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

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DEPARTMENT OF HUMAN ANATOMY, FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

NOVEMBER, 2016
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 nuts 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 210-230 grams 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 fraction of 19.2, 38.4 and 57.6 mg/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 those used 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 entries into 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 showed statistical significant decrease in superoxide 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 ($p > 0.05$) and decrease ($P > 0.05$) 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.
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<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>CC</td>
<td>Central chromatolysis</td>
</tr>
<tr>
<td>CDPC</td>
<td>Clumped Degenerating Pyramidal Cells</td>
</tr>
<tr>
<td>DN</td>
<td>Degenerating neuron</td>
</tr>
<tr>
<td>DPC</td>
<td>Degenerating Pyramidal cells</td>
</tr>
<tr>
<td>ECA</td>
<td>Entry into close arm</td>
</tr>
<tr>
<td>EOA</td>
<td>Entry into open arm</td>
</tr>
<tr>
<td>EOS</td>
<td>Eosinophils</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione concentration</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HD</td>
<td>Head dip</td>
</tr>
<tr>
<td>IU</td>
<td>International unit</td>
</tr>
<tr>
<td>LCDPC</td>
<td>Layer of Clumped Degenerating Pyramidal Cells</td>
</tr>
<tr>
<td>LYM</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>NEU</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>NPC</td>
<td>Normal pyramidal cells</td>
</tr>
<tr>
<td>PCV</td>
<td>Packed Cell Volume</td>
</tr>
<tr>
<td>PK</td>
<td>Pyknosis</td>
</tr>
<tr>
<td>PC</td>
<td>Pyramidal cells</td>
</tr>
<tr>
<td>S</td>
<td>Seconds</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of Mean</td>
</tr>
<tr>
<td>SC</td>
<td>Stellate cells</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide Dismutase</td>
</tr>
<tr>
<td>TBARs</td>
<td>Thiobabituric acid reaction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>TOA</td>
<td>Time spent in open arm</td>
</tr>
<tr>
<td>TCA</td>
<td>Time spent in close arm</td>
</tr>
<tr>
<td>V</td>
<td>Vacuolation</td>
</tr>
<tr>
<td>WAA</td>
<td>Weight after administration</td>
</tr>
<tr>
<td>WBA</td>
<td>Weight before administration</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cells</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>%WC</td>
<td>Percentage weight change</td>
</tr>
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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 Sterculioideae. 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 is an 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 in identifying 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, aqueous extract and di-chloromethane caffeine fraction extract administration of *Cola nitida* on the cerebrum and hippocampus of adult Long Evans rats.

1.4.2 Objectives of the Study

The objectives of this study were to:

I. Investigate the effect of *Cola nitida* on spatial learning and memory, and anxiety-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 neurobehaviours of 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 (Lowore 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 Igbo’s 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).
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 and Nigeria. 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 and Fatima, 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 hunger because of 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
gastrointestinal 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 (C₈H₁₀N₄O₂) 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).
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 lemniscal 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, touch sensation, thinking, perceiving, producing and understanding language. Most information processing occurs in the cerebral cortex. The cerebral cortex is divided into lobes of which each has a specific function (Kandel et al., 2000).

**Motor Function:** The cerebrum directs the conscious or volitional motor functions of the body. These functions originate within the primary motor cortex and other frontal lobe motor areas where actions are planned. Upper motor neurons in the primary motor cortex send their axons to the brainstem and spinal cord to synapse on the lower motor
neurons, which innervate the muscles. Damage to motor areas of cortex can lead to certain types of motor neuron disease (Shipp, 2007). This kind of damage results in loss of muscular power and precision rather than total paralysis. The motor area of classical description is located in the precentral gyrus on the superior lateral surface of the hemisphere and in the anterior part of the precentral lobule; the medial surface. This corresponds to area 4 of Broman and possibly part of area 6, which lies in the precentral gyrus, the specific region in the area responsible for movement of specific part of the body (Kandel et al., 2000).

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

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

### 2.4 The Hippocampus

The hippocampus is 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 it is 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).
Plate III: 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 over and over the 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 soaked in 50% methanol for 75 hours, the mixture was filtered and concentrated to 1/10th 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 extract 19.2, 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

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>N</th>
<th>TREATMENT</th>
<th>DURATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>Normal feed and distilled water</td>
<td>21 days</td>
</tr>
<tr>
<td>B1</td>
<td>4</td>
<td>19.2mg/kg caffeine extract from Cola nitida</td>
<td>21 days</td>
</tr>
<tr>
<td>B2</td>
<td>4</td>
<td>38.4mg/kg caffeine extract from Cola nitida</td>
<td>21 days</td>
</tr>
<tr>
<td>B3</td>
<td>4</td>
<td>57.6mg/kg caffeine extract from Cola nitida</td>
<td>21 days</td>
</tr>
<tr>
<td>C1</td>
<td>4</td>
<td>300mg/kg aqueous extract of Cola nitida</td>
<td>21 days</td>
</tr>
<tr>
<td>C2</td>
<td>4</td>
<td>600mg/kg aqueous extract of Cola nitida</td>
<td>21 days</td>
</tr>
<tr>
<td>C3</td>
<td>4</td>
<td>900mg/kg aqueous extract of Cola nitida</td>
<td>21 days</td>
</tr>
<tr>
<td>D1</td>
<td>4</td>
<td>10% Cola nitida supplemented feed w/w</td>
<td>21 days</td>
</tr>
<tr>
<td>D2</td>
<td>4</td>
<td>20% Cola nitida supplemented feed w/w</td>
<td>21 days</td>
</tr>
<tr>
<td>D3</td>
<td>4</td>
<td>30% Cola nitida supplemented feed w/w</td>
<td>21 days</td>
</tr>
</tbody>
</table>

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 latency and 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.
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.
3.8 Animal Sacrifice

All animals were humanely sacrificed on the 22nd day 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 bottles at 4°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 rpm for five minute using microhematocrit centrifuge. The value read hematocrit reader was expressed in percentage.

3.9.2 Haemoglobin (Hb) Determination

A spencer hematogobinometre chamber was charge with a drop of blood and the color was matched against the standard color of hematogobinometre. 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 in WBC 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) and Bradbury (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 photomicrograph of 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 $H_2O_2$: 0.6 ml of $H_2O_2$ was dissolved in little quantity of distilled water and made up to
100 ml. it was stored at 4°C. 0.01M phosphate buffer: 1.2g of NaH₂P0₄ and 1.4lg of Na₂HPO₄ was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 10.2. Diethylamine/Acetic acid solution 5 % Potassium heptaoxochromate (VI) K₂Cr₂O₇ was mixed with glacial acetic acid in the ratio 1:3, and was stored in brown bottle at room temperature. 0.9 ml of 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 H₂O₂ 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 Dichrominate/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 bases of this assay. 0.05 M phosphate buffer: 6.97 g of diphosphate K₂HPO₄ and 1.36 g of KH₂PO₄ 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 Na₂CO₃ and 4.2g of NaHC0₃ 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 0.3ml of 0.3 mM adrenaline. The
reference mixture contains 2.5 ml of 0.05 M Carbonate buffer. 0.3ml of 0.3 mM adrenaline and 0.20 ml of distilled water. Absorbance was measured every 30 up to 150 s at 480nm. 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, (0.25M sucrose solution) were treated with 2ml of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.37%, 0.25N HCL and 15% TBA) and placed in water bath for 1 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 Ellman (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 150μl of serum or tissue homogenate (in phosphate -saline PH 7.4), 1.5ml of 10% TCA was added and centrifuged at 1 500g for 5mm. 1 ml of supernatant was treated with 0.5 ml of Ellman’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 Microsoft Excel 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 48 hours. 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 group also 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 $> 0.05$) 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 $> 0.05$) 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*.

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

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

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

*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 \( (p > 0.05) \) 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*.

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

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 > 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 eosinophil count were not significantly increased when compared with the control. There was significant increase (p > 0.05) in lymphocytes in the groups treated with 20 and 30% of caffeine fraction and significant decrease (p > 0.05) in neutrophils in the 20 and 30% of the aqueous extract groups when compared with the control. (Table 4.4).
<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENT</th>
<th>PCV%</th>
<th>Hb g/dl</th>
<th>WBCx10^9/L</th>
<th>NEU%</th>
<th>LYM%</th>
<th>EOS%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal saline</td>
<td>39.00 ± 9.00</td>
<td>11.50 ± 2.70</td>
<td>7.05 ± 1.05</td>
<td>39.50 ± 2.50</td>
<td>59.00 ± 1.00</td>
<td>1.50 ± 0.50</td>
</tr>
<tr>
<td>B1</td>
<td>19.2 mg/kg caffeine extract from <em>Cola nitida.</em></td>
<td>47.50 ± 1.50</td>
<td>14.05 ± 0.25</td>
<td>9.95 ± 1.35</td>
<td>29.50 ± 0.50</td>
<td>69.00 ± 1.00</td>
<td>1.50 ± 0.50</td>
</tr>
<tr>
<td>B2</td>
<td>38.4 mg/kg caffeine extract from <em>Cola nitida.</em></td>
<td>48.00 ± 5.00</td>
<td>13.85 ± 0.85</td>
<td>9.80 ± 0.40</td>
<td>20.50 ± 1.50*</td>
<td>77.00 ± 1.00*</td>
<td>2.50 ± 0.50</td>
</tr>
<tr>
<td>B3</td>
<td>57.6 mg/kg caffeine extract from <em>Cola nitida.</em></td>
<td>48.50 ± 5.50</td>
<td>13.75 ± 0.95</td>
<td>10.05 ± 2.05</td>
<td>19.50 ± 0.50*</td>
<td>77.50 ± 0.50*</td>
<td>2.50 ± 0.50</td>
</tr>
<tr>
<td>C1</td>
<td>300 mg/kg aqueous extract of <em>Cola nitida.</em></td>
<td>31.00 ± 1.00</td>
<td>8.95 ± 0.50</td>
<td>6.90 ± 0.40</td>
<td>28.00 ± 5.00</td>
<td>72.00 ± 5.00</td>
<td>2.00 ± 0.00</td>
</tr>
<tr>
<td>C2</td>
<td>600 mg/kg aqueous extract of <em>Cola nitida.</em></td>
<td>43.50 ± 1.50</td>
<td>13.00 ± 0.40</td>
<td>6.65 ± 0.45</td>
<td>18.00 ± 2.00*</td>
<td>79.00 ± 2.00*</td>
<td>2.00 ± 0.00</td>
</tr>
<tr>
<td>C3</td>
<td>900 mg/kg aqueous extract of <em>Cola nitida.</em></td>
<td>39.50 ± 0.50</td>
<td>11.45 ± 0.35</td>
<td>6.70 ± 0.60</td>
<td>17.00 ± 3.00*</td>
<td>81.50 ± 1.50*</td>
<td>2.00 ± 0.00</td>
</tr>
<tr>
<td>D1</td>
<td>10% <em>Cola nut</em> supplemented feed.</td>
<td>44.00 ± 2.00</td>
<td>12.95 ± 0.45</td>
<td>8.10 ± 0.20</td>
<td>29.50 ± 1.50</td>
<td>68.00 ± 2.00</td>
<td>2.00 ± 1.00</td>
</tr>
<tr>
<td>D2</td>
<td>20% <em>Cola nut</em> supplemented feed.</td>
<td>49.00 ± 1.00</td>
<td>14.35 ± 0.05</td>
<td>7.10 ± 0.30</td>
<td>32.50 ± 2.50</td>
<td>66.00 ± 2.00</td>
<td>1.50 ± 0.50</td>
</tr>
<tr>
<td>D3</td>
<td>30% <em>Cola nut</em> supplemented feed.</td>
<td>47.00 ± 1.00</td>
<td>13.8 ± 50.15</td>
<td>7.15 ± 1.05</td>
<td>28.00 ± 6.00</td>
<td>70.50 ± 4.50</td>
<td>2.00 ± 0.00</td>
</tr>
</tbody>
</table>

*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 \( \leq 0.05 \)) 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 \( \leq 0.05 \)) in all the other treatment groups when compared with the control. The result of superoxide dismutase (SOD) activity showed a statistical significant decrease (p \( \leq 0.05 \)) in the caffeine and aqueous extract groups and a non-significant decrease (p \( \leq 0.05 \)) was observed in the crude extract groups (Table 4.5).
Table 4.4: Oxidative Stress Parameters of rats administered *Cola nitida*.

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

*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.
4.8 Histological Studies

The results of histological studies 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, clumped cells 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 (Plates XXIX, XXX, XXXI and XXXII), with less stain uptake (XXXIII)some normal stellate and pyramidal cells, and vacuolation (Plate XXXIV), normal pyramidal cells and clumped 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 had normal 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 of cerebral cortex (layer II and III) of rat treated with 300mg/kg aqueous extract of *Cola nitida* showing: Vacuolation (V), Some normal Stellate cells (SC) and Pyramidal cells (PC), (H and E × 250).
Plate IX: Section of cerebral 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).
Plate X: Section of cerebral 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 with 10% *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 supplemented with 30% *Cola nitida* showing: Vacuolation (V), Clumped cells (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 administered 38.4mg/kg caffeine extract showing: Clumped cells (CC), Pyknosis (PK) (H and E × 250).
Plate XVI: Section of cerebral cortex (layer II and III) of rat administered 57.6mg/kg caffeine extract showing: Degenerating neuron (DN), Vacuolation (V), Clumped Cells (CC) (H and E x 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 *Cola nitida* 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 *Coluanitia* 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).
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 extract showing; Layer of Clumped Degenerating Pyramidal Cells (CDPC) (H and E × 250).
Plate XX: Section of cerebral cortex (layer II and III) of rat from control group 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 with 600 mg/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 with 900mg/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 nitid* 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), Clumped cells (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 extract showing; Degenerating neuron (DN), Stellate cell (SC) (Toluidine blue × 250).
Plate XXXVIII: Section hippocampus (CA3 region) from control group showing 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 Pyramidal Cells (CDPC), (Toluidine blue × 250).
Plate XL: Section of hippocampus (CA3 region) of rat treated with 600mg/kg aqueous extract of *Colanitida* showing: Clumped Degenerating Pyramidal Cells (CDPC) (Toluidine blue × 250)
Plate XLI: Section of hippocampus (CA3 region) of rat treated with 900mg/kg aqueous extract of *Cola nitida* 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) (Toluidine blue × 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 extract showing Clumped Pyramidal Cell (CPC) (Toluidine blue × 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 for group 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 containing supplement of *Cola nitida*. It is known that chronic 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 in body 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 of Nmajuet *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 an increased 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
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 of Oyedeji 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 nitida* 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 study resulted in the following:

1. Non-significant increase in weight ($p > 0.05$) 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


Appendices

Appendix I: Calculation of the extract dose.

**Extracts used** = Aqueous extract, di-chloromethane caffeine fraction and 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**

\[
\frac{10 \times 192 \text{mg/kg}}{100} = 19.2 \text{mg/kg}
\]

\[
\frac{20 \times 192 \text{mg/kg}}{100} = 38.4 \text{mg/kg}
\]

\[
\frac{30 \times 192 \text{mg/kg}}{100} = 57.6 \text{mg/kg}
\]

19.2mg 
\[
\frac{\text{1000g}}{X \text{mg}} \leftarrow 222.75 \text{g} = 4.28 \text{mg}
\]

38.4mg 
\[
\frac{\text{1000g}}{X \text{mg}} \leftarrow 222.50 \text{g} = 8.54 \text{mg}
\]

57.6mg 
\[
\frac{\text{1000g}}{X \text{mg}} \leftarrow 222.25 \text{g} = 12.80 \text{mg}
\]

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

10 x 3000 = 300 mg/kg was used as the low dose of Aqueous extract
100

20 x 3000 mg/kg = 600 mg/kg was used as the medium dose of Aqueous extract
100

30 x 3000 mg/kg = 900 mg/kg was the low dose of Aqueous extract.
100

300mg → 1000g
X 222.25g = 66.68 mg

600mg → 1000g
X 221.50g = 132.90 mg

900mg → 1000g
X 221.50g = 199.35 mg

The animals in groups C1, C2 and C3 received 66.68, 132.90 and 199.35 mg/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.