	2.95 ± 0.46	3.30 ± 2.04*	1.87 ± 0.24
C3	900mg/kg aqueous extract of *Cola nitida.*	2.53 ± 0.40	3.74 ± 1.90	3.29 ± 1.20	1.98 ± 0.40
D1	10% Cola nut supplemented feed.	2.14 ± 0.38	2.54 ± 0.45	1.92 ± 1.04	1.82 ± 0.33
D2	20% Cola nut supplemented feed.	2.99 ± 0.31	1.66 ± 0.16	2.18 ± -0.01	1.94 ± 0.26
D3	30% Cola nut supplemented feed	2.70 ± 0.59	1.85 ± 0.27	1.18 ± 1.30	1.62 ± 0.15

*p <0.05 indicates significant difference compared to control SEM: Standard Error of Mean

s = mean time in seconds n = 4

A= Control.B(B1-B3) = Caffeine fraction group. C(C-C3)= Aqueous extract group D(D1-D3) = Cola nut supplement group.

4.5 Elevated plus maze

The result of the effect of extracts of *Cola nitida* on anxiety-like behaviours in elevated plus maze showed no significant alteration or change ($p \square 0.05$) in all the parameters observed when compared with the control. There was a non -significant increase in the meantime spent in the open arm, increased number of rearing and head dip as the dose of the caffeine and aqueous extract groups increases (Table 4.3)

Table 4.2:Anxiety-like behaviours of rats administered*Cola nitida*.

Groups	TREATMENT	EOA	ECA	TOA (s) /5min Mean ±SEM	TCA (s) /5min Mean ±SEM	Rearing	HD
A	2 ml distilled water	2.67 ± 0.67	5.25 ± 0.63	99.88 ± 44.18	190.31 ± 33.32	9.00 ± 1.78	3.50 ± 0.86
B1	19.2 mg/kg caffeine extract from *Cola nitida*.	3.25 ± 1.44	3.00 ± 0.58	91.90 ± 45.41	208.32 ± 45.38	10.25 ± 0.75	4.75 ± 2.75
B2	38.4mg/kg caffeine extract from *Cola nitida*.	1.25 ± 0.48	2.00 ± 0.71	44.60 ± 34.57	258.58 ± 28.37	7.75 ± 1.70	2.00 ± 0.00
B3	57.6mg/kg caffeine extract from *Cola nitida*.	3.75 ± 1.93	3.50 ± 2.02	142.46 ± 46.94	157.51 ± 46.94	10.50 ± 2.66	6.50 ± 1.85
C1	300mg/kg aqueous extract of*Cola nitida* .	3.00 ± 1.22	5.50 ± 0.29	65.96 ± 8.23	249.54 ± 17.59	10.75 ± 1.80	3.75 ± 0.75
C2	600mg/kg aqueous extract of*Cola nitida.*	5.25 ± 1.80	6.75 ± 2.32	85.44 ± 26.98	214.46 ± 26.97	12.25 ± 2.75	4.50 ± 1.32
C3	900mg/kg aqueous extract of*Cola nitida.*	6.00 ± 2.52	7.00 ± 2.00	167.13 ± 24.73	126.18 ± 25.04	16.33 ± 3.67	8.00 ± 2.51
D1	10% Cola nut supplemented feed.	2.00 ± 0.71	4.67 ± 0.67	137.08 ± 56.87	183.55 ± 33.61	8.00 ± 2.55	5.00 ± 0.58
D2	20% Cola nut supplemented feed.	1.67 ± 0.67	3.50 ± 0.96	100.06 ± 61.39	212.51 ± 50.80	6.00 ± 1.58	5.00 ± 1.41
D3	30% Cola nut supplemented feed.	1.50 ± 0.50	3.50 ± 0.65	46.30 ± 37.56	274.10 ± 19.42	11.7±1.43	4.33 ± 1.45

SEM: Standard Error of Mean s = mean time in seconds

A= Control, B= Caffeine fraction group, C= Aqueous extract group, C= Cola nut supplement group. EOA: Entry in to open arm, ECA: Entry in to close arm, TOA: Time spent in open arm, TCA: Time spent in close arm, HD: Head dip.

4.6 Haematological indices

The haematological assessment revealed that there was no statistically significant increase($p \square 0.05$) in the packed cell volume (PCV) for the caffeine fraction, aqueous extract and supplement groups when compared with the control. Slight increase in white blood cell (WBC) count was observed in all the caffeine fraction groups and 10% Cola nut supplement groups, haemoglobin (Hb) and eosinophilcount were not significantly increased when compared with the control. There was significant increase($p \square 0.05$) in lymphocytes in the groups treated with 20 and 30% of caffeine fraction and significant decrease ($p \square 0.05$) in neutrophils in the 20 and 30% of the aqueous extract groups when compared with the control.(Table 4.4).

GROUPS	TREATMENT	PCV%	Hb g/dl	WBCx10^9/L	NEU%	LYM%	EOS%
A	Normal saline	39.00 ± 9.00	11.50 ± 2.70	7.05 ± 1.05	39.50 ± 2.50	59.00 ± 1.00	1.50 ± 0.50
B1	19.2 mg/kg caffeine extract from *Cola nitida.*	47.50 ± 1.50	14.05 ± 0.25	9.95 ± 1.35	29.50 ± 0.50	69.00 ± 1.00	1.50 ± 0.50
B2	38.4mg/kg caffeine extract from *Cola nitida.*	48.00 ± 5.00	13.85 ± 0.85	9.80 ± 0.40	20.50 ± 1.50*	77.00 ± 1.00*	2.50 ± 0.50
B3	57.6mg/kg caffeine extract from *Cola nitida.*	48.50 ± 5.50	13.75 ± 0.95	10.05 ± 2.05	19.50 ± 0.50*	77.50 ± 0.50*	2.50 ± 0.50
C1	300mg/kg aqueous extract of *Cola nitida* .	31.00 ± 1.00	8.95 ± 0.50	6.90 ± 0.40	28.00 ± 5.00	72.00 ± 5.00	2.00 ± 0.00
C2	600mg/kg aqueous extract of Cola nitida.	43.50 ± 1.50	13.00 ± 0.40	6.65 ± 0.45	18.00 ± 2.00*	79.00 ± 2.00*	2.00 ± 0.00
C3	900mg/kg aqueous extract of *Cola nitida*.	39.50 ± 0.50	11.45 ± 0.35	6.70 ± 0.60	17.00 ± 3.00*	81.50 ± 1.50*	2.00 ± 0.00
D1	10% Cola nut supplemented feed.	44.00 ± 2.00	12.95 ± 0.45	8.10 ± 0.20	29.50 ± 1.50	68.00 ± 2.00	2.00 ± 1.00
D2	20% Cola nut supplemented feed.	49.00 ± 1.00	14.35 ± 0.05	7.10 ± 0.30	32.50 ± 2.50	66.00 ± 2.00	1.50 ± 0.50
D3	30% Cola nut supplemented feed.	47.00 ± I.00	13.8 ± 50.15	7.15 ± 1.05	28.00 ± 6.00	70.50 ± 4.50	2.00 ± 0.00

Table 4.4: Haematological indices of rats administered*Cola nitida*.

*P<0.05 indicates significant difference compared to control, SEM= Standard Error of Mean, A= Control, B= Caffeine fraction group, C= Aqueous extract group) CG= cola nut supplement group, PCV= Packed Cell Volume in percentage, WBC= white blood cells, Hb= Haemoglobin g/dl,

NEU= neutrophils, LYM= lymphocytes, EOS= eosinophils.

4.7 Oxidative stress

The result of glutathione concentration (GSH) showed a statistical significant increase ($p < 0.05$) only in the 10% aqueous extract, non- significant increase was observed in all other treated groups when compared with the control. There was no statistical significant increase in all the treated groups for catalase (CAT) activity when compared with the control. Malondialdehyde (MDA) concentration result showed non-statistical significant decrease in both caffeine fraction and aqueous extract 20% groups and a non-significant increase ($p > 0.05$) in all the other treatment groups when compared with the control. The result of superoxide dismutase (SOD)activity showed a statistical significant decrease ($p < 0.05$) in the caffeine and aqueous extract groups and a non-significant decrease($p > 0.05$)was observed in the crude extract groups (Table 4.5).

Table 4.4: Oxidative Stress Parameters of rats administered*Cola nitida*.

GROUPS	**TREATMENT**	**GSH (µg/ml)**	**CAT (U/ml)**	**MDA (nmol/ml)**	**SOD (U/ml)**
A	2 ml distilled water	44.15 ± 0.85	6.0 ± 1.70	158.60 ± 36.80	11.11 ± 0.89
B1	19.2 mg/kg caffeine extract from *Cola nitida*.	55.85 ± 4.15	6.90 ± 1.40	272.50 ± 47.20	8.00 ± 1.33
B2	38.4mg/kg caffeine extract from *Cola nitida*.	46.65 ± 1.65	8.65 ± 1.75	101.45 ± 15.45	6.23 ± 0.45*
B3	57.6mg/kg caffeine extract from *Cola nitida*.	57.50 ± 4.20	8.25 ± 0.75	311.00 ± 3.60	7.11 ± 1.33*
C1	300mg/kg aqueous extract of *Cola nitida* .	73.30 ± 10.0*	6.35 ± 1.15	271.30 ± 168.20	6.23 ± 0.45*
C2	600mg/kg aqueous extract of *Cola nitida.*	57.50 ± 2.50	11.35 ± 3.85	135.15 ± 7.85	5.78 ± 0.00*
C3	900mg/kg aqueous extract of *Cola nitida.*	39.15 ± 0.85	11.30 ± 3.00	408.45 ± 48.25	6.79 ± 0.11*
D1	10% cola nut supplemented feed.	43.35 ±0.00	6.70 ± 0.80	187.75 ± 111.65	10.22 ± 0.00
D2	20% cola nut supplemented feed.	43.35 ± 6.65	9.00 ± 2.90	179.40 ± 18.50	10.22 ± 0.00
D3	30% cola nut supplemented feed.	48.35 ± 1.65	9.80 ± 1.90	216.70 ± 59.10	9.78 ± 0.45

*P<0.05 indicates significant difference compared to control SEM= Standard Error of Mean

A= Control, B= Caffeine fraction group, C= Aqueous extract group, D= cola nut supplement group, GSH= Glutathione concentration µg/ml microgram per mil CAT= Catalase, MDA= Malondialdehyde, SOD= Superoxide Dismutase, U= international unit.

4.8Histological Studies

The results of histologicalstudies showed histological changes in the tissues studied. Animals in control (distilled water)group had normal histology of the cerebral cortex with normal Pyramidal cells and Stellate cell(Plate VII), while animals administered aqueous extracts of *Cola nitida* had some normal cells and vacuolation (Plate VIII), clumped cells and some normal pyramidal cells (Plate IX), neuronal degeneration and pyknosis (Plate X) in their cerebral cortices. The animals that were administered crude cola nut had vacuolation and some normal stellate cells (Plate XI)vacuolation and pyknosis (Plates XII),clumped cells, vacuolation and pyknosis (Plate XIII) in their cerebral cortices, while animals given caffeine fraction of *Cola nitida*had some normal pyramidal cells and vacuolation (Plate XIV), pyknosis and clumped cells (Plate XV), neuronal degeneration, clumpedcells and vacuolation (Plate XVI) in their cerebral cortices.

The histological studies of the hippocampus showed that animals in the control group had normal appearance of the hippocampus with normal pyramidal cells (Plate XVIII), while animals administered aqueous extract had some normal and clumped pyramidal cells (Plates XIX and XX) and clumped degenerating pyramidal cells (Plate XXI) in the hippocampus. The animals given crude*Cola nitida* had clumped pyramidal cell (Plates XXII and XXIII), clumped degenerated pyramidal cells (Plate XXIV), while animals administered caffeine fraction of *Cola nitida*groups had clumped pyramidal cells (Plates XXV and XXVI),clumped degenerating pyramidal cells (Plate XXVII).

The results from toluidine blue stain showed histological changes in the tissues studied. Animals in control group have normal histology of the cerebral cortex with normal pyramidal and stellate cells with normal stain uptake (Plate XXVIII), some normal

stellate and pyramidal cells, and clumped cells (PlatesXXIX, XXX,XXXI andXXXII), with less stain uptake (XXXIII)some normal stellate and pyramidal cells, and vacuolation (Plate XXXIV), normal pyramidal cells andclumped cells (Plate XXXV),clumped cells (Plate XXXVI), normal stellate cells and degenerating neuron(Plate XXXVII).

The histological studies of the hippocampus using toluidine blue special stain showed that animals in the control group hadnormal pyramidal cells (Plate XXXVIII),clumped degenerating pyramidal cells (Plates XXXIX and XL), degenerating pyramidal cell (Plates XLI) clumped pyramidal cell(XLII and XLIII),clumped degenerating pyramidal cell (Plate XLIV), clumped pyramidal cell (Plates XLV and XLVI), clumped degenerating pyramidal cell (Plate XLVII,).

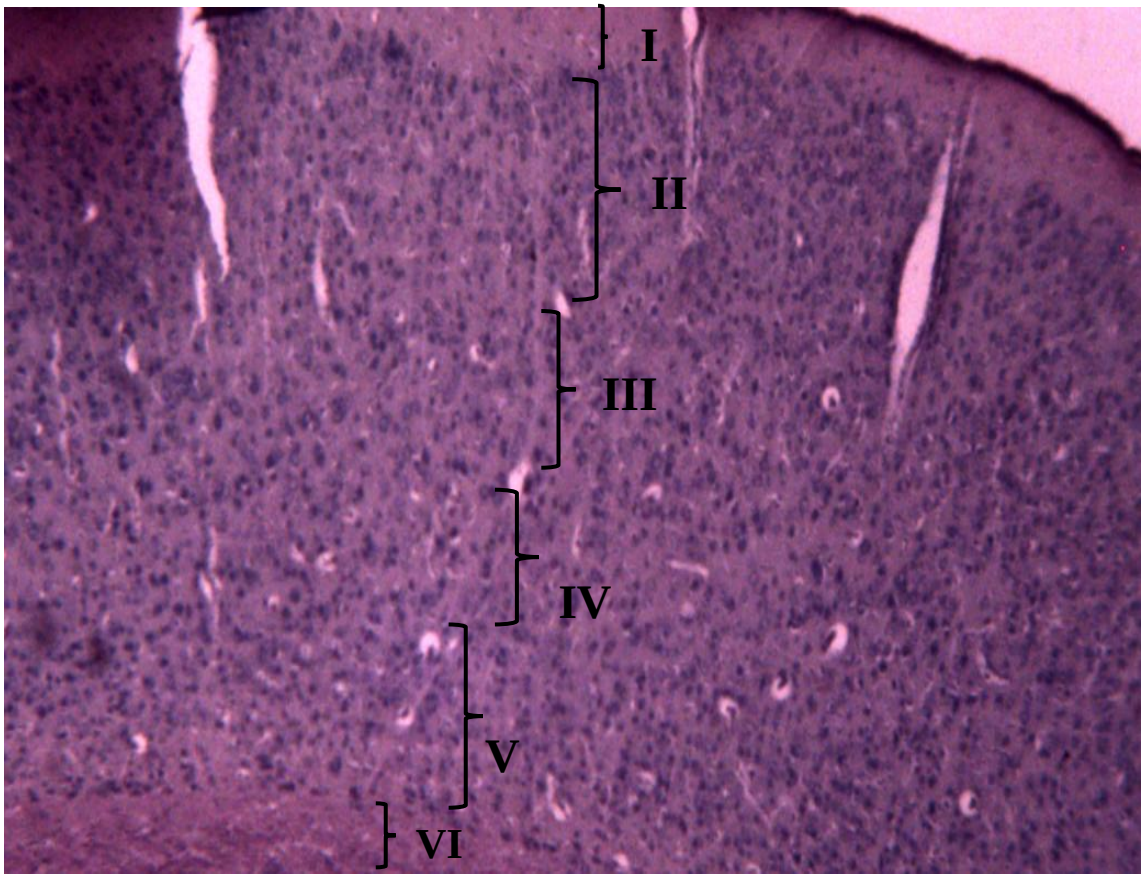


Plate VI: Section of cerebral cortex of rat showing the cell layers from Molecular (I), Outer granular (II), Outer pyramidal (III), Internal granular (IV), Internal pyramidal (V) and Multiform(VI). (H and E × 40)

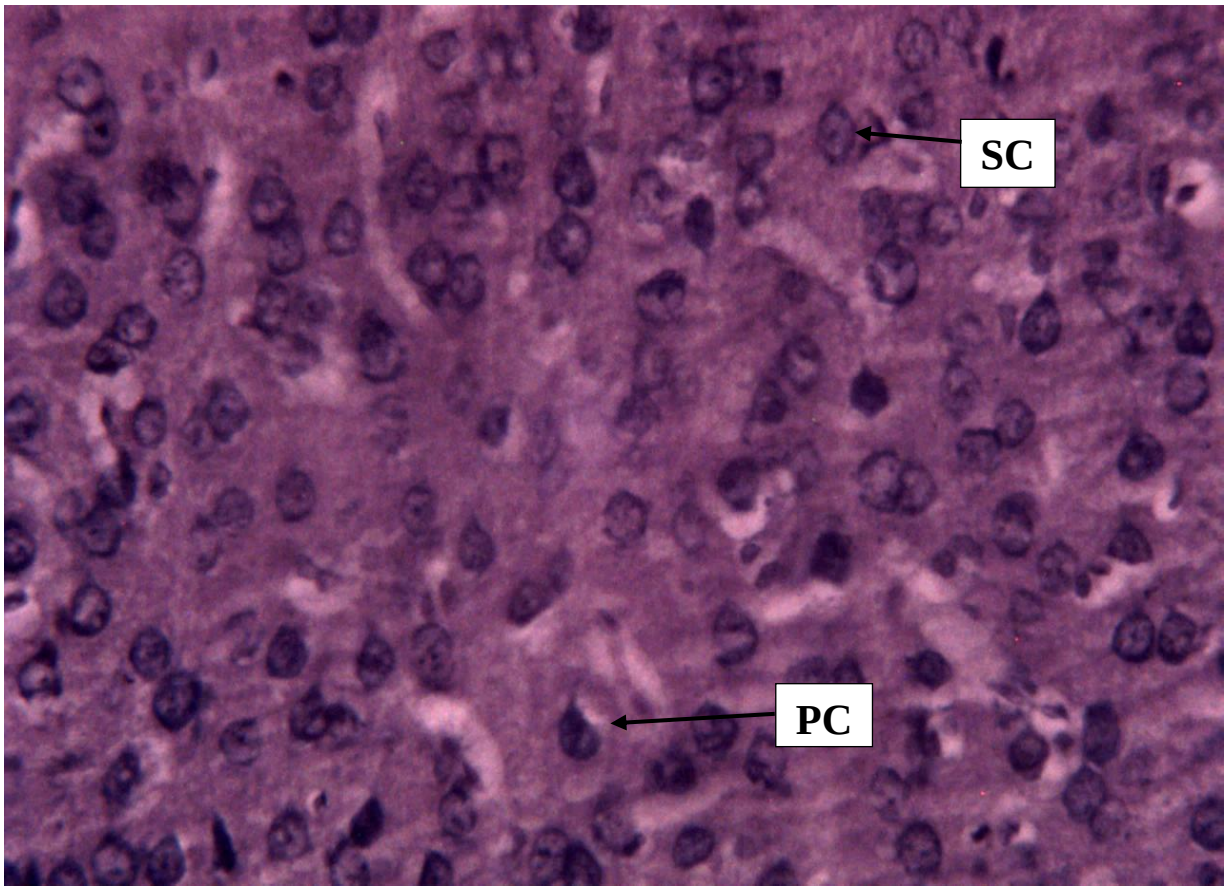


Plate VII: Section of cerebral cortex (layer II and III) of rat from control group showing; Normal Stellate cells (SC) and Pyramidal cells (PC), (H and E × 250).

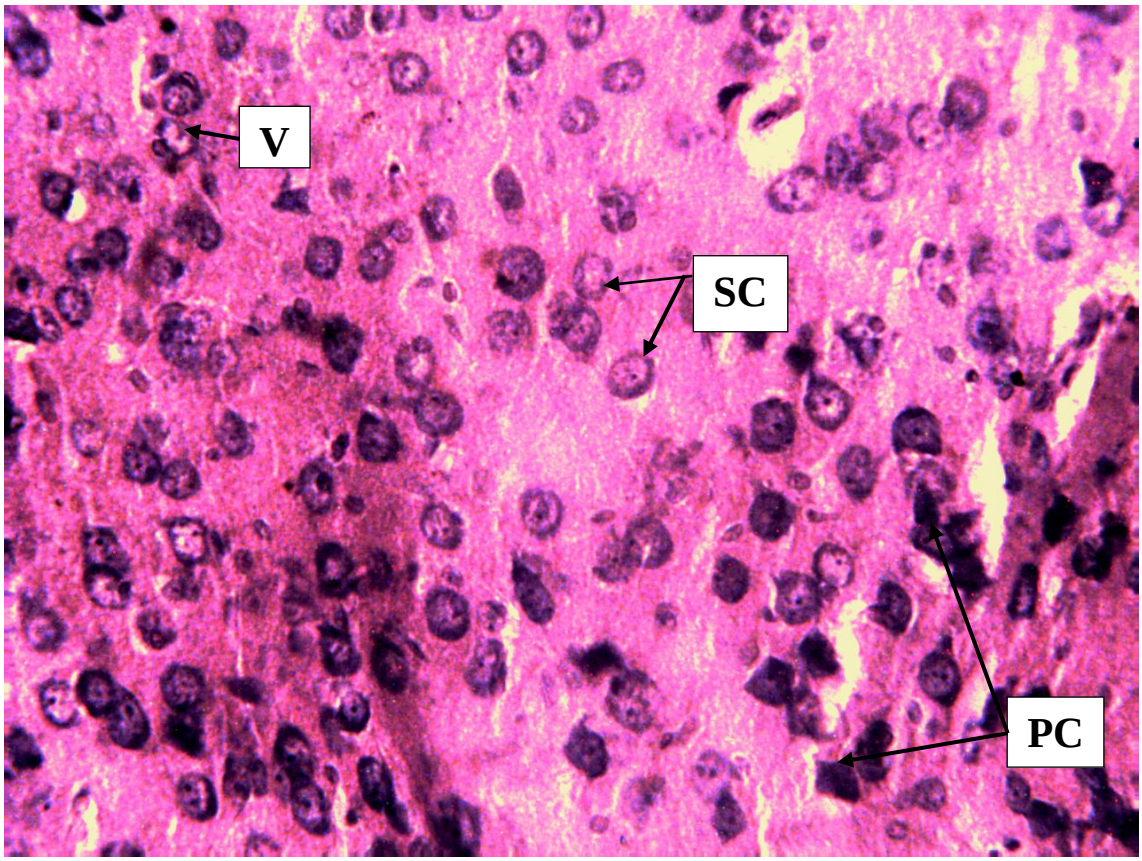


Plate VIII: Section ofcerebral cortex (layer II and III) of rat treated with 300mg/kg aqueous extract of *Colanitida*showing;Vacuolation(V), Some normalStellate cells (SC) and Pyramidal cells (PC), (H and E × 250).

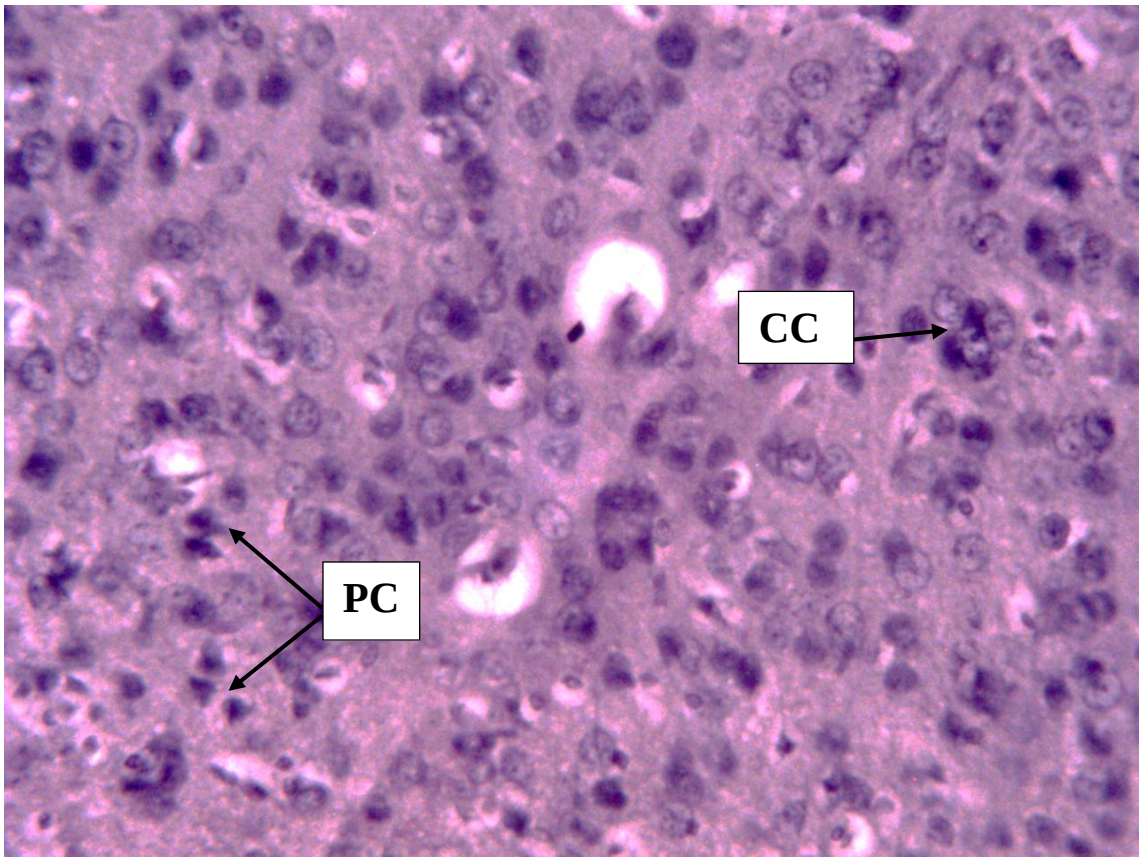
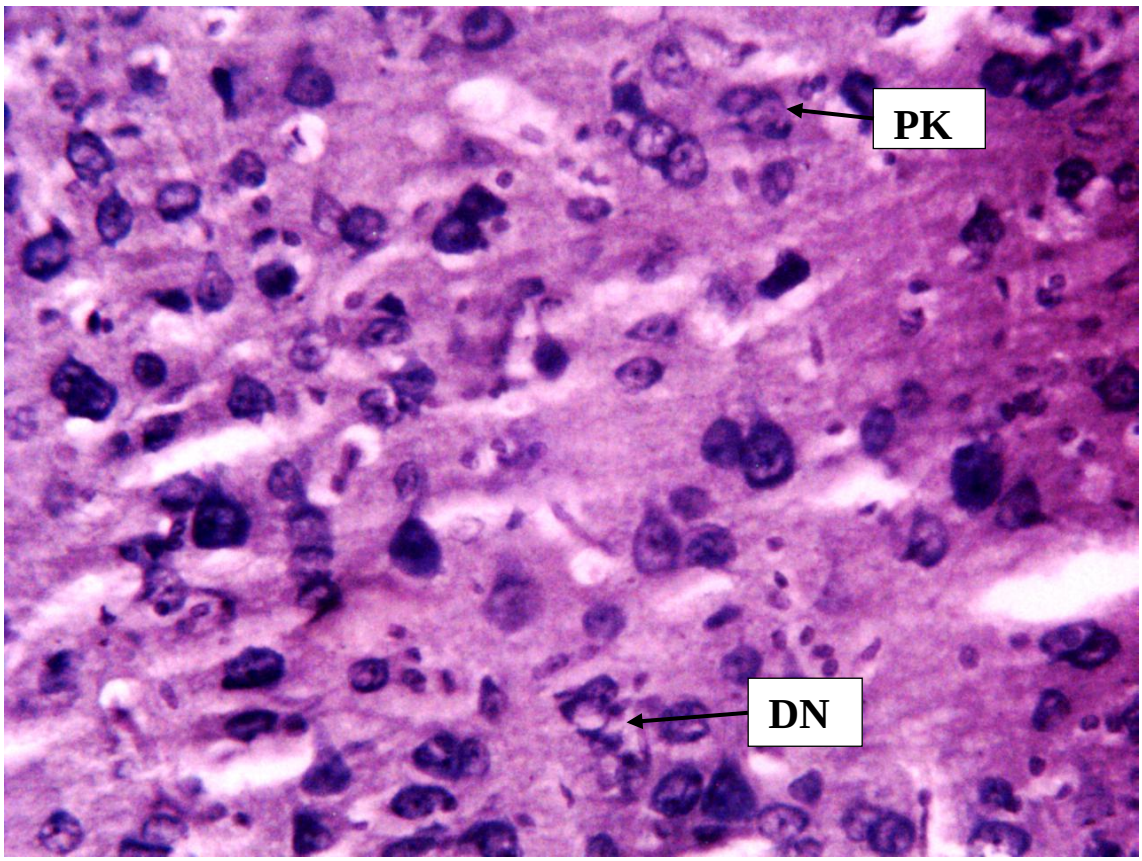


Plate IX: Section ofcerebral cortex(layer II and III) of rat treated with 600mg/kg aqueous extract of *Colanitida* showing; Pyramidal cells (PC),Clumped cells (CC) (H and E × 250).



PlateX: Section ofcerebral cortex (layer II and III)of rat treated with 900mg/kg aqueous extract of *Colanitida* showing; Degenerating neuron(DN), Pyknosis (PK) (H and E × 250).

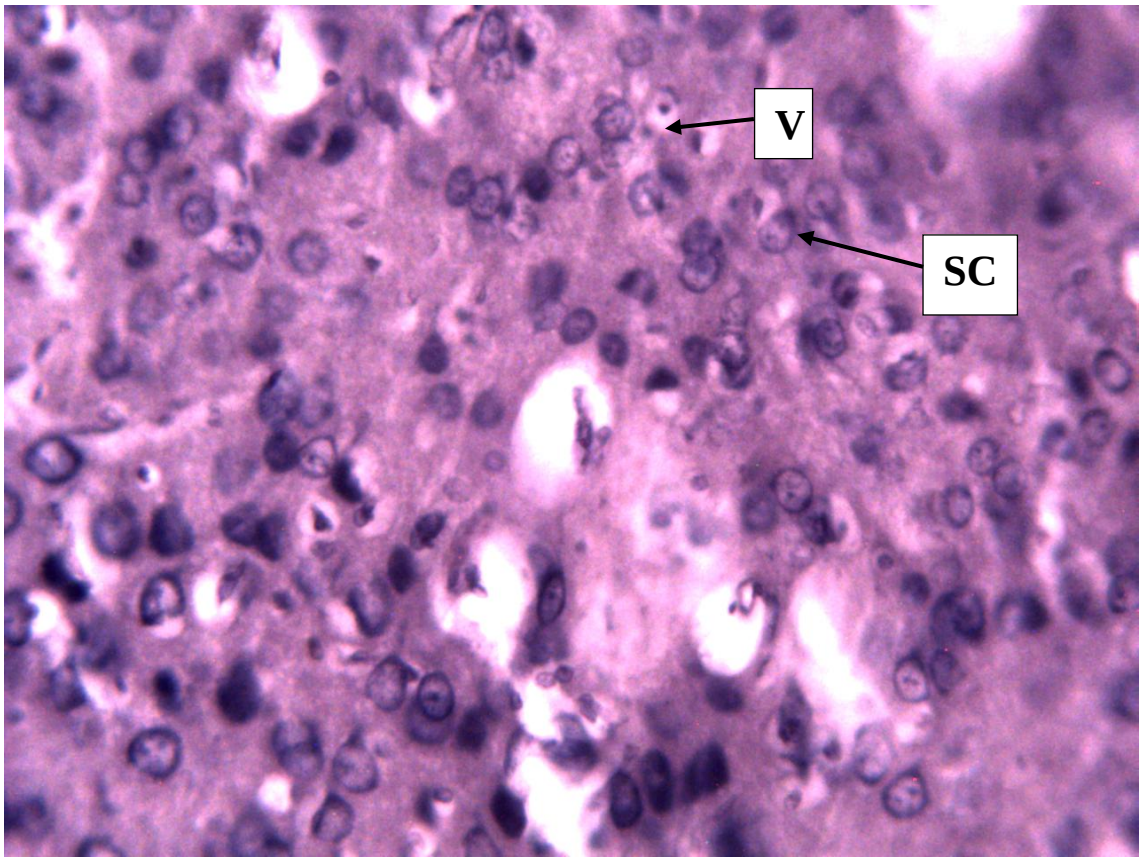


Plate XI: Section of cerebral cortex (layer II and III)of rat supplemented with10% *Cola nitida*showing; Stellate cell (SC), Vacuolation (V), (H and E ×250).

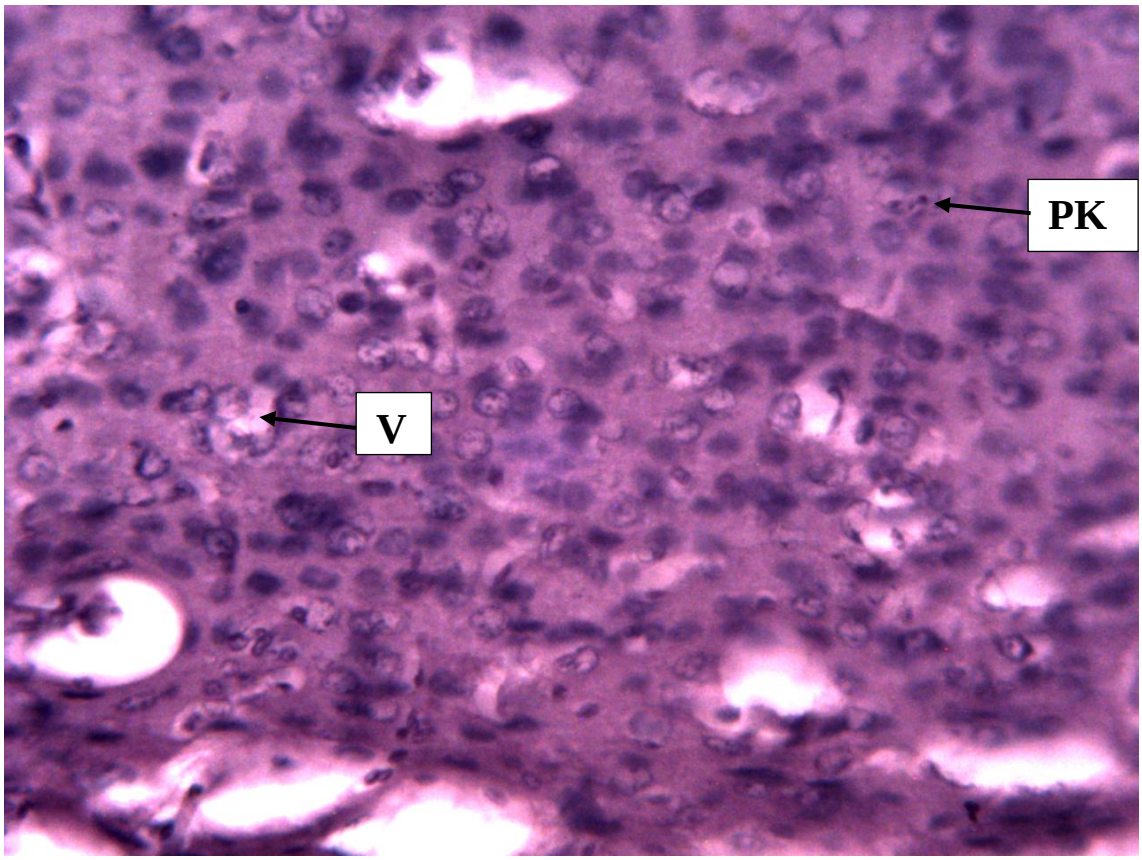


Plate XII: Section of cerebral cortex (layer II and III) of rat supplemented 20% *Cola nitida* showing;Vacuolation (V), Pyknosis (PK). (H and E × 250).

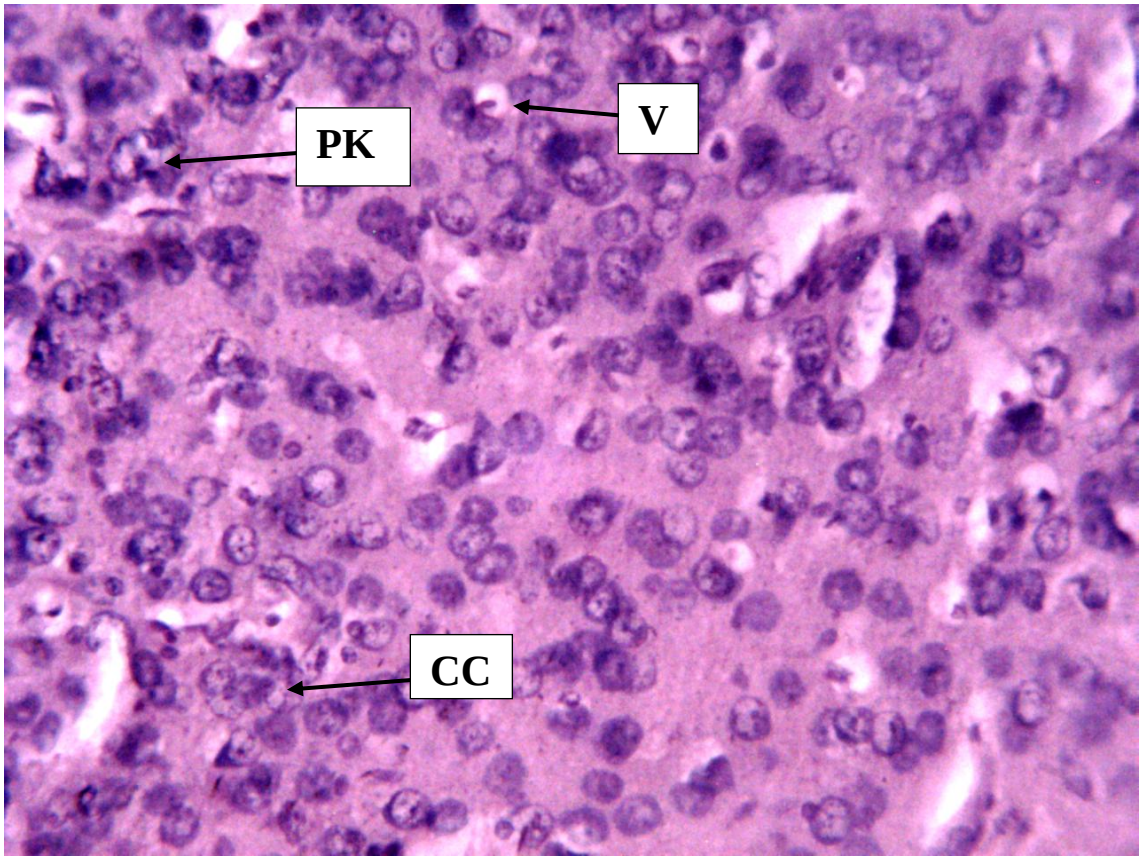


Plate XIII: Section of cerebral cortex (layer II and III) of rat feed supplementedwith 30% *Cola nitida* showing; Vacuolation (V), Clumpedcells (CC), Pyknosis (PK) (H and E × 250).

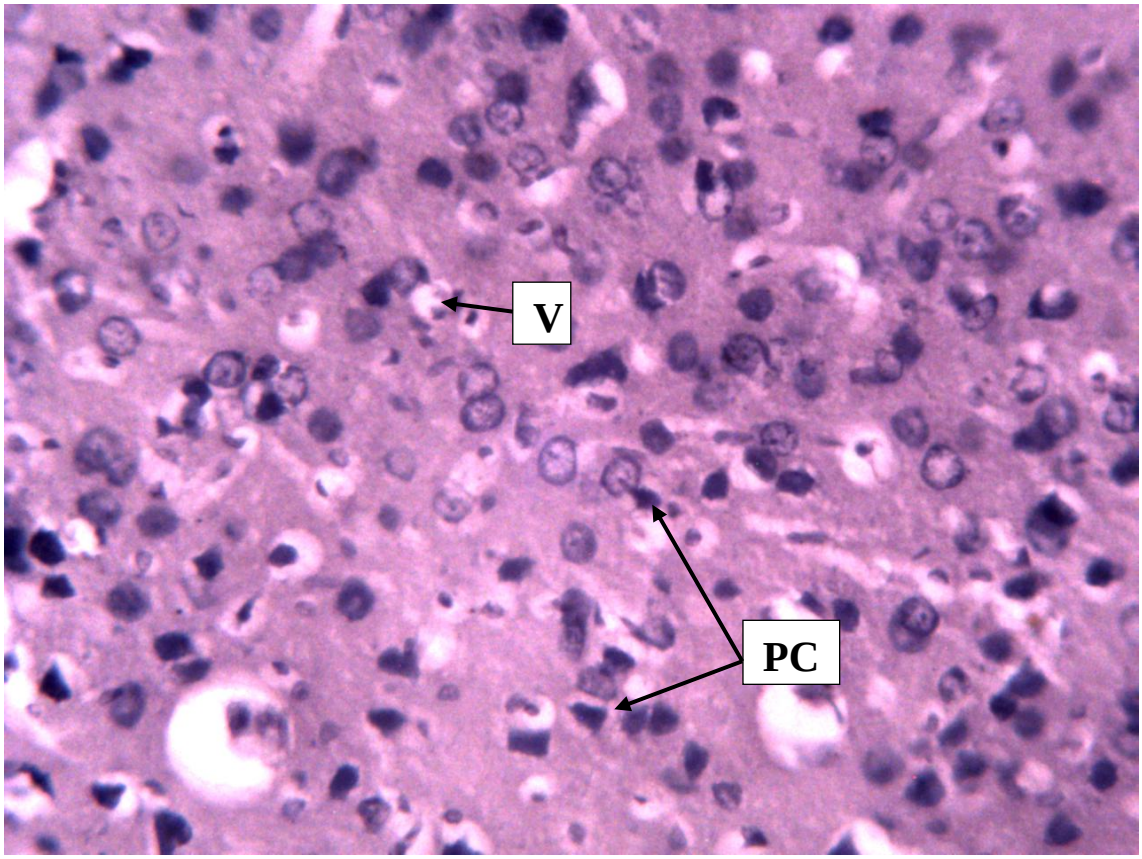


Plate XIV: Section of cerebral cortex (layer II and III)of rat administered 19.2mg/kg caffeine extract showing; Vacuolation (V), pyramidal cells (PC).(H and E × 250).

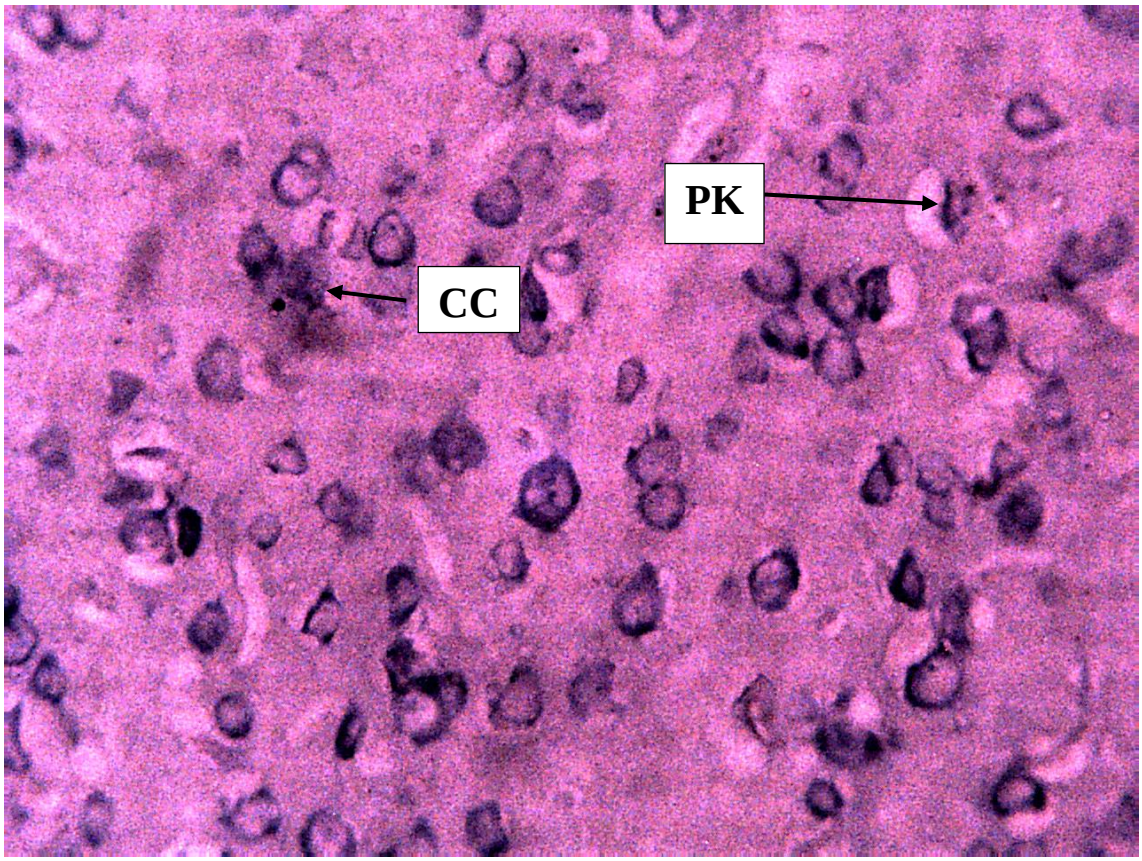


Plate XV: Section of cerebral cortex (layer II and III) of rat administered38.4mg/kg caffeine extractshowing; Clumped cells (CC), Pyknosis (PK) (H and E × 250).

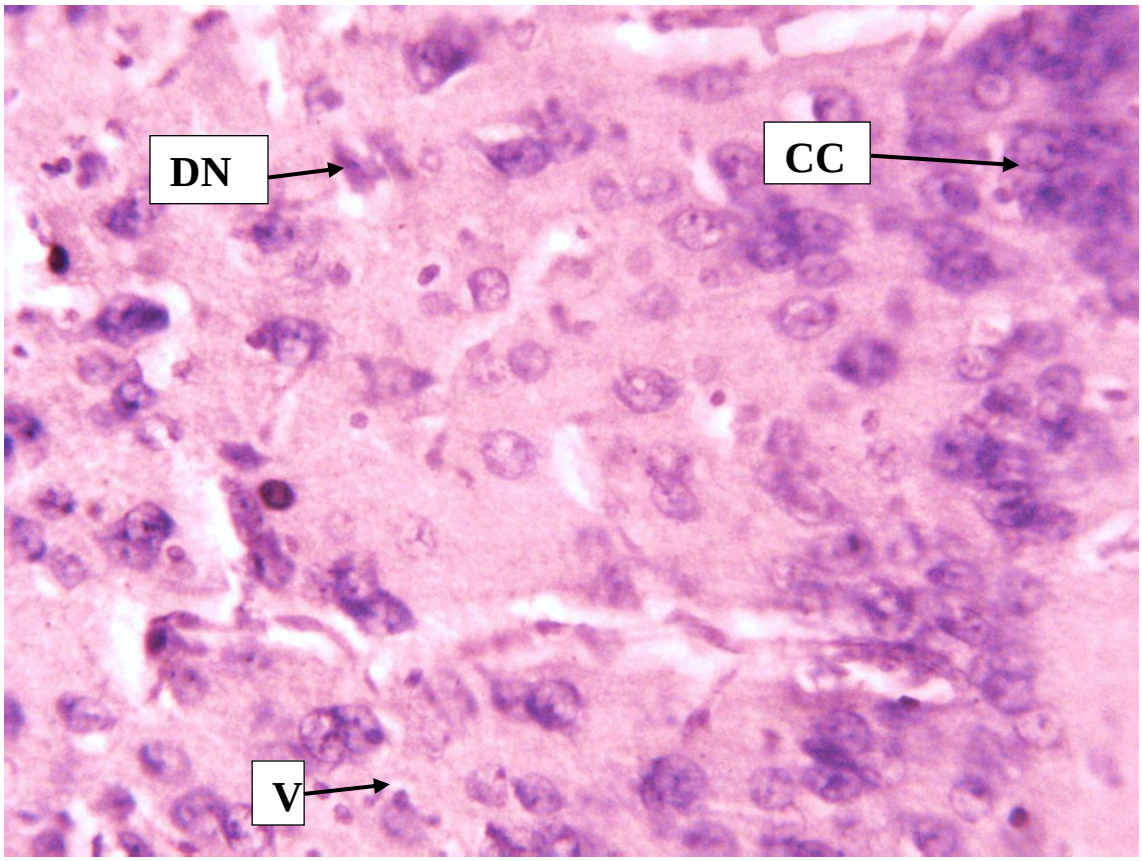


Plate XVI: Section of cerebral cortex (layer II and III)of rat administered57.6mg/kg caffeine extractshowing; Degenerating neuron (DN), Vacuolation (V),Clumped Cells (CC)(H and E × 250).

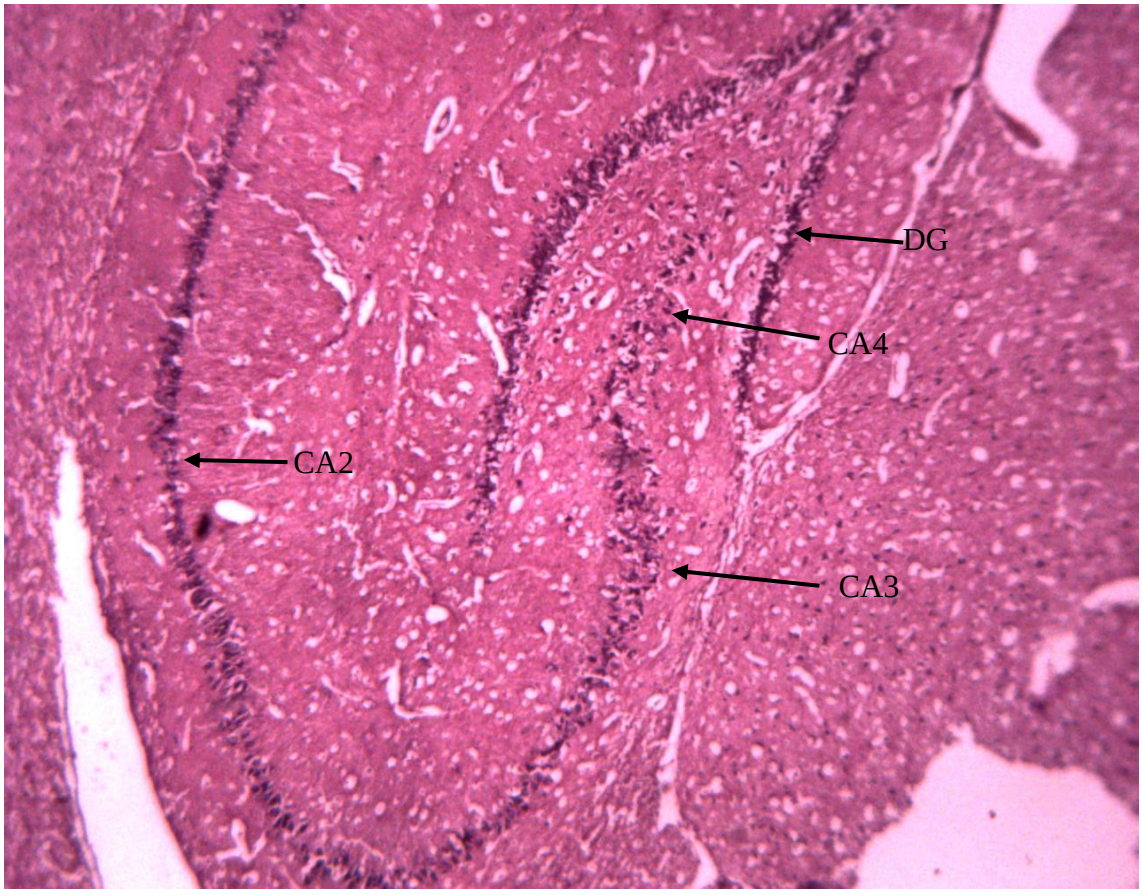


Plate XVII: Section of hippocampus of rat showing CA2, CA3, CA4 regions and the dentate gyrus. (H and E × 40)

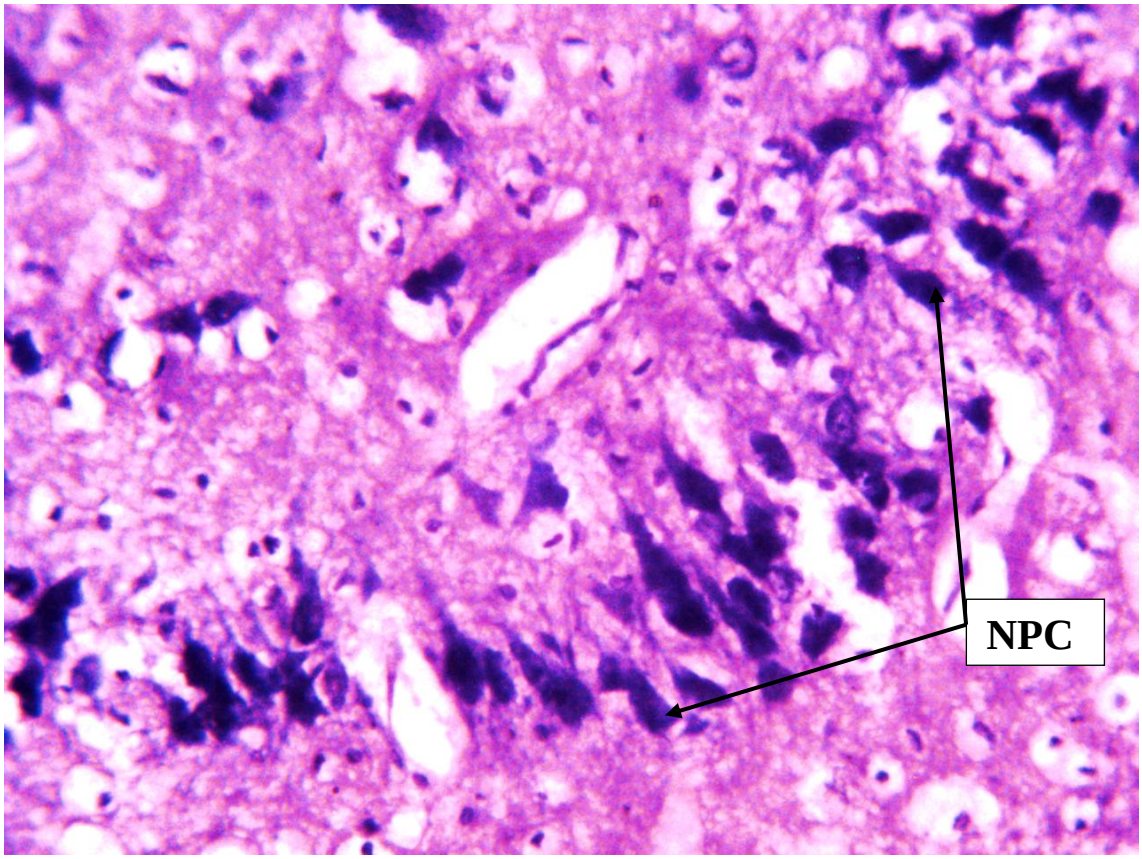


Plate XVIII: Section of hippocampus (CA3 region) of rat from control group showing; Normal pyramidal cells (NPC). (H and E × 250).

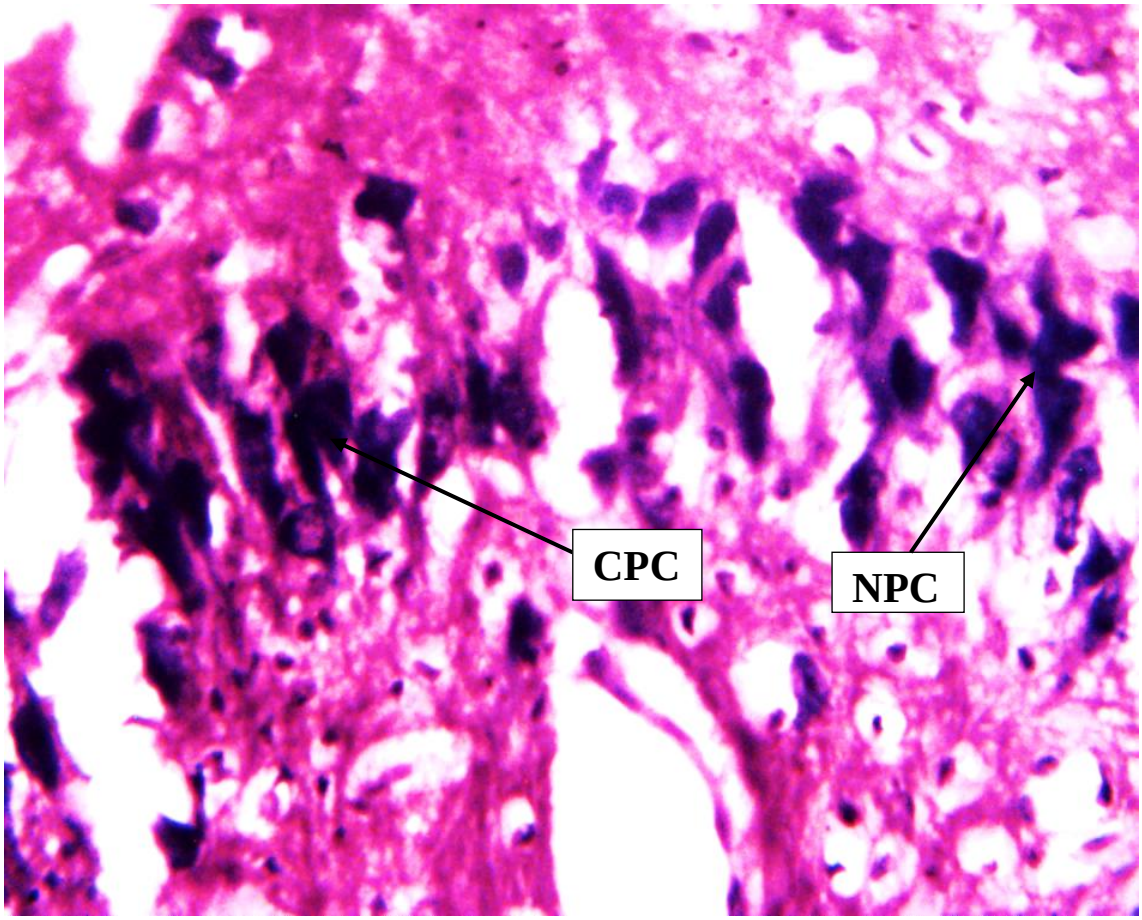


Plate XIX: Section of hippocampus (CA3 region) of rat treated with 300mg/kg aqueous extract of *Colanitida* showing; Clumped Pyramidal cells (CPC), Normal pyramidal cells (NPC). (H and E × 250).

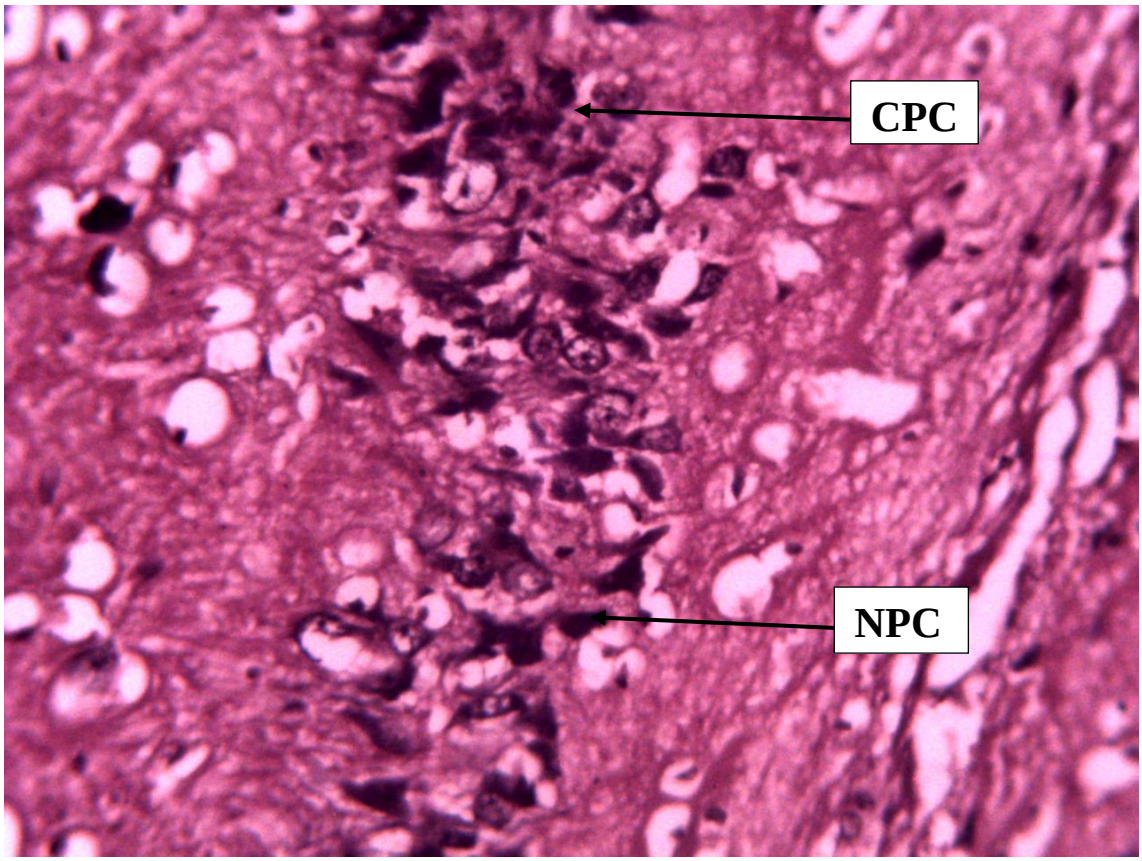


Plate XX: Section of hippocampus (CA3 region) of rat treated with 600mg/kg aqueous extract of *Colanitida*showing; Clumped Pyramidal cells (CPC), Normal pyramidal cells(NPC). (H and E × 250).

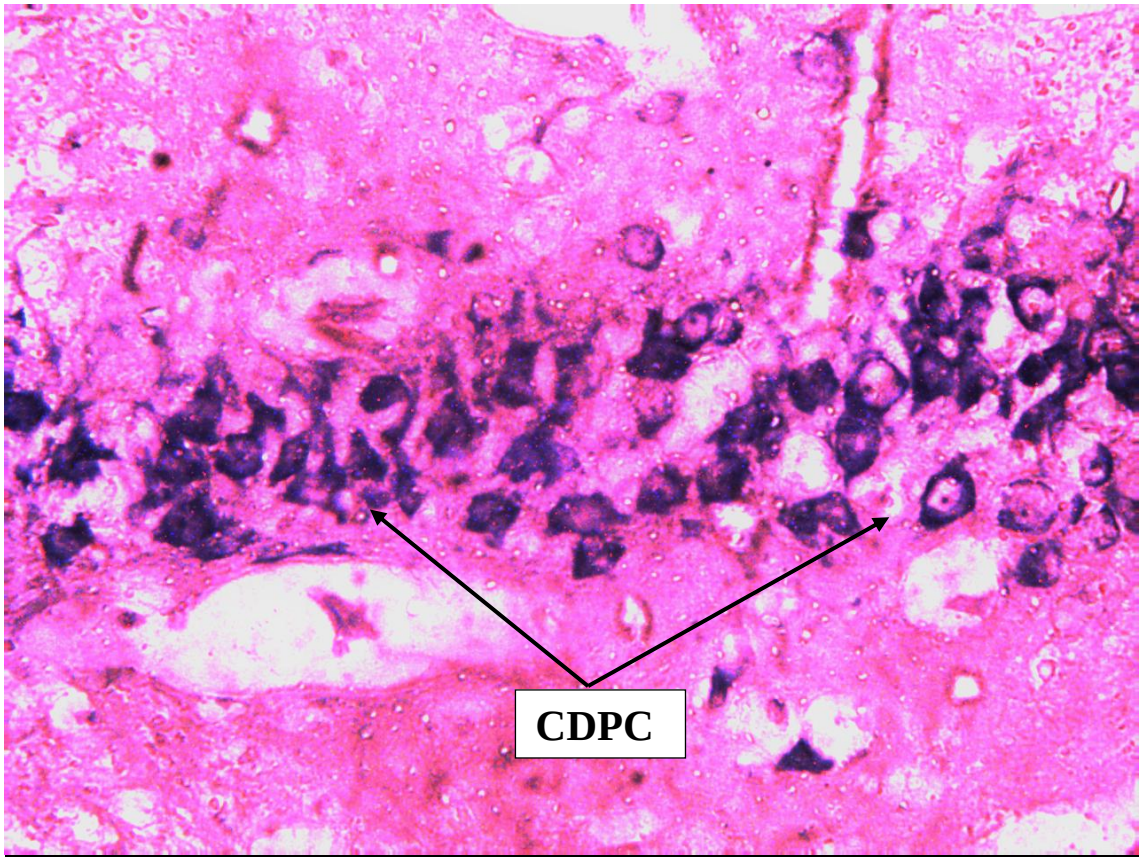


Plate XXI: Section of hippocampus (CA3 region) of rat treated with 900mg/kg aqueous extract of *Colanitida* showing; Clumped Degenerating Pyramidal Cells (CDPC) (H and E × 250).

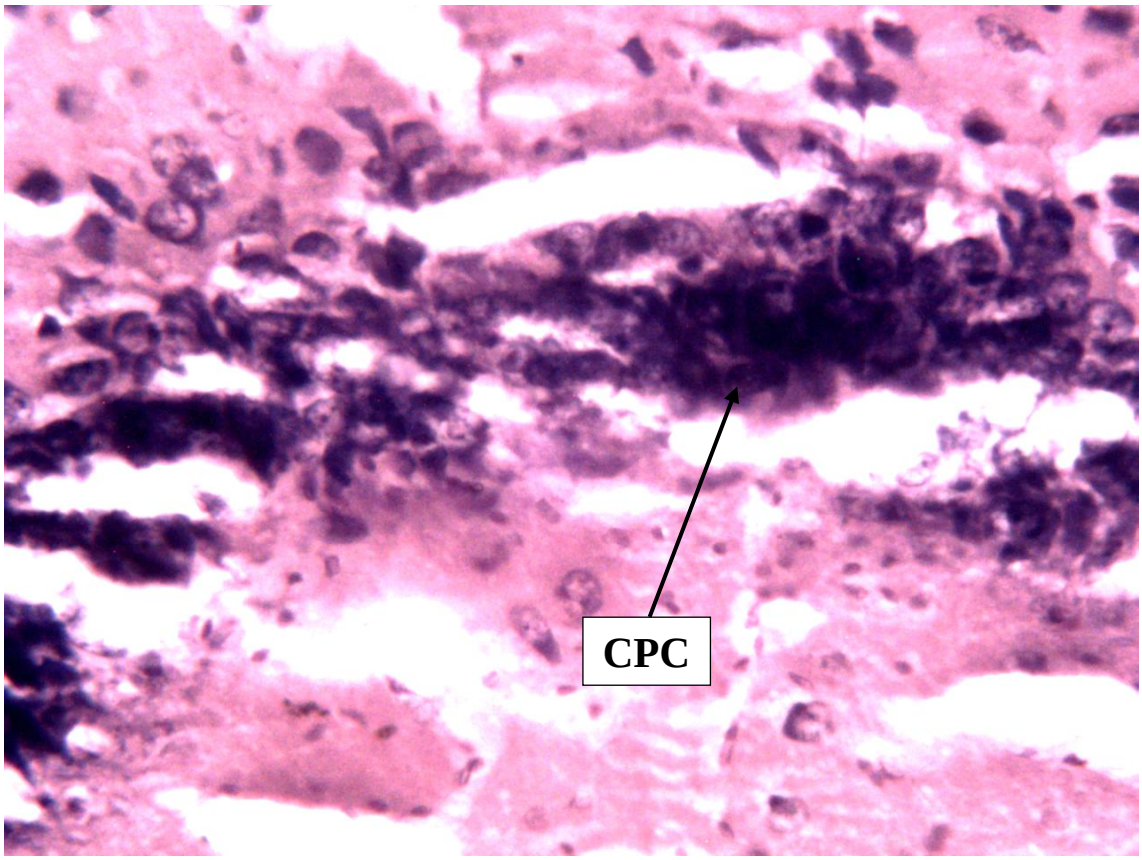


Plate XXII: Section of hippocampus (CA3 region) of rat 10% supplemented with *Cola nitida*showing;Degenerating Pyramidal Cell (CPC). (H and E × 250).

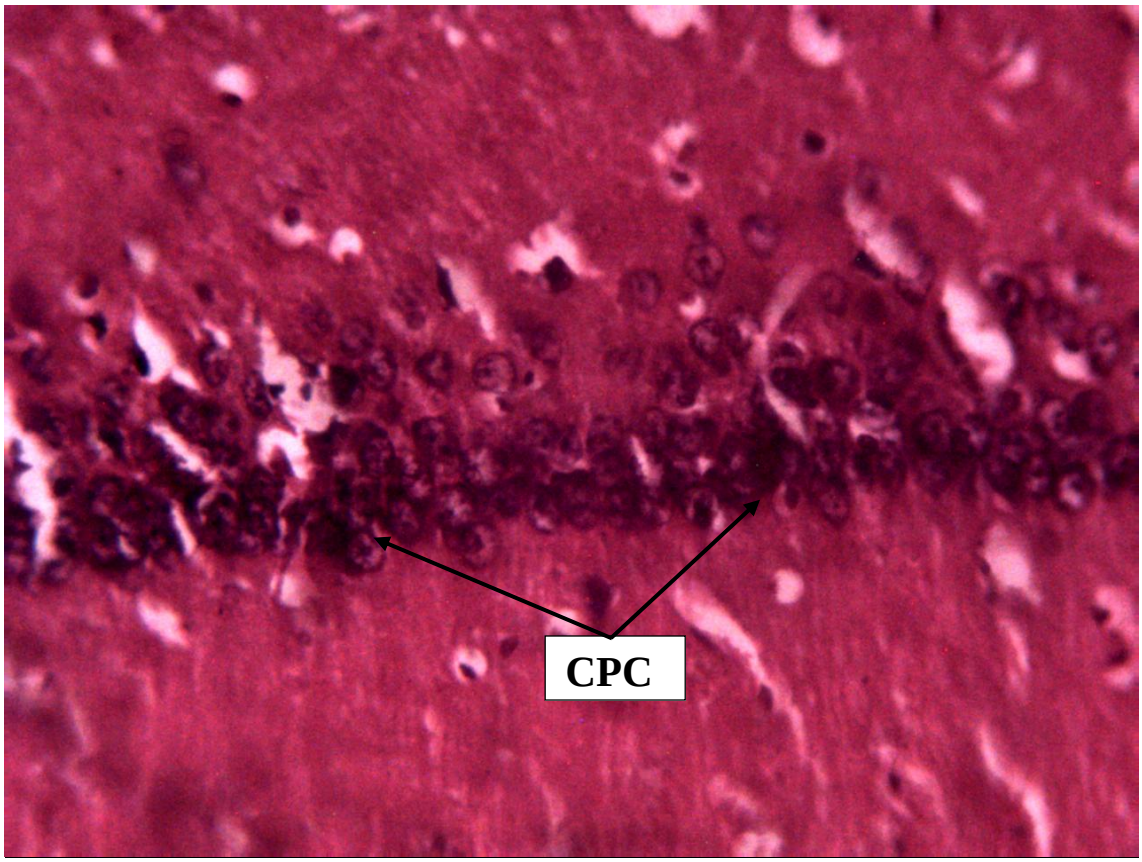


Plate XXIII: Section of hippocampus (CA3 region) of rat supplemented with 20% *Cola nitida* showing; Clumped Pyramidal Cell (CPC). (H and E × 250).

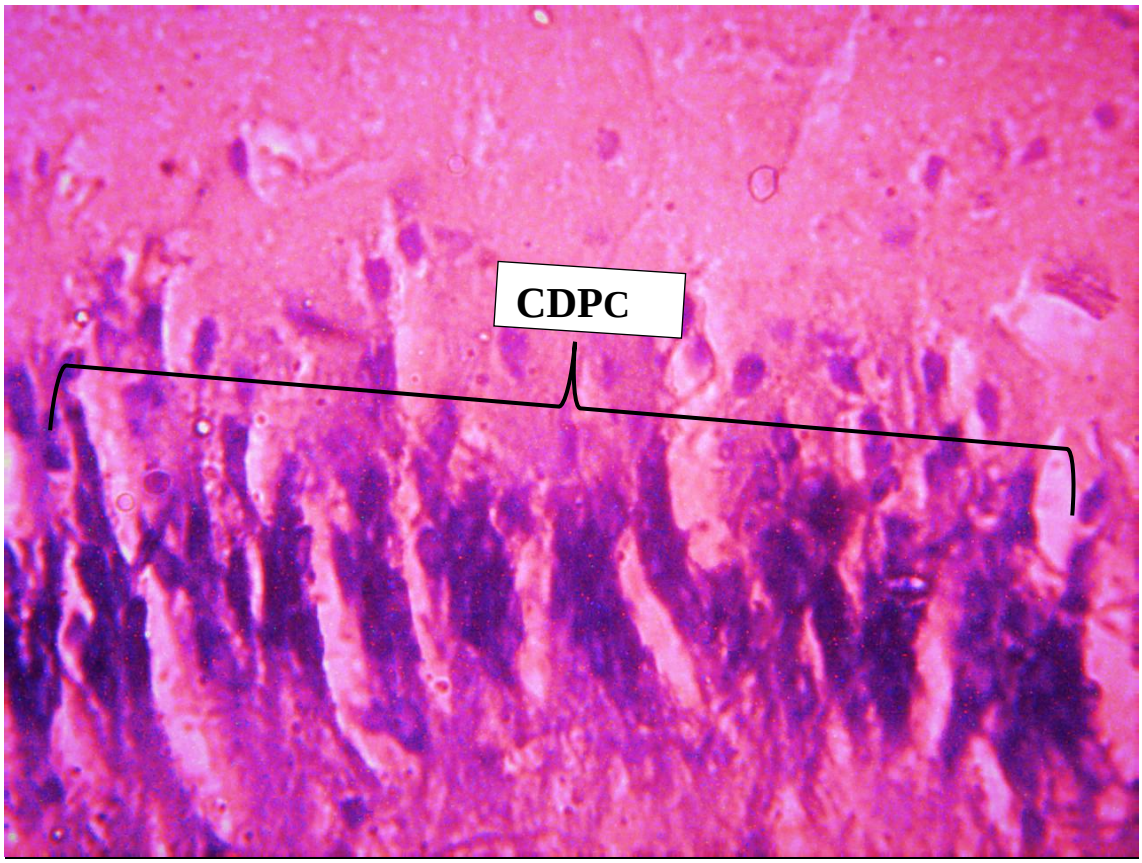


Plate XXIV: Section of hippocampus (CA3 region)of rat supplemented with 30% *Cola nitida* showing;Clumped Degenerating Pyramidal Cells (CDPC) (H and E × 250).

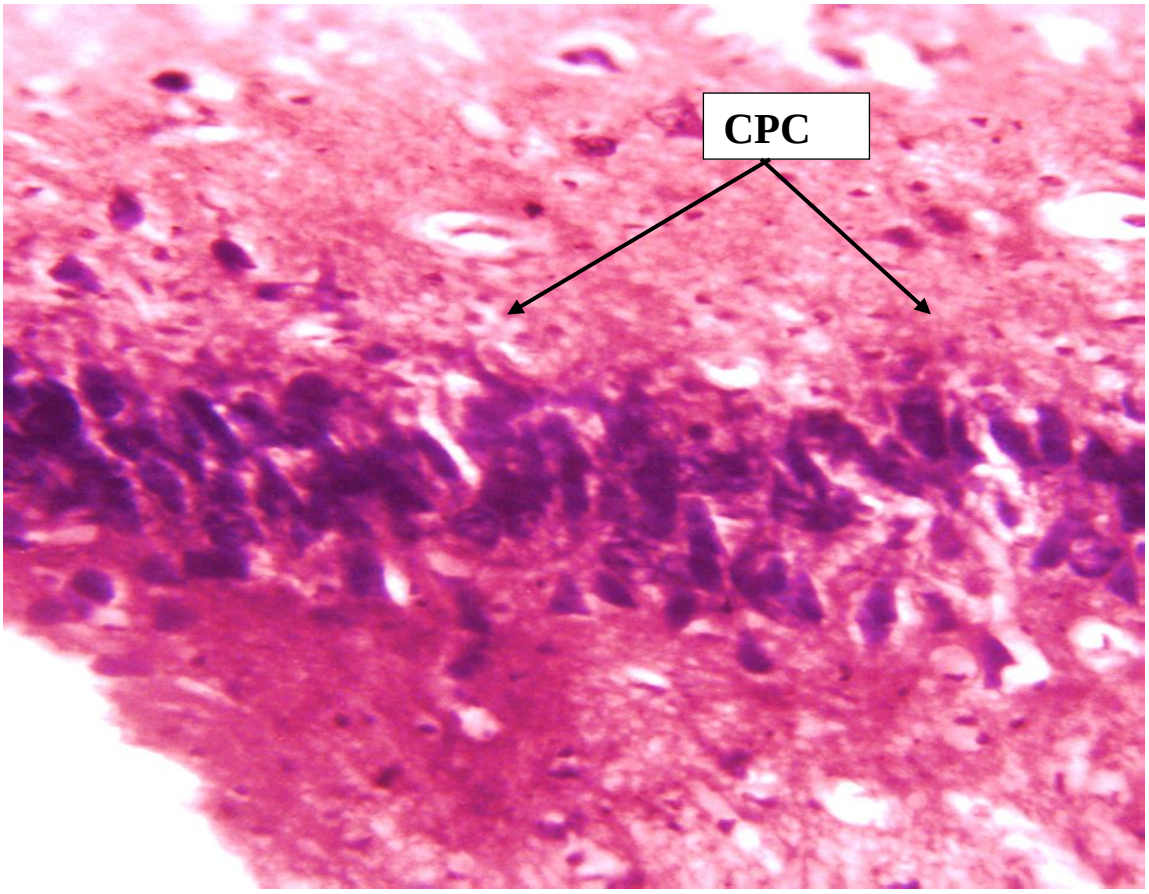


Plate XXV: Section of hippocampus (CA3 region) of rat administered 19.2mg/kg caffeine extract showing; Clumped Pyramidal Cells(CPC) (H and E × 250).

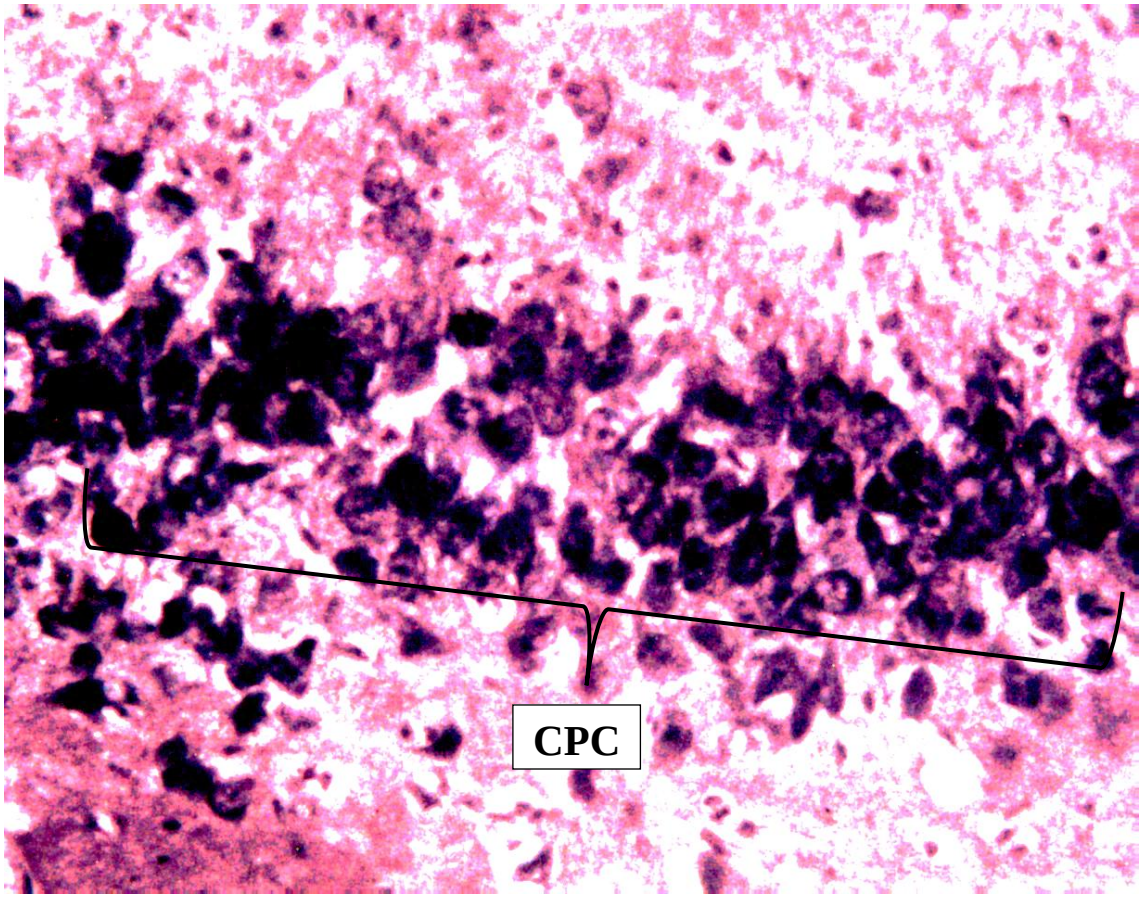

CPC

Plate XXVI: Section of hippocampus (CA3 region)of rat administered 38.4mg/kg caffeine extract showing; Clumped Pyramidal Cells (CPC).(H and E × 250).

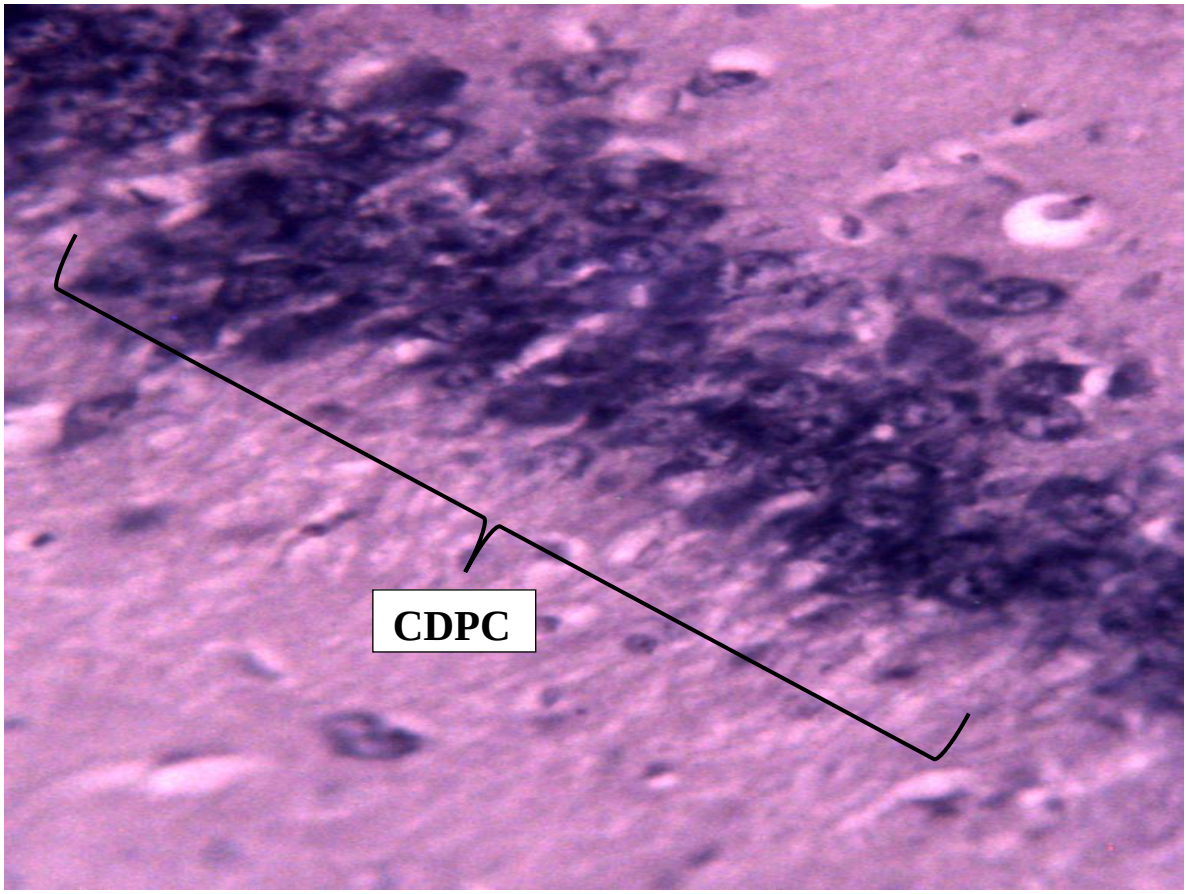
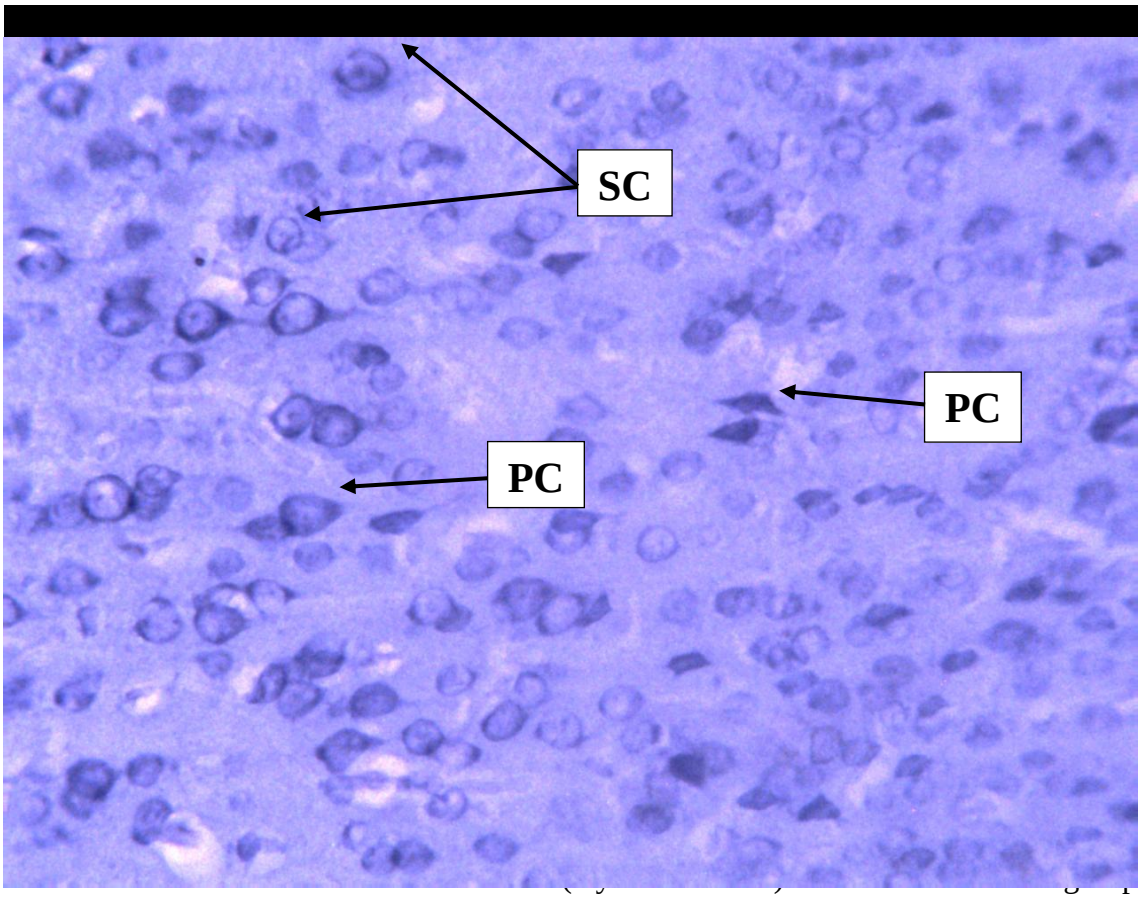


Plate XXVII: Section of hippocampus(CA3 region) of rat administered 57.6mg/kg caffeine extractshowing; Layer of Clumped Degenerating Pyramidal Cells (CDPC) (H and E × 250).



showing;PC (Pyramidal cell), SC (Stellate cell) (Toluidine blue × 250)

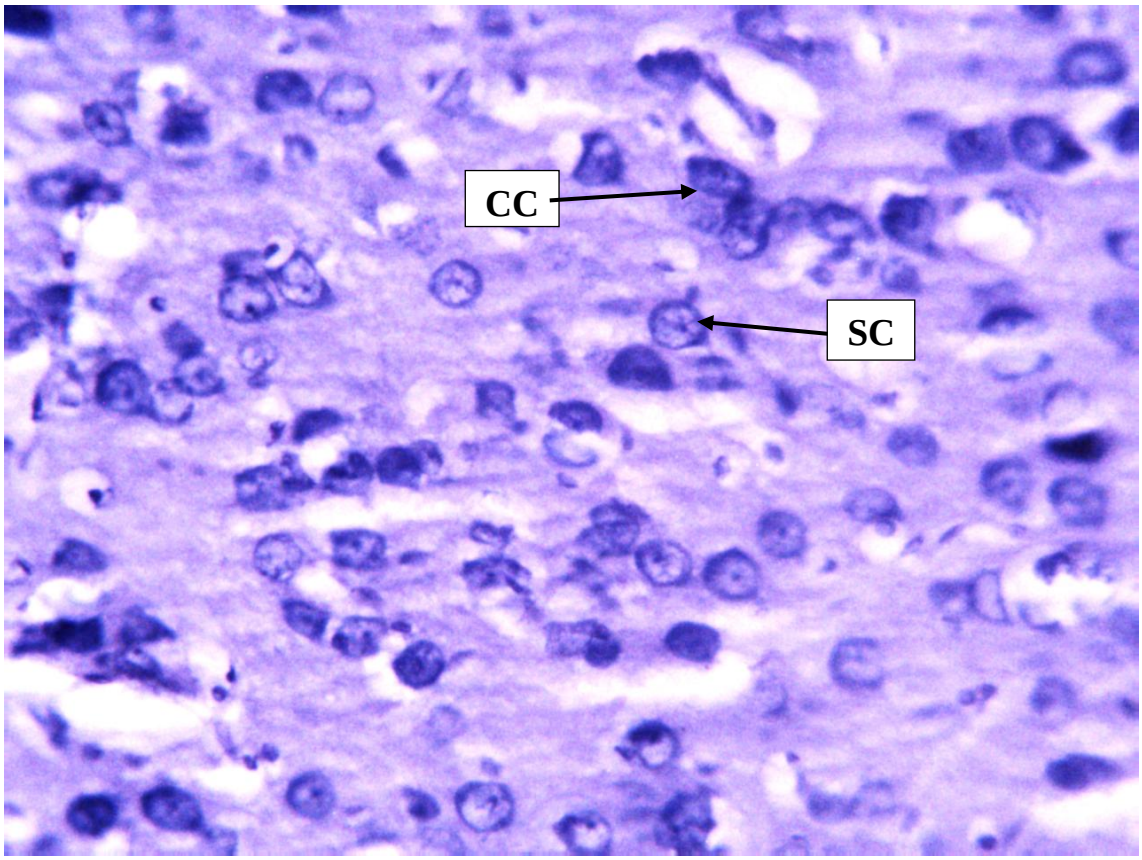


Plate XXIX: Section of cerebral cortex (layer II and III) of rat treated with 300mg/kg aqueous extract of*Colanitida* treated group showing; Stellate cell (SC),Clumped cells(CC) (Toluidine blue × 250)

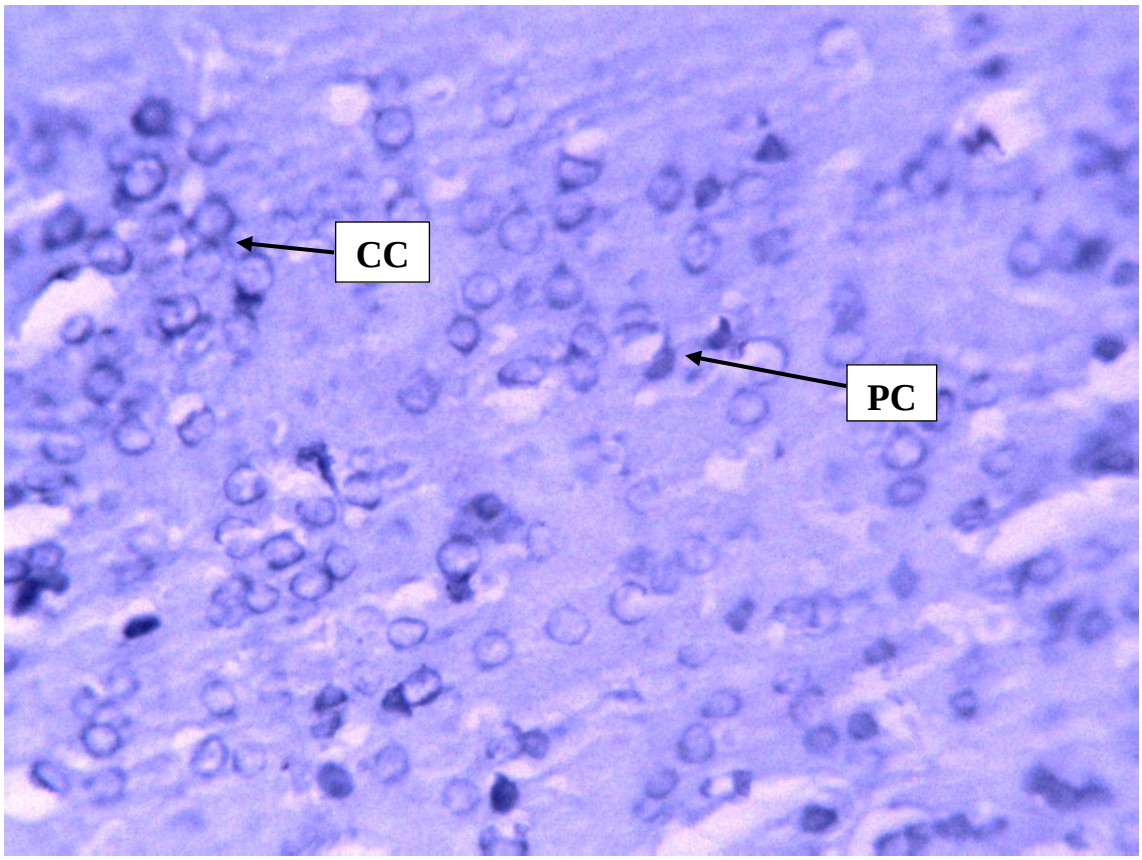


Plate XXX: Section of cerebral cortex (layer II and III) of rat treated with600mg/kg aqueous extract of*Cola nitida* showing; Clumped cells (CC),Pyramidal Cell (PC). (Toluidine blue × 250).

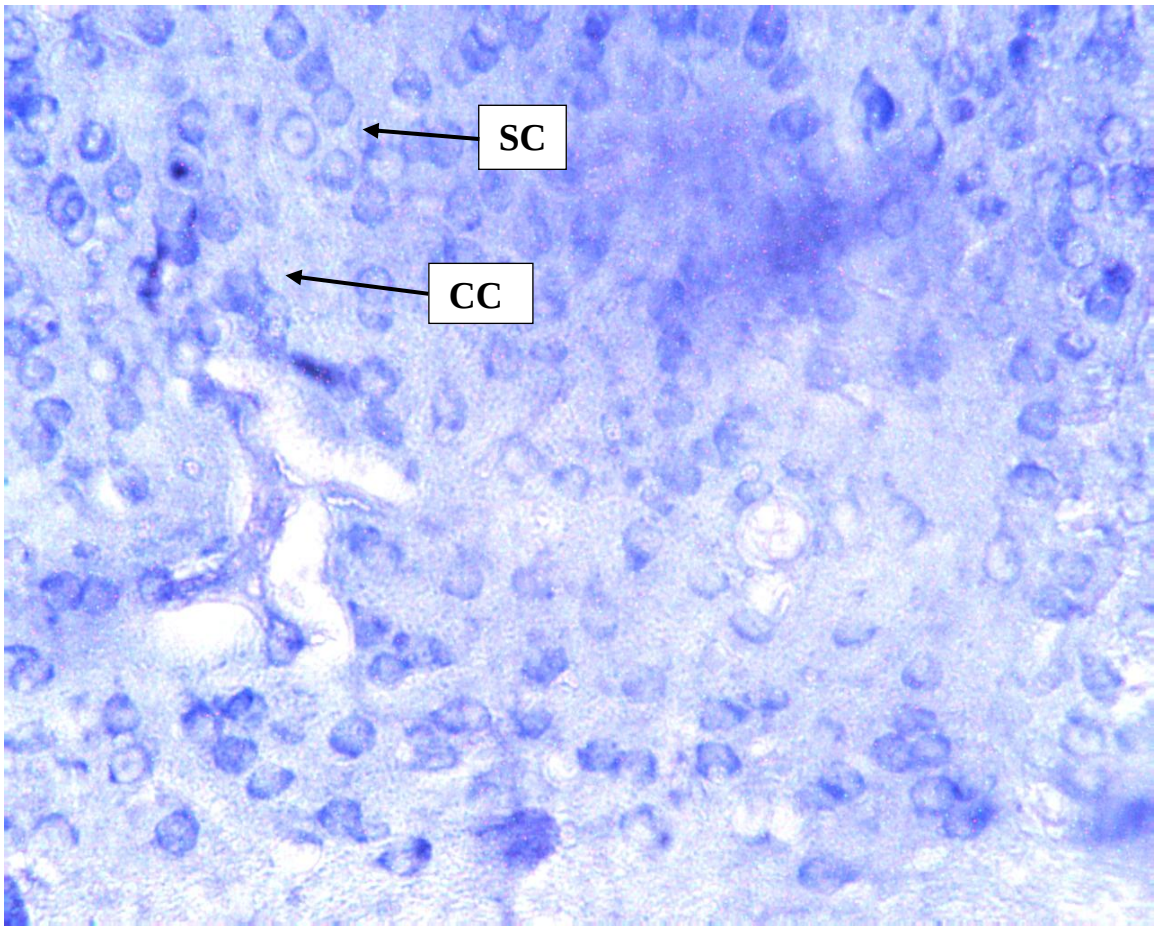


Plate XXXI: Section of cerebral cortex (layer II and III) of rat treated with900mg/kg aqueous extract of*Colanitida* treated group showing; Stellate cell (SC), Clumped cells (CC) (Toluidine blue × 250).

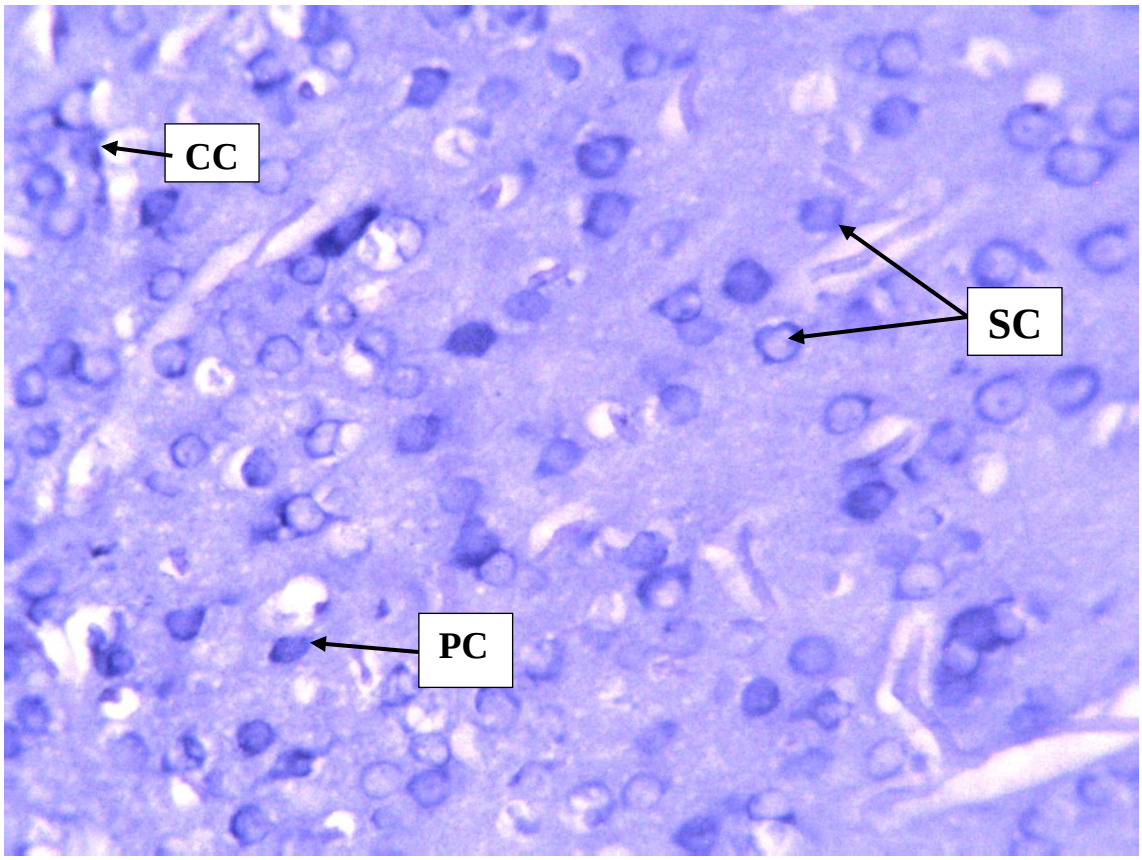


Plate XXXII: Section of cerebral cortex (layer II and III) of rat supplemented with 10% *Cola nitida*showing; Pyramidal cell (PC), Stellate cells (SC), Clumped cells (CC) (Toluidineblue × 250).

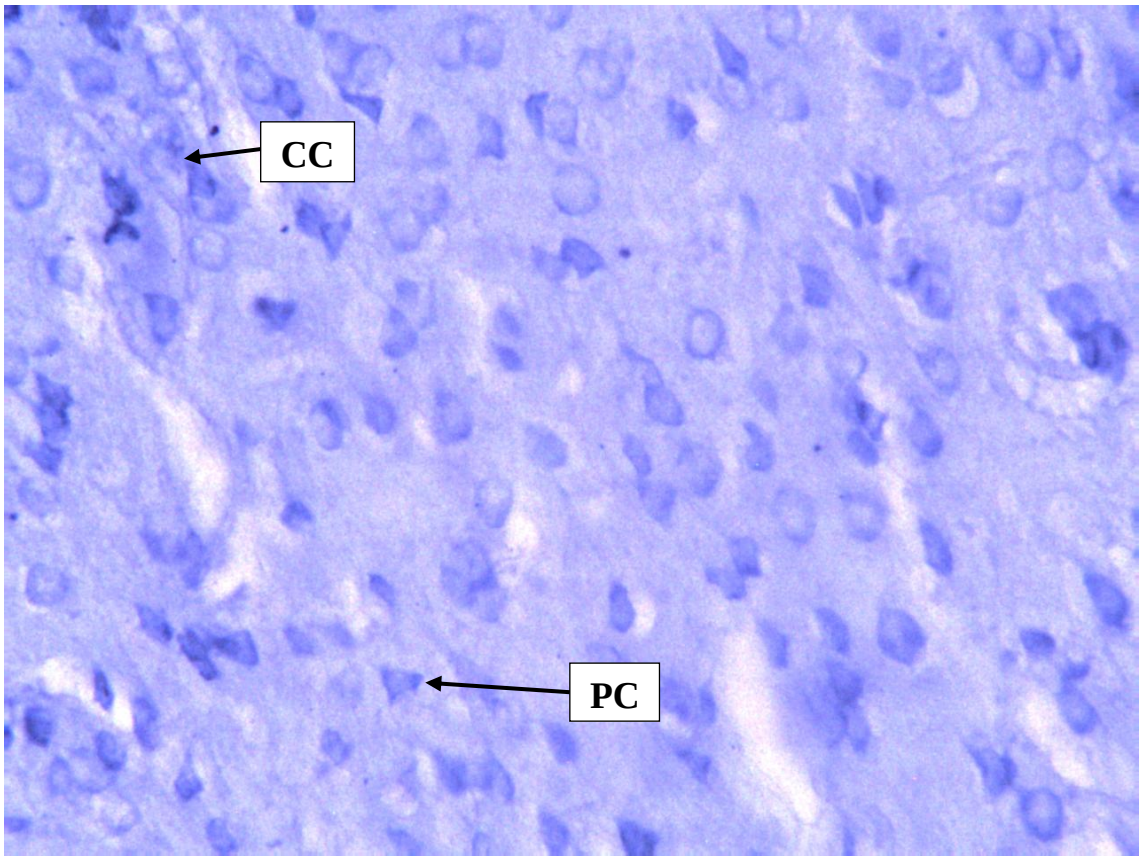


Plate XXXIII: Section of cerebral cortex (layer II and III) of rat supplemented with 20% *Cola nitida*showing; Pyramidal cell (PC), Clumped cells (CC) (Toluidine blue × 250).

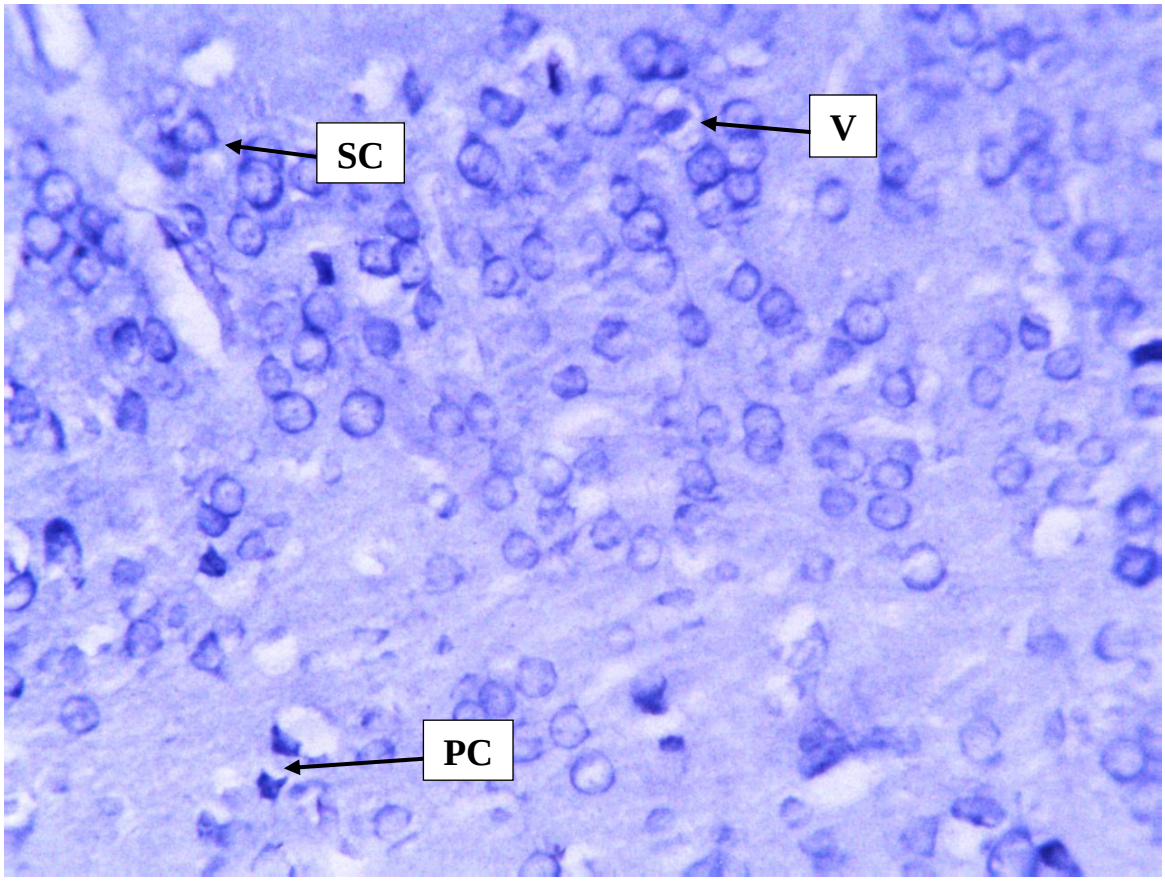


Plate XXXIV: Section of cerebral cortex (layer II and III) of rat supplemented with 30% *Cola nitida*showing; Vacuolation (V), Stellate cell (SC), Pyramidal cell (PC) (Toluidine blue × 250).

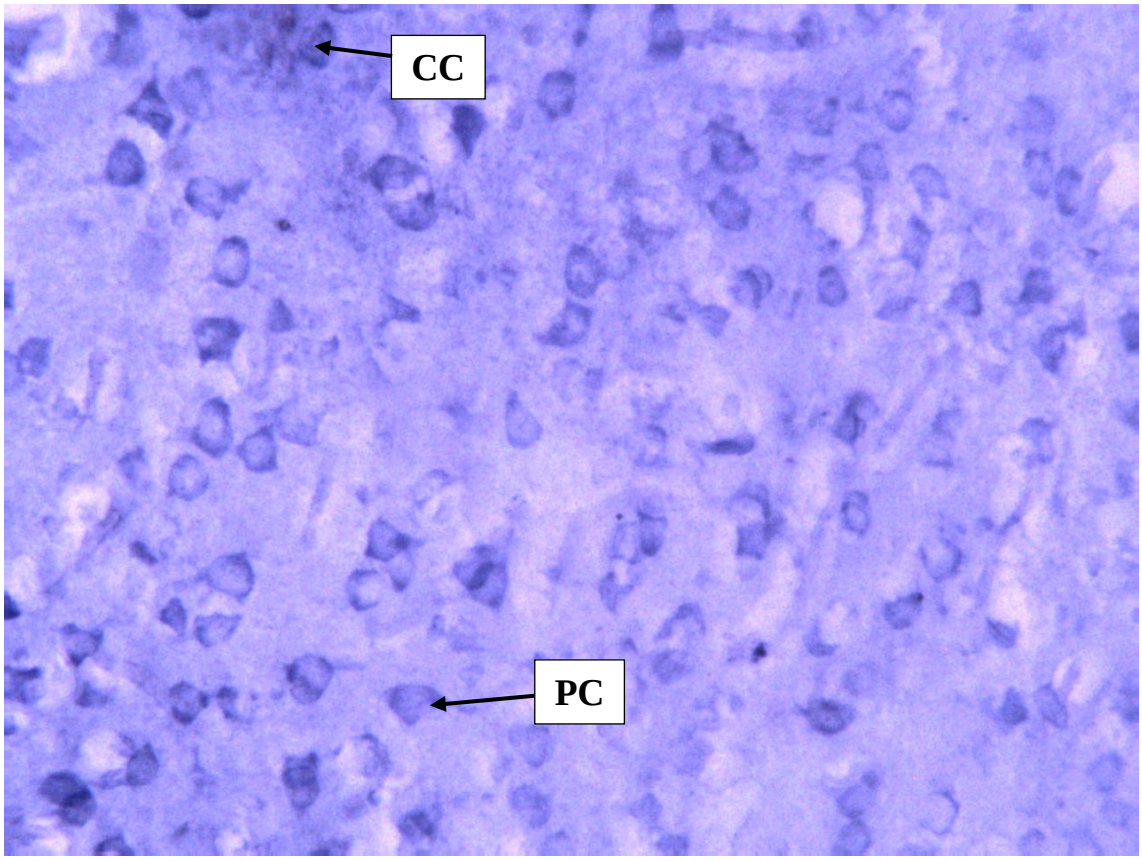


Plate XXXV: Section of cerebral cortex (layer II and III) of rat administered with 19.2mg/kg caffeine extract showing; Pyramidal cell (PC), Clumpedcells (CC) (Toluidine blue × 250).

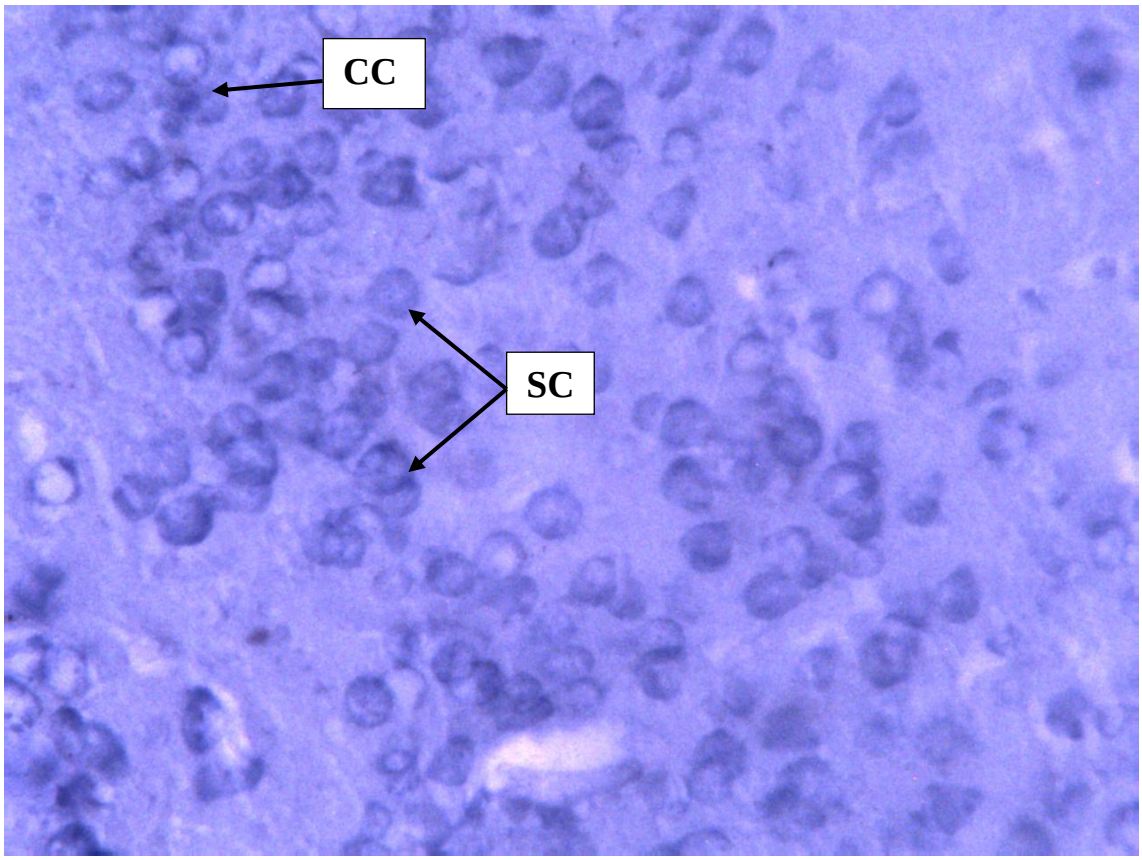


Plate XXXVI: Section of cerebral cortex (layer II and III) of rat administered 38.4mg/kg caffeine extract showing; Stellate cell (SC),Clumped cells (CC) (Toluidine blue × 250).

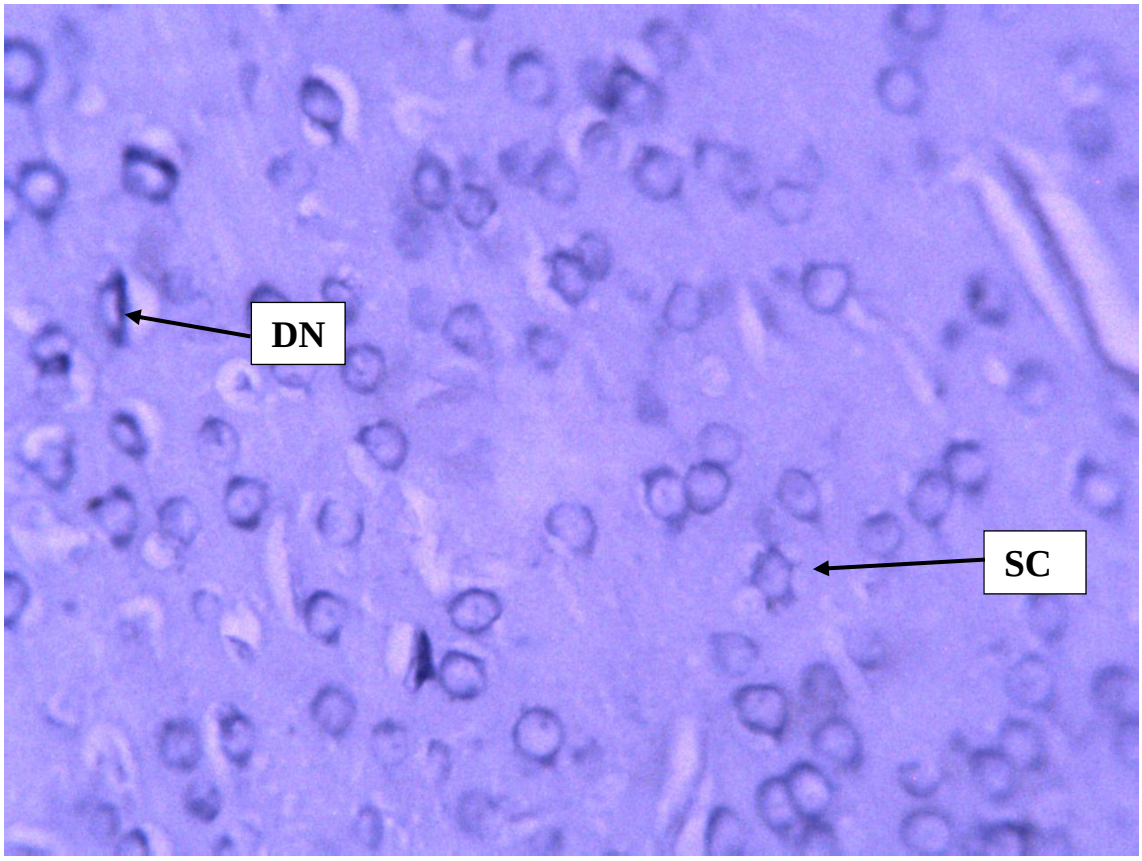


Plate XXXVII: Section of cerebral cortex(layer II and III) of rat administered 57.6mg/kg caffeine extractshowing; Degenerating neuron (DN), Stellatecell(SC) (Toluidine blue × 250).

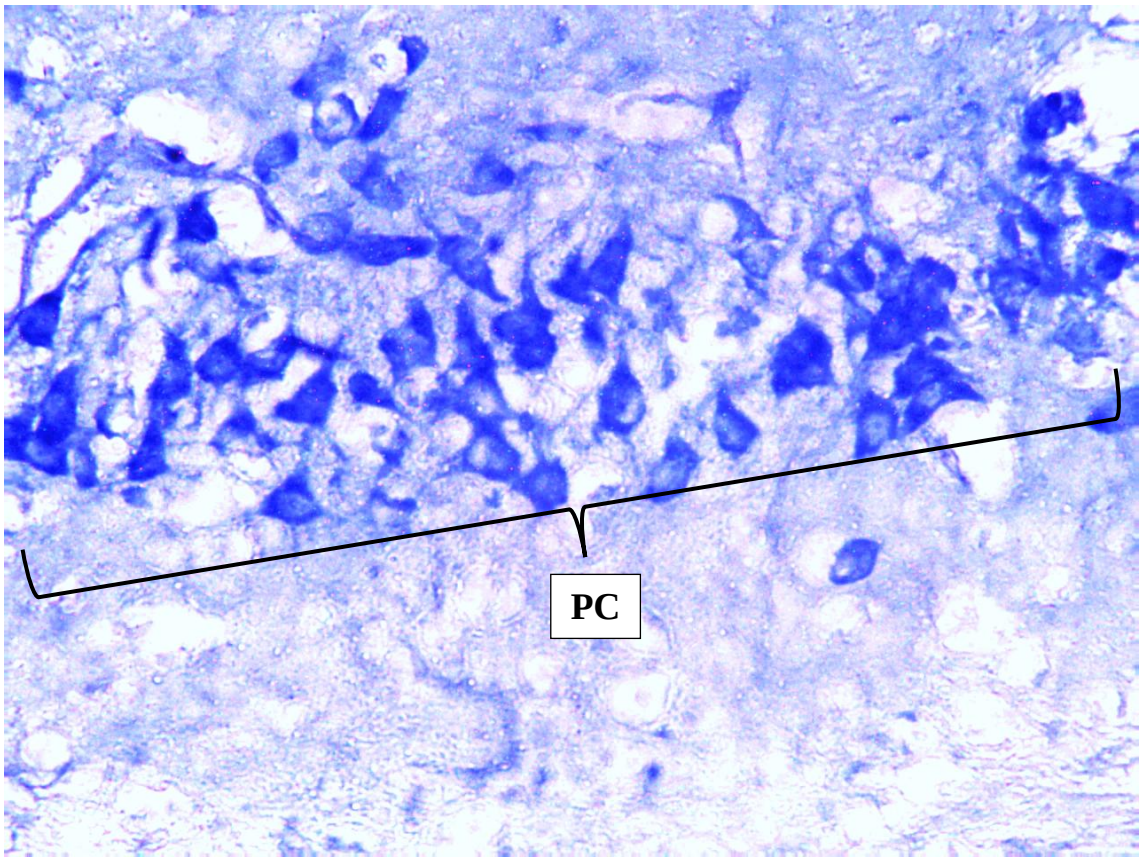


Plate XXXVIII: Section hippocampus (CA3 region) from control groupshowing; Pyramidal Cells (PC),(Toluidine blue × 250).

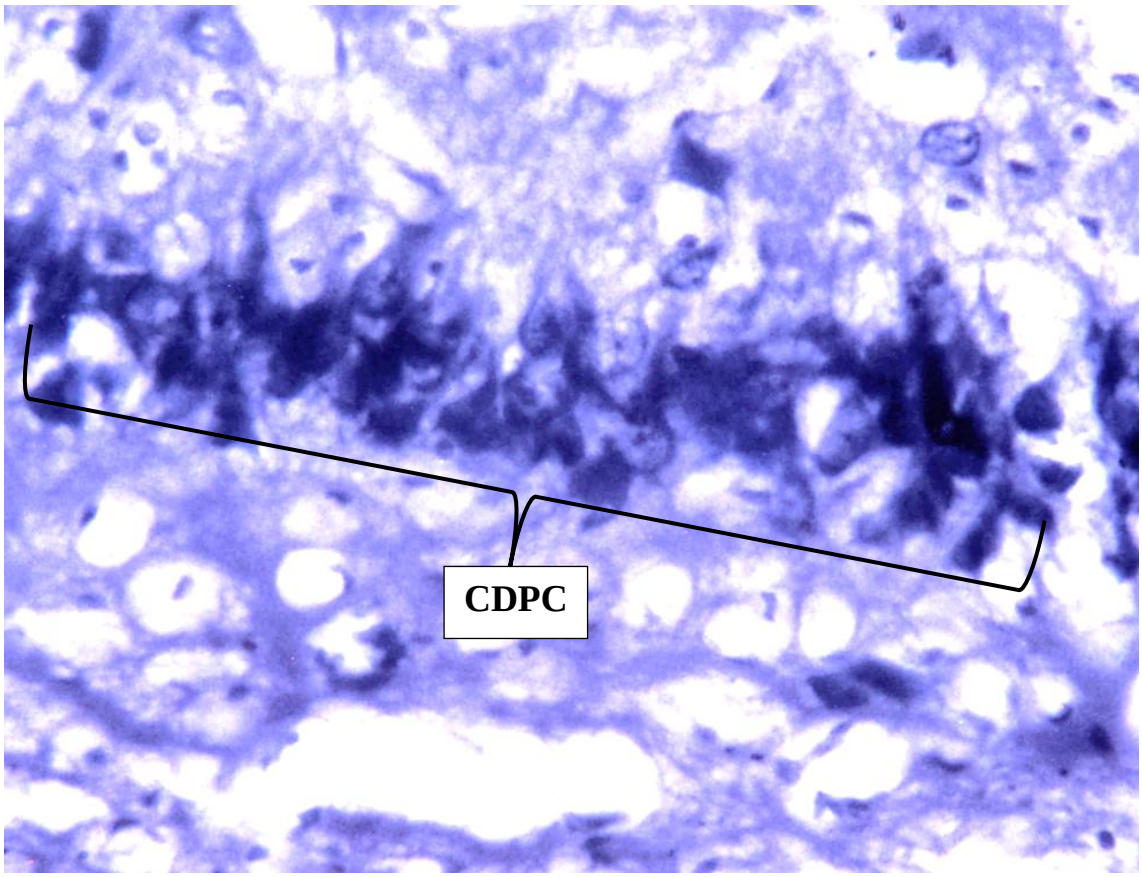


Plate XXXIX: Section of hippocampus (CA3 region) of rat treated with 300mg/kg aqueous extract of*Cola nitida* showing; Clumped Degenerating PyramidalCells (CDPC), (Toluidine blue × 250).

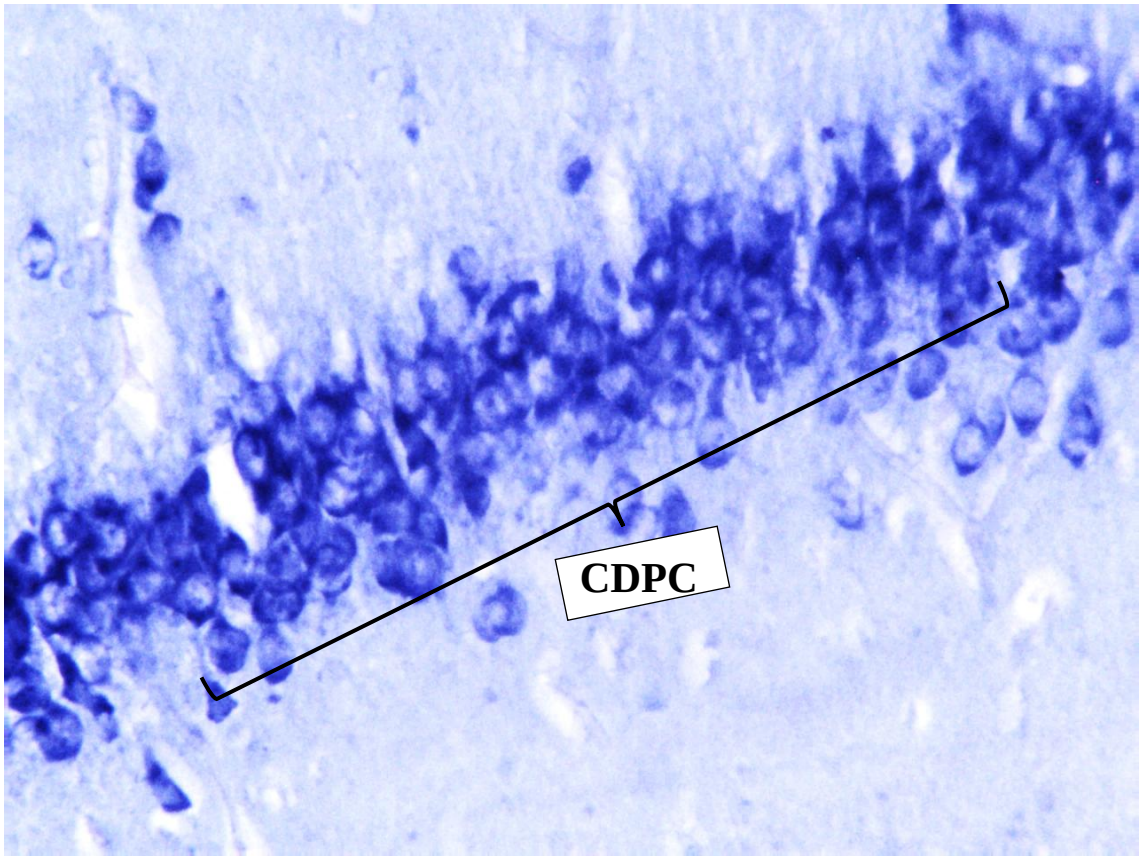


Plate XL: Section of hippocampus (CA3 region) of rat treated with600mg/kg aqueous extract of*Colanitida* showing; Clumped Degenerating Pyramidal Cells(CDPC) (Toluidine blue × 250)

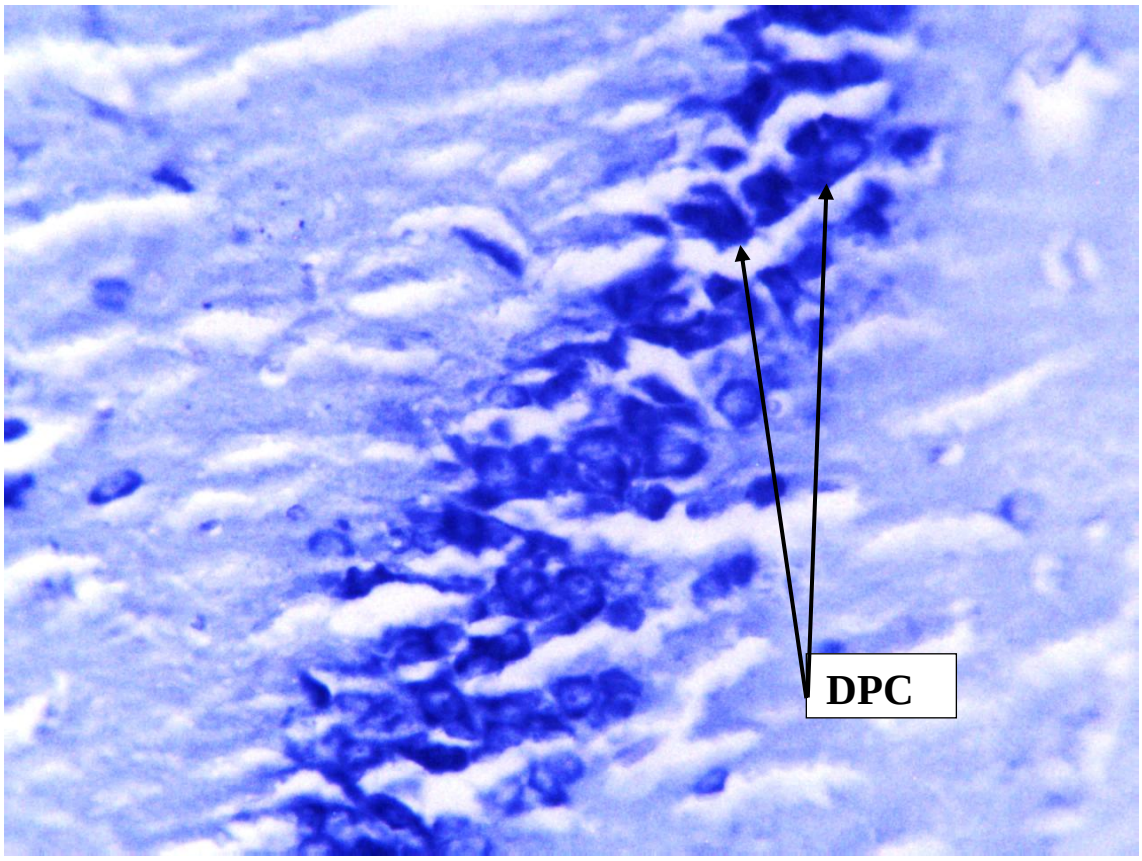


Plate XLI: Section of hippocampus (CA3 region) of rattreated with 900mg/kg aqueous extract of*Colanitida* showing; Degenerating Pyramidal Cell (DPC), (Toluidine blue × 250)

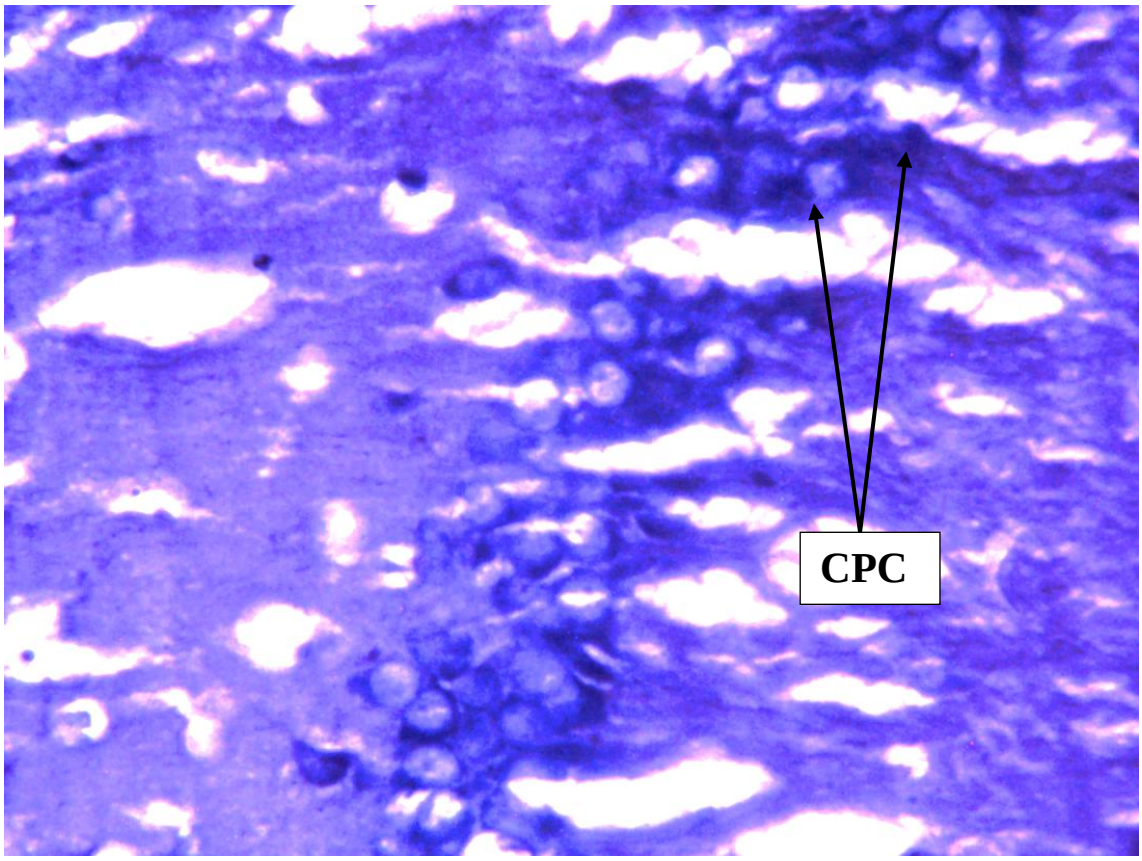


Plate XLII: Section of hippocampus (CA3 region) of rat supplemented with 10% *Cola nitida* showing; Clumped Pyramidal Cell (CPC) (Toluidine blue × 250).

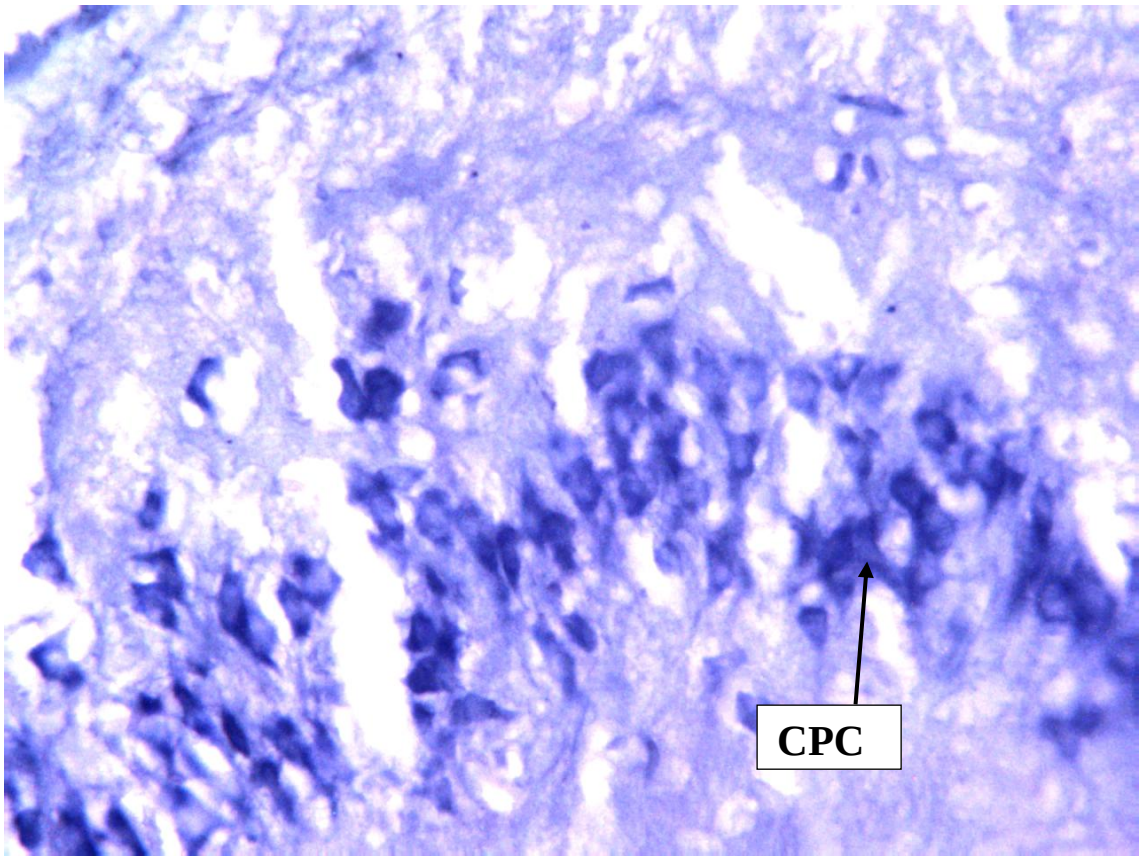


Plate XLIII: Section of hippocampus (CA3 region)supplemented with 20% *Cola nitida* showing;Clumped Pyramidal Cell (CPC) (Toluidine blue × 250)

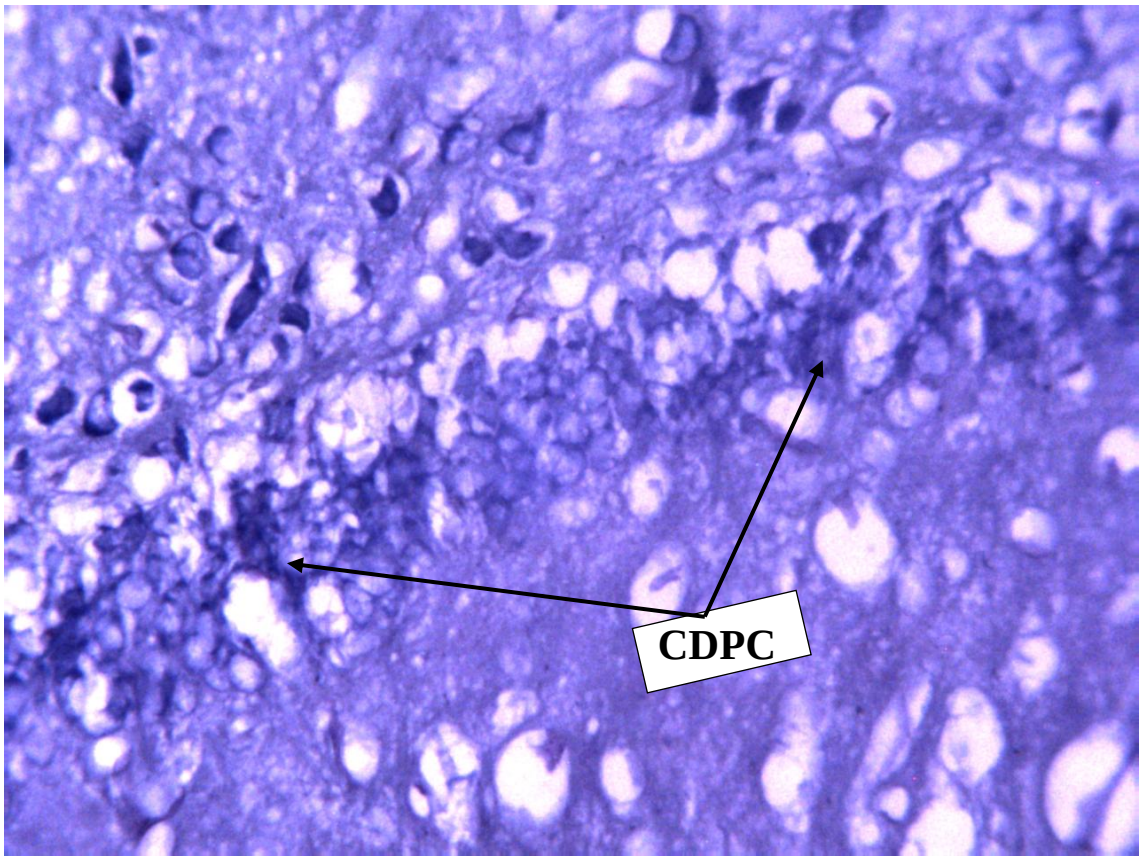


Plate XLIV: Section of hippocampus (CA3 region) of rat supplemented with 30% *Cola nitida*showing; Clumped Degenerating Pyramidal Cell (CDPC)
(Toluidineblue × 250)

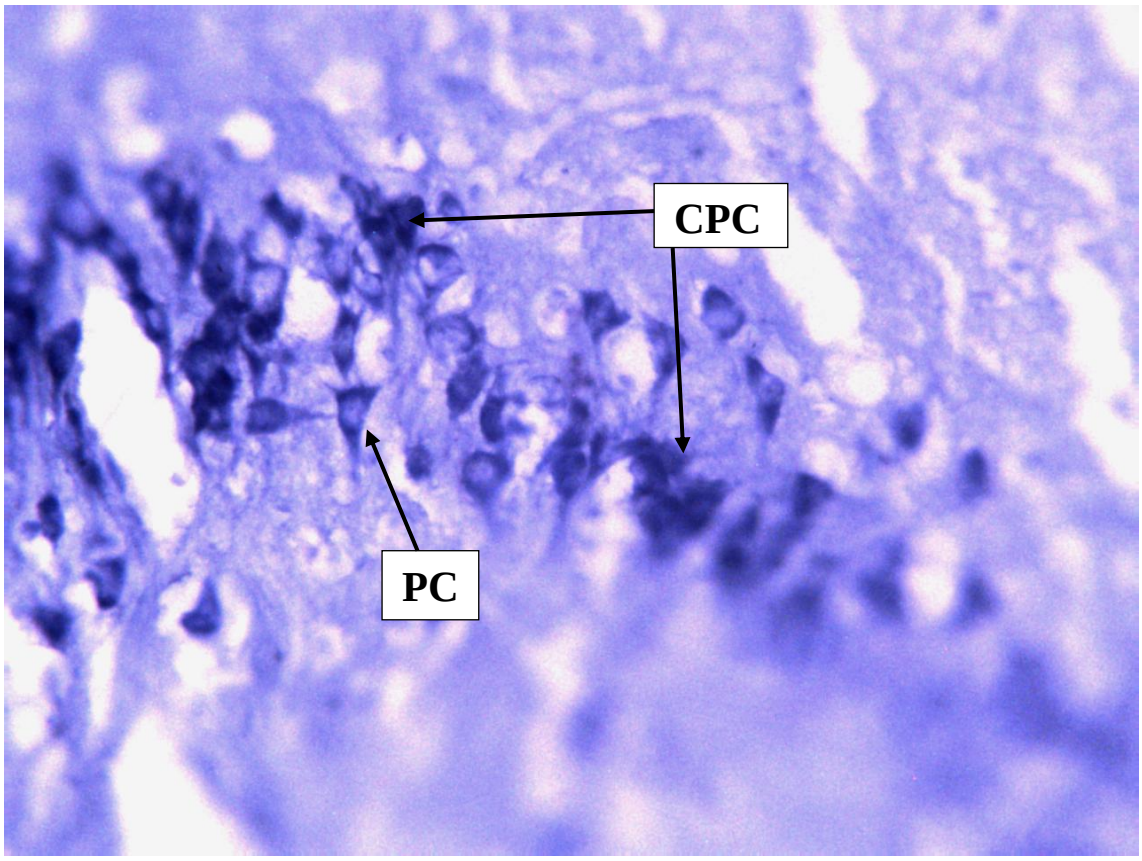


Plate XLV: Section of hippocampus (CA3 region) of rat administered 19.2mg/kg caffeine extract showing; Clumped Pyramidal Cell (CPC), Pyramidal cell(PC) (Toluidine blue × 250).

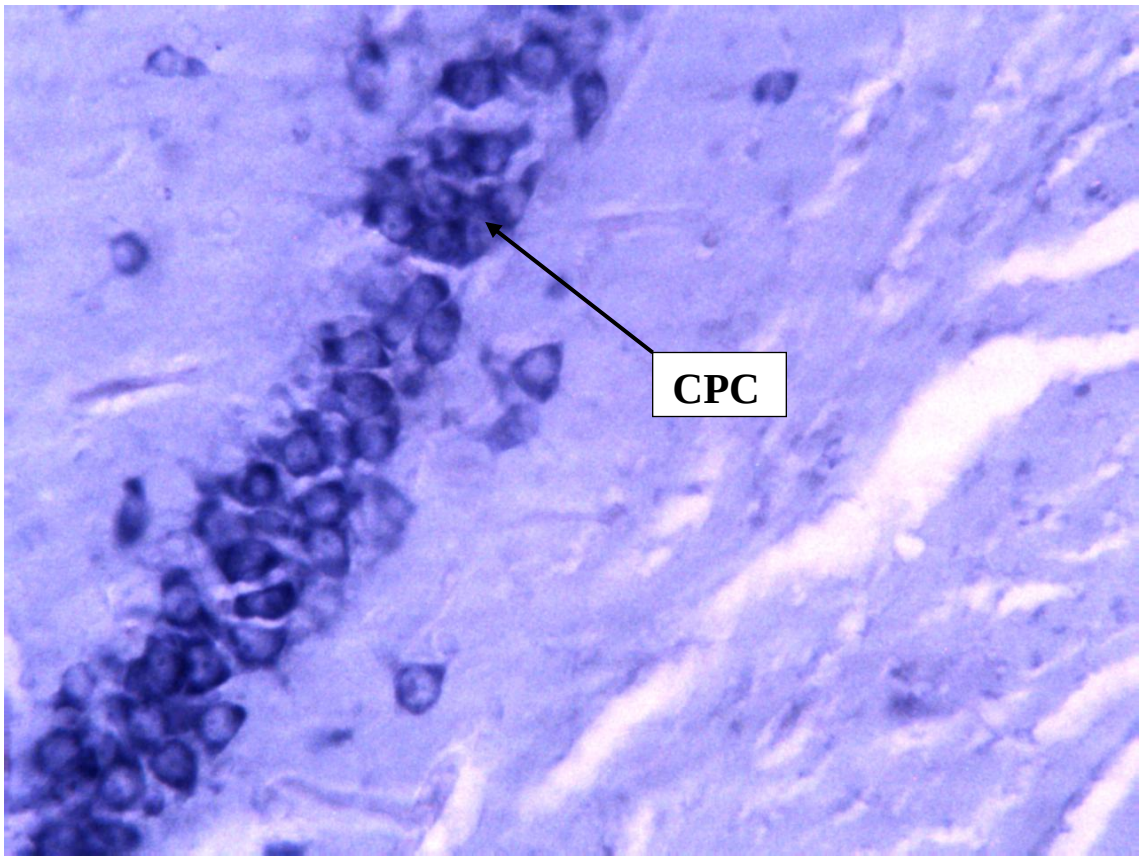


Plate XLVI: Section of hippocampus (CA3 region) of rat administered 38.4mg/kg caffeine extractshowing; Clumped Pyramidal Cell (CPC) (Toluidine blue × 250).

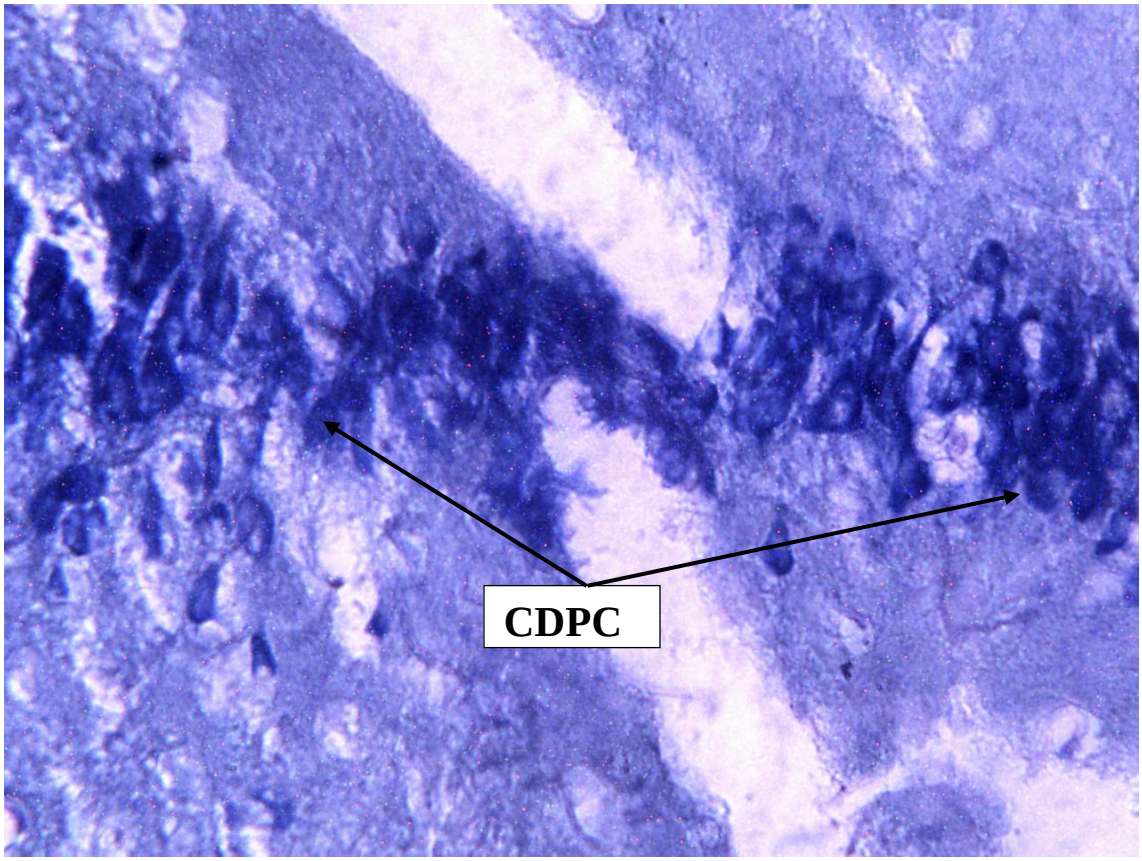


Plate XLVII: Section of hippocampus (CA3 region) of rat administered57.6mg/kg caffeineextractshowing; Clumped Degenerating Pyramidal Cell (CDPC) (Toluidineblue X 250).

CHAPTER FIVE

5.0 Discussion

The administration of *Cola nitida* for 21 days resulted in a non- significant decrease in body weight of the rats in the *Cola nitida*supplement groups. This finding agrees with the work of Agbai *et al*.(2013) who reported a non-significant loss of body weight in rats treated with extract of *Cola nitida*.The loss of weight in the present study forgroup administered crude *Cola nitida* may be as a result of bitter taste of cola nut, thereby

causing decrease in appetite, and subsequently, reduce intake of feed containingsupplement of *Cola nitida*.It is known thatchronic consumption of cola nut and caffeine diets caused decrease food intake and body weight (Umoren*et al*.,2009). Similarly, *Cola nitida* extract had been reported to bring a decrease in total body weight and increase in the absolute weight of some organs (Ikegwuonu *et al*., 1981). Thus, Changes in body weight have been used as an indicator of adverse effect of drugs and chemicals (Mukinda and Syce, 2007).

Result from present study showed non- significant increase inbody weight of animals treated with aqueous and caffeine extracts. This finding is in consonance with the work of Agbai and Ugwu(2012) who reported a non- significant increase in the weight of animals treated with aqueous extract of *Cola nitida*. The slight weight gained by the caffeine treated groups in this study is in contrast with the findings ofNmaju*et al.* (2014) who reported a decreased weight in the coffee diet-fed group of mice when compared with the control. On the other hand, other researchers have also shown that coffee intake decreased body weight by decreasing food intake via some indirect mechanism that is not well understood (Fisone *et al*., 2004).

This hidden platform version of the Morris water maze tests for visuo-spatial learning and memory is hippocampus dependent (McDonald and White, 1994).

The results of spatial learning and memory using Morris water maze test in the present study showed that animals in the control group had an increased mean latency time to locate the hidden platform in the Morris water maze test at the third week of the experiment, and the treated groups had decreased time although no significant difference was recorded. This may be related to the effect of caffeine and cola nut as central nervous system stimulant, therefore increasing the activity in all the treated

groups. This result agrees with the findings of Nmaju *et al*.(2014) who reported a significant decrease in the swim latency for the coffee diet-fed mice when compared to the control. The result in this study is also in line with the report of Alan(2009), who showed that since caffeine penetrates the blood-brain barriers, thus, it is assumed that the central stimulant effects that enhance alertness and counteract feelings of fatigue are due to its action. The results of the study on the effects of cola nut extracts is also in consonance with the reports of Graham and Spriet(1991) who observed that trained runners showed a 44% increase in "race-pace" endurance, as well as 51% increase in cycling endurance, after a dosage of 9 milligrams of caffeine per kilogram of body weight. Thus, the brain is unique for its ability to add to its stock of information by acquiring information, (learning), retaining and retrieving the information (memory) as appropriate (Osim, 2003).

The result of the effect of extracts of *Cola nitida* on anxiety-like behaviours in elevated plus maze showed a non-significant increase in the mean latency time spent in the open arm of the elevated plus maze. There was anincreased number of rearing and head dips as the dose of the aqueous extract increases. The elevated plus-maze (EPM) has been used and proven as a model for assessing anxiety and fear (Lister, 1987; Brown *et al.*, 1999). This test is based on the natural aversion of rodents for space and heights. Therefore, when exposed to the elevated plus-maze, fearful and anxious animals will avoid the open arm and spend most time in the closed arm (Trullas *et al.*, 1993). This method was used to assess the effect of cola nut extracts on anxiety-like behaviours in Long Evans rats. The increase in the mean latency time spent in open arm, increased rearing and head dip in the caffeine fraction and aqueous extract groups in this study suggests decrease in anxiety, this is in agreement with the reports of Elizabeth *et*

al.(2011) who stated a non-significant difference in the duration of entry into the elevated plus maze between cola nut and caffeine diet-fed mice. This implies that there was probably no increase anxiety in the treated groups in this study, especially the caffeine treated group. This finding is in contrast with the work of Neil (1978), who showed that excessive consumption of caffeine caused mixed depressive state in psychiatric patients. Greden(1978) also reported depressive syndrome as associated with caffeine. On the other hand, the reports of Mrvos *et al*.(1989) showed that large amounts of caffeine intake can induce anxiety severe enough to necessitate clinical attention. This does not agree with the results of this study since the higher dose caffeine treated group showed a decrease in anxiety. It could also mean that the large intake of caffeine-induced anxiety may probably be dependent on the type of animal species used and the frequency of intake.

Anxiety and fear are emotions controlled by the limbic system and hypothalamus (Guyton, 2006). The exact mechanism whereby administration of *Cola nitida* and its constituent (caffeine) reduces anxiety and fear is uncertain. However, it is known that impairment of the amygdala reduces fear in animals (Guyton, 2006). It is therefore likely that cola nuts and its constituent (caffeine) may be impairing the amygdala.

The haematology result showed that there were no significant changes in the packed cell volume, white blood cell count, hemoglobin and eosinophil of the treated groups and the control. This suggests that the extracts do not have noticeable effect on these blood parameters. This finding agrees with the work of Oyedeji *et al.*(2013)who reported a non-significant change in blood parameters albino rats treated with aqueous extract of *Cola nitida*. The significant increase in lymphocytes and neutrophils in the caffeine and

aqueous extract groups when compared with the control may indicate an increased ability of the immune cells to attack and destroy toxins and invading bacteria, viruses and other injurious agents through phagocytosis. This is in contrast with the work ofOyedeji *et al.*(2013)who reported a significant reduction in neutrophil value as a result of the effect of aqueous extract of *Cola nitida* (cola nut) on haematological and plasma biochemical parameters in male albino rats.

Excessive free radicals in the body system are known to cause oxidative stress, resulting in some pathological conditions which are fast becoming a challenge that needs urgent attention. Oxidative stress has been observed to induce a cellular redox imbalance. The result of this study showed a significant increase of GSH (glutathione concentration) only in *Cola nitida* aqueous extract treated group with a lower concentration when compared with the control. High GSH levels have been associated with protection against peroxidative damage, this suggests that low dose of *Cola nitida* extracts confers more antioxidant potentials than the higher dose. The non-significant increase in the malondialdehyde (MDA) level in all the treated groups when compared with the control shows that lipid peroxidation was increased, suggesting that the extracts may not cause excessive production of free radicals. This is in line with the work of Mahdavil *et al.* (2012) who reported that 5 mg/kg caffeine supplementation had no significant effect on plasma MDA levels after Wingate test in athletes. Conversely, the work done by Abreu *et al*. (2011) showed that chronic caffeinated coffee and caffeine ingestion reduced lipid peroxidation and increased the concentration of reduced glutathione in brain membranes of rats. The study of Ayebe *et al*. (2012) demonstrated that *Cola nitida* administration caused a decrease in the levels of MDA in rabbits. Malondialdehyde is a product of lipid peroxidation, this means that the Cola nut extracts effect though not significant

does not cause lipid peroxidation. The non- significant effect of cola nut and caffeine extracts on the brain tissue MDA level in this study may be due to the doses and the type of plant used, it may also be as a result of other constituents of cola nut in the crude and aqueous extracts like tannins, sugar and minerals.

The result of this study showed a significant decrease in the caffeine and aqueous extract groups for superoxide dismutase activity. This decrease may be related to inflammation and degenerative changes observed in the brain of rats in this study. Superoxide dismutase has been reported to have powerful anti-inflammatory activity and it tends to decrease due to destruction of proteins in massive reaction of antioxidant (Rujito *et al*., 2015).

Catalase concentration in the treatment groups when compared with the control was also non significantly increased in the aqueous extract groups when compared to the caffeine fraction and the crude extract groups. Catalase is considered as a primary antioxidant as it is involved in the direct elimination of reactive oxygen species. It is known that catalase scavenges hydrogen peroxide generated by superoxide dismutase converting it to water and oxygen (Vatassery, 1998).

In general, the histological changes observed in the cerebrum and hippocampus were neuronal degeneration, vacuolation, clumped cells, and pyknosis. The pyramidal cells in the hippocampus revealed changes, such as clumping and degeneration of pyramidal cells when compared with the control in both histological stains (haematoxylin and eosin and toluidine special stain). This finding is related to the work of Buraimoh *et al.* (2014) who reported a neuro-degenerative effect on the cerebellum of Wistar rats after

the administration of ethanol extract of *Cola nitida* for fifteen days. The neuro-degenerative and histo-morphological changes revealed in the cerebral cortex can affect the functional areas of the cerebral cortex which deals with skilled movement, ability to speak, ability to appreciate pains and temperature.

Stimulation or inhibition of hippocampal neurons may affect learning and memory. The destruction of the pyramidal cells as observed in this study implies that activity from the brain region that projects into the pyramidal layer of the hippocampus will also be lost such as memory and learning ability (Wolf, 2009; Quirino, 2012). These alterations can consequently lead to memory impairments which could be as a result of neuronal degeneration in all the treated groups, which could have resulted in the non- significant decrease in the time taken for the rats treated with *Cola nititda* extracts to reach the escape platform in Morris water maze test as observed in this study. The destruction of the neuronal cells also justifies the significant decrease in the activity of superoxide dismutase enzyme.

CHAPTER SIX

6.0 Conclusion and Recommendations

6.1 Conclusion

The administration of *Cola nitida* and its active ingredient (caffeine) for 21 days from the present studyresultedin the following:

1. Non- significant increase in weight($p \square 0.05$)in the aqueous extract and caffeine extract group and decreased weight in animals fed with cola nut supplement.

2. Decrease in time taken to find the hidden platform for all the treated groups in spatial learning and memory test using Morris water maze; and decrease in anxiety in Elevated plus maze test.
3. Non-significant increase ($p > 0.05$) of the glutathione concentration (GSH) and malondialdehyde (MDA) in the aqueous extract and caffeine fraction groups
4. Significant decrease ($p < 0.05$) in superoxide dismutase (SOD) in the groups treated with aqueous extract and caffeine fraction.
5. Degeneration and clumping of cerebral and hippocampal cells in all the treated groups which may lead to eventual cell death.

6.2 Recommendations for Further Research

1. It is recommended that further work should be carried out on stereology to support the histological findings.
2. Ultrastructural studies should be carried out using electron microscope.
3. Immunohistochemistry for specific antibodies peculiar for proteins of interest to the cerebrum and hippocampus should be assayed.
4. Neurotransmitters of interest to the cerebrum and hippocampus should be assayed.

6.3 Contribution to knowledge

1. Cola nitida has been able to cause increased physical activity and decreased anxiety as assed using elevated plus maze ($p < 0.05$) A (EOA = 2.67 ± 0.67, TOA = 99.88 ± 44.18, Rearing = 9.00 ± 1.78) C2 (EOA = 5.25 ± 1.80, TOA =

85.44 ± 26.98, Rearing = 12.25 ± 2.75) C3 (EOA = 6.00 ± 2.52, TOA = 167.13 ± 24.73, Rearing = 16.33 ± 3.67).

2. It caused decrease in Superoxide dismutase (SOD) activity (p□ 0.05) A (11.11 ± 0.89), B2 (6.23 ± 0.45), B3 (7.11 ± 1.33), C1 (6.23 ± 0.45), C2 (5.78 ± 0.00), C3 (6.79 ± 0.11)

References

Abreu, R.V., Silva-Oliveira, E.M., and Moraes, M.F.D. (2011). Chronic and caffeine ingestion effects on the cognitive function and antioxidant system of rat brains. *Pharmacological Biochemistry and Behaviour*, 99:659–664

Adeniyi, B.A., Groves, M.J. and Gangadharam, P.R. (2004). In vitro anti-mycobacterial activities of three species of Cola plant extracts (Sterculiaceae). *International Journal of Phytotherapy Research*, 18(5):414–418.

Adewale-Somadhi, A. (2004). *Practitioner's Handbook for the IFA Professional*. Ile Orunmila Communication p.1

Agbai, E.O. and Ugwu, N. F. (2012). Dose-Dependent Effect of Aqueous *Cola Nitida* Rubra Extracts onReproductive Functions in Female Albino Wistar Rats at Elele. *Journal of Biological Science and Bio-conservation*,4:87 ISSN 2277-0143.

Alan, W. C. (2009). Abrief history of the British Pharmacological Society. *Journal of Toxicology*, 1:147

Alaribe, A.A.A., Ejezie, G.C. and Ezedinachi E.N.U. (2003). The role of kola nut (Cola nitida) in the etiology of malaria morbidity. *Pharmacological Biology*, 41(6):458-462.

Amaral, D., Lavenex, P., Andersen, P., Morris, R., Bliss, T. and O'Keefe J, (2006). "Hippocampal Neuroanatomy". *The Hippocampus Book*. Oxford University Press. Chapter 3,ISBN 9780195100273.

Arash, F., Athena, A., Vahid, E. and Mathew, E.D. (2014). Tactile perception and working memory in rats and humans. *Proceedings of the National Academy of Sciences*, 82(2): 101-122.

Asogwa, E.U., Anikwe, J.C. and Mokwunye, F.C. (2006). Kola production and utilisation for economic development. *African Science Journal*, 7(4):217-222.

Atawodi S.E., Mende, P., Pfundstein, B., Preussmann, R. and Spiegelhalde,r B. (1995). Nitrosable amines and nitrosamide formation in natural stimulants: *Cola acuminata,Colanitida* and *Garcina cola*. *Food Chemistry Toxicology*, 33:625-630.

Ayebe, E. K., Yapi, H. F., Edjeme, A. A., Meite, S., M'boh, M. G., Yapo, A. F., Monnet, D., Djama, A. J. and Nguessan, J. D. (2012).In vivo, in vitro Antioxidant Activity Assessment and Acute Toxicity of Aqueous Extract of *Cola Nitida* (Sterculiaceae). *Asian Journal of Biochemical and Pharmaceutical Research*, 4 (2) :149 ISSN: 2231-2560

Beattie, G.B. (1970). Soft drink flavours: Cola for kola flavours. *The FlavourIndustry*. Pp390 – 394.

Bradbury, P. (1977). Histological Methods. In: Hewer's Textbook of Histology for Medical Students 9th Ed. ELBS, pp 431-450, OCLC 6565 Kilgour Place, Dublin OH USA 43017.

Brinker, F., (2001). Cola. In Herb Contraindications and Drug Interactions, third ed. Eclectic Medical Publications, Sandy, OR, pp. 71–74.

Brown, R.E., Corey, S.C. and Moore, A.K. (1999). Differences in Measures of Exploration and fear in MHC-Cogenic C57BL/6J and B6H-2K mice. *Behaviour. Genetics, 29*, 263-271.

Bruce, R. D. (1985). An up-and-down procedure for acute toxicity testing. *Fundamentals of Applied Toxicology,* 6: 151-157.

Buraimoh, A.A.,Sumanu, A. and Ojo, S.A. (2014). Histological observation of the cerebellum of Wistar rat following administration of ethanolic extract of *Cola nitida. Annals of Experimental Biology*, 2(1): 58-62.

Burdock, G. A., Carabin, I. G. and Crincoli, C. M. (2009). Safety assessment of kola nut extract as a food ingredient. *Journal of Food and Medical Toxicology*, 47: 1725-32.

Cardoso-Lopes, E M., Bentes de Paula, D M., Barbo, F E., Souza, A., Terumi, C (2009). Chemical composition, acetyl cholinesterase inhibitory and antifungal activities of *Pera glabrata* (Schott) baill. *Brazilian Journal of Botany* (Sao Paulo), 32:4.

Chaurasia B.D. (2004) Human Anatomy Regional and Applied, Dissection and Clinical. Fourth Edition*:* Satish Kumar Jain for CBS Publishers and Distributors, 4596/1-A, 11 Darya Ganj, New Delhi - 110 002 (India). P.365.

Chukwu L.U., Odiete W.O., Briggs L.S. (2006). Basal Metabolic Responses and Rhythmic Activity of Mammalian Hearts to Aqueous Kola nut extracts. *African Journal of Biotechnology*,5(5):484–486.

Conrad, C.D. (2008). Chronic stress-induced hippocampal vulnerability the glucocorticoid vulnerability hypothesis. *Revised Neuroscience,*19: 395–411.

Culling, C. F. A. (1963). Handbook of Histopathological technique 2nd. Ed Butterworth's,
London.

Davis, J. M. and Bailey, S. P. (2003). Central nervous system effects of caffeine and adenosine on fatigue. *American Journal of Physiology*, 284(2): 399-404.

Elizabeth, B. U., Osim E. E. and Udoh P. B. (2011). The comparative effects of chronic consumption of kola nut (*cola nitida*) and caffeine diets on exploration, anxiety and fear in Swiss white mice *International Research Journal of Pharmacy and Pharmacology*, 1(5) pp. 093-099. ISSN: 2251-0176

Ellman, G. L. (1959). Tissue sulfhydril group, *Arch BiochemistryBiophysiology*, 82:70-7.

Epega, A. A. (2003). *Obi Divination*. Athelia Henrietta Press. pp. 1–2.

Erbe, L. and Lawrence, E. (2014). "Cola". *Encyclopedia Americana* (Grolier Online).

Erowid.Org, (2015). Erowid Psychoactive Vaults' LD_{50} and Material Safety Data Sheets. Retrieved 23 March, from *http:// erowid.org/psychoactives/health/psychoactives LD_{50}s. html.*

Esimone, M.U., Charles, O.,Adikwu, C.S., Nwosu, F., Okoye, B.C. and Odimegwu, D.C. (2007). *Scientific Research and Essay*, 2(7): 232-237.

Evans, W.C. (1996). Trease and Evans Pharmacognosy. WB Saunders company Ltd, London-Philadelhia-Toronto-Sydnay-Tokio., P.612.

Food and agricultural Organization, (1982). Fruit bearing forest trees. Forestry Paper No.34. FAO, Rome. Pp 177.

Fisone G, Borgkvist A, Usiello A (2004). "Caffeine as a psychomotor stimulant: mechanism of action". *Cellular and Molecular Life Science*,61 (7–8): 857–72.

Fridovich, I. and Beyer W. F. Jr. (1989). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *AnalyticalBiochemistry*, 161(2):559-66.

Gray, L., Conor, D. C., and Christine, M. G. (2014). Pharmacological enhancement of memory or cognition in normal subjects. *Frontiers in Systems Neuroscience*, 8: 90.

Graham, T.E. and Spriet, L.L. (1991). "Performance and metabolic responses to a high caffeine dose during prolonged exercise". *Journal of Applied Physiology, 71(6),* 2292-8.

Greden, J.F., Fountaine, P., Lubestky,M. and Chamberlin, K. (1978). Anxietyand depression associated with caffeinism among psychiatric inpatients *American Journal of Psychiatry*, 135: 963-966.

Guyton, A.C. and Hall, J.E. (2006). Textbook of Medical Physiology 11th ed.). Published by Elseveir, a division of Reed Elsevier India Private Limited, Sri Pratap Udyog, 274, Captain Gaur Marg, Sriniwaspuri, New Delhi. 110-065, Idia.

Haustein A. (1971). La noix de Cola:coustumes et rites de quelques ethais de cote d'Ivore. Anthropos, 69:457–49.

Huang, Z. L., Qu, W. M., Eguchi, N., Chen, J. F., Schwarzschild, M. A., Fredholm, B. B., Urade, Y. and Hayaishi, O. (2005). "Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine". *Nature Neuroscience*,8(7): 858–9.

Ibu, J.O., Iyama, A.C., Ijije, C.T., Ishmael, D., Ibeshi, M. and Nwokediuko, S. (1986). The effect of *Cola acuminata* and *Cola nitida* on gastric acid secretion. *American Journal of Gastroenterology Surgery*,124:39–45.

Igwe, S.A., Dakunyili, D.N.and Ikonne, E.U. (2007). Ocular effects of acute ingestion of *Cola nitida* (Linn) on healthy adult volunteers. *South African Optomology.* 66(1):19–23.

Ikegwuonu, F.I., Aire, T.A., and Ogwuegbu, S.O. (1981) Effects of kola-nut extract administration on the liver, kidney, brain, testis and some serum constituents of the rat.*Journal of Applied Toxicology,* 1(6):292–294.

Jayeola C.O. (2001). Preliminary studies on the use of kola nuts (*Cola nitida*) for soft drink production. *Journal of Food Technology Africa,* 6(1): 25-26.

Julien, R.M. (2005). A Primer of drug action. 10th ed. New York: Worth Publishers-Medical- 690 pages.

Kandel, E. R., Schwartz, J. H. and Jessell, T. M. (2000). *Principles of Neural Science Fourth Edition*. United State of America: McGraw-Hill. p. 324. ISBN 0-8385-7701-6

Karau, M., Kihunyu, J., Kathenya, N., Wangai, L., Kariuki, D. and Kibet, R. (2010). "Determination of caffeine content in non-alcoholic beverages and energy drinks using Hplc-Uv method". *African Journal of Drug and Alcohol Studies* (Centre for Research and Information on Substance Abuse),9(1): 15–21.

Kataria, S., Mehra, L. and Badiye A. (2012).A Study of Pharmacokinetics of Sodium Thiopental in Rats byHistological Examination of Kidney. *Research Journal of Pharmaceutical Sciences*,1(2), 1-6.

Kouame, K., and Scande, M. (2006). *Cola nitida* (vent) Schott and Endl. *Seed Leaflet,Forest and Landscape*. (111):1–2.

Labark,S. and Cabeza, R. (2006). Cognitive neuroscience of emotional memory. *Nature Reviews Neuroscience,* 7: 54–64.

Lebbie, A.R. and Guries R.P. (1995). Ethnobotanical value and conservation of sacred groves of the Kpaa Mende in Sierra Leone. *Economy Botany,* 49(3): 297-308.

Lim, T.K. (2012). *Edible Medicinal and Non-Medicinal Plants: Fruits*., Springer Science Business Media B.V. 3:175-182.

Lister, R.G. (1987). The Use of a Plus-Maze to Measure Anxiety in Mouse. *Psychopharmacology,*92:180-185.

Liu, Li., Jiong D., Charles, M., Junying G., Gang., Hu, M. and Xiao, K. (2011) Pre-training affects Morris water maze performance with different patternsbetween control and ovariectomized plus d-galactose-injected mice. *Behavioral Brain Research,* 217:1- 27.

Lopez-Garcia, E., Rob, M. D., Swapnil, R., Walter, C. W., Manson, J. E. and Hu, F. B. (2006). Changes in caffeine intake and long-term weight change in men and women. *American Journal of Clinical Nutrition,* 83 (3): 674-680.

Lovejoy, R. and Paul, S. (1980). Kola in the history of West Africa**.** *Cahiers d'Etudes Africaines,*20: 1-2, 97.

Lown, J.W. (1993). 'Discovery and Development of anthracycline Antitumour, Anti biotics''. *Royal Society of Chemistry*, P165.

Lowor, S. T., Aculey, P. C. and Assuah, M. K. (2010). Analysis of some quality indicators in cured. *Cola nitida* (Vent) *Agriculture and Biology Journal of North America*. 1(6):1206–1214.

Mahdavi1, R., Daneghian, S., Homayouni, A. and Jafari, A. (2012)Effects of Caffeine Supplementation on Oxidative Stress, Exercise-Induced Muscle Damage and Leukocytosis*Pharmaceutical Science*, 18(3): 177-182.

McDonald, R.J.and White, N.M. (1994). Parallel information processing in the water maze: Evidencefor independent memory systems involving dorsal striatum and hippocampus. *Behavioural Neural Biology*, 61:260–270.

McGuffin, M., Hobbs, C., Upton, R., and Goldberg, A. (1997). Cola acuminata (P. Beauv.) Schott. & Endl. In: Botanical Safety Handbook. CRC Press, Boca Raton, FL (Pp. 34– 35, 172, 176, 185).

Moore, K. L., Dally, A.F., Agur, A.M.R. (2010). Clinically Oriented Anatomy, Lippincott Williams and Wilkins, a Wolters Kluwer business Philadelphia USA. p.878.

Mrvos, R.M., Reilly, P.E., Dean, B.S. and Krenzelok, E.P. (1989). Massive caffeine ingestion resulting in death". *Journal of Veterinary and Human Toxicology, 31 (6)*: 571-2.

Muhammad, S. and Fatima, A. (2014). Studies on phytochemical Evaluation and antibacterial properties of two varieties of kola nut *(Cola nitida)* in Nigeria. *Journal of Bioscience and Medicines,*2:37-42.

Mukinda, J.T. and Syce, J.A. (2007). Acute and Chronic toxicity of the aqueous extract of artemesia arfa in rodents. *Journal of Ethnopharmacology*, 112:138-144.

Neil, J.F. (1978).Caffeinism complicating hypersomic depressive episodes. *Comprehensive Psychiatry, 19*: 337.

Newall, C.A., Anderson, L.A. and Philipson, J.D. (1996). Herbal Medicine: A Guide for Health CareProfessionals London. The Pharmaceutical Press, Pp.199- 200.

Ncube, N.S., Afolayan, A.J. and Okah, A.I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin:current methods and future trends. *African Journal of Biotechnology*, 7 (12):1797-1806.

Niehaus, W. G. and Samuelson, B. (1968). Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *European journal Biochemistry*, 6: 126-130.

Nmaju, A. U., Bisong S. A., Nwankwo, A. A., Joshua, I. E.and Osim, E. E. (2014). Comparative Effects of *Garcinia kola* andCoffee Diets on Learning and Memory in Mice *British Journal of Medicine and Medical Research*, 4(2): 731-74.

Obika, L.F.O., Babatunde, E.O., Akoni, F.A., Adeeko, A.O., Nsaho, J., Reza, H. and Williams, S.A. (1996). Kola nut (*kola nitida*) enhances anti-diuretic activity in young dehydrated subjects. *International Journal of Phytotherapy Research*, 10: 563-568.

Odebunmi, E.O., Oluwaniyi, O.O., Awolola, G.V. and Adediji, O.D.(2009) Proximate and nutritional composition of kola nut (*Cola nitida*), bitter cola (*Garcinia cola*) and alligator pepper (*Afromomum melegueta*). *African Journal of Biotechnology*, 8(2):308–310.

Ogutuga, D.B.A. (1975). Chemical composition and potential commercial uses of kola nut*Cola nitida.* Ghana. *Journal of Agricultural Science*, 8: 121-125.

Oladukun, A.O., (1989). Nut weight and nutrient content of *Cola acuminate*(Sterculiaceae). *Economy Botany*, 47: 17-22.

Osim, E.E.and Udia, P.M. (1993). Effects of consuming a kolanut (*Cola nitida*) diet on mean arterial pressure in rats. *International Journal Pharmacognosy* 31:193–197.

Osim, E.E. (2003). Neurophysiology, 2nd edition. Calabar, El Sapphire Limited. Pp.198.

Oyedeji, K. O. Bolarinwa, A. F. and Alamu, Y.S. (2013). Effect of Aqueous Extract of *Cola Nitida* (Kola Nut) On Haematological and Plasma Biochemical Parameters in Male Albino Rats. *Journal of Pharmacy and Biological Sciences*,4 (6): 45-48.

Puntel, R. L., Nogueira, C. W., and Rocha, J. B.T. (2005). Krebs cycle intermediates modulatethiobarbituricacid reactive species (TBARS) production in rat brain *in vitro*. *Neurochemistry Research*; 30:225-235.

Quirino Cordeiro Júnior, M.D. Marcília de Araújo Medrado Faria, M.D.and Renério Fráguas Júnior, M.D.(2012). Depression, Insomnia, and Memory Loss in a Patientwith Chronic Intoxication by Inorganic Mercury.*The Journal of Neuropsychiatry and Clinical Neurosciences*,191-205

Rajagopalan, R., Kode, A., Penumatha, S.V., Kallikat, N.R., and Venugopal, P.M. (2004). Comparative effect of curcumin and an analog of curcurmin on alcohol and PUFA induced oxidative stress. *Journal of Pharmaceutical Science*, 83:2747-2752.

Rodgers, R.J. (1997). Animal models of "anxiety": where next? *Behavioural. Pharmacology*, 8: 477-496.

Rujito, L. Mulatsih, S. and Sofro, A.S. (2015). Status of superoxide Dismutase in Transfusion dependent thalassemia. *North American Journal of Medical science*, 7(5):194-8.

Russell, S. (2007). Caffeine consumption in pregnant women and its effects on infant health, *Health Psychology Home Page.*

Saladin, K. (2010). Anatomy and Physiology: The Unity of Form and Function, 5th Ed. New York: McGraw-Hill Companies Inc, Print.

Schalm, O.W., Jain, N.C. and Carroll, E.J.(1975). Veterinary haematology. 3rd ed. Lea and Febiger, Philadelphia. U.S.A. Pp.123-136.

Sharma, S., Rakoczy, S. and Brown-Borg, H. (2010). Assessment of spatial memory in mice. *Life Sciences,* 87(17-18): 521-536.

Shipp, S. (2007). "Structure and function of the cerebral cortex". *Current Biology*,17 (12): R443–9.

Sinha, A. K. (1972). Calorimetric Assay of Catalase. *Analytical Biochemistry*, 47: 389-394.

Togola, A., Austarheim, I., Diallo, A., Theisis, A. and Paulsen, B.S. (2008). Ethnopharmacological uses of Erythrina senegalensis, a comparison of three areas in Mali and link between traditional knowledge and modern biological science, *Journal of Ethnobiology Ethnomedicine,* 4: 6.

Tripathi, L. and Tripathi J.N. (2003). Role of biotechnology in medicinal plants. *Tropical Journal of Pharmaceutical Research*, 2(2): 243-253.

Trullas, R. and Skolnick, P. (1993). Differences in fear motivated behaviours among inbred mouse strains. *Journal of Psychopharmacology*,111:323-331.

Umoren, E. B., Osim, E. E. and Udoh, P. B. (2009). The comparative effect of chronic consumption of kola nut (*Cola nitida)* and caffeine diets on locomotor behaviour and body weights in mice. *Niger Journal of Physiological Science*, 24(1):73-78.

Urzúa, Z. X.., Huerta, T. M., Trujillo-Hernández, B., Ríos-Silva, M., Onetti, C., Ortiz-Mesina, M. and Sánchez-Pastor, E. (2012). Effects of chronic caffeine administration on blood glucose levels and on glucose tolerance in healthy and diabetic rats. *The Journal of International Medical Research,*40: 2220 – 2230

Utu-Baku, A.B., Obochi, G. O., Inah, S. A., Atangwho, J. I., Ugon, E. A., Ogbeche, J. O., Idenyi, J. N. and Ukah, D. A. (2009). The influence of alcohol kola nut constituent on liver Aspartate, amino transferse and Alanine aminotransferase enzymes on Albino rats.*International Journal of Biological Chemistry*, 3(1): 25-29.

Vatassery, G.T. (1998). Vitamin E and other endogenous anti-oxidants in the central nervous system. *Geriatrics,* 53 (1): 25-27.

Wolf, U. Rapoport, M.J. and Schweizer, T.A. (2009). "Evaluating the affective component of the cerebellar cognitive affective syndrome". *Journal of Neuropsychiatry Clinical Neuroscience*, 21 (3):245–53.

World Health Organization (2000). Guidelines to promote proper use of alternative medicine Fact Sheet No. 164 and 204, October, 2000.

World Agroforestry Centre. (2015). Agroforestry database. http://www.worldagroforestry.org/sea/Products/AFDbases/af/index.asp.Accessed August, 2015.

Appendices

Appendix I: Calculation of the extract dose.

Extracts used = Aqueous extract, di-chloromethane caffeine fractionand supplement.

LD_{50} of Aqueous extract = 3000mg/kg body weight

LD_{50} of caffeine extract = 192mg/kg body weight

10%, 20%, and 30% of the LD_{50} were used as low, medium and high dose respectively.

Dose of extracts used

caffeine extract

$\frac{10 \times 192}{100}$mg/kg =19.2mg/kg

$\frac{20 \times 192}{100}$mg/kg =38.4mg/kg

$\frac{30 \times 192}{100}$mg/kg = 57.6mg/kg

19.2mg ~~1000g~~ ▶

Xmg ⟶ 222.75g = 4.28mg

38.4mg~~1000g~~ ▶

Xmg 22~~2.50g~~ ▶ 8.54mg

57.6mg ~~1000g~~ ▶

Xmg222~~.25g~~ ▶12.80mg

The animals in groups B1, B2 and B3 received 4.28, 8.54 and 12.80mg/kg average group weight respectively.

Aqueous extract

$\frac{10 \times 3000}{100}$ = 300 mg/kg was used as the low dose of Aqueous extract

$\frac{20 \times 3000}{100}$ mg/kg= 600mg/kg was use as the medium dose of Aqueous extract

$\frac{30 \times 3000}{100}$mg/kg = 900 mg/kg was the low dose of Aqueous extract.

300mg ⟶ 1000g

X 22.25g = 66.68mg

600mg 1000g

X 221.50g = 132.90mg

900mg 1000g

X 221.50g = 199.35mg

The animals in groups C1, C2 and C3 received 66.68, 132.90 and 199.35mg/kg average group weight respectively.

D1 received 10g *Cola nitida* + 90g chicken mesh

D2 received 20g *Cola nitida*+ 80g chicken mesh

D3 received 30g *Cola nitida* + 90g chicken mesh

Group A animals were used as control and were administered distilled water orally.