SOME NEUROPHARMACOLOGICAL PROPERTIES OF METHANOLIC STEM BARK EXTRACT OF *FICUS INGENS* (MIQUEL) MIQUEL IN MICE AND CHICKS

BY

RAYMOND OGBONNA OFFIAH

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,

FACULTY OF PHARMACEUTICAL SCIENCES,

AHMADU BELLO UNIVERSITY, ZARIA.

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By

Raymond Ogbonna OFFIAH (BSc Biochem, UNIZIK, 1996)

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MAY, 2013

DECLARATION

I declare that the work in this thesis entitled "SOME NEUROPHARMACOLOGICAL PROPERTIES OF METHANOLIC STEM BARK EXTRACT OF *FICUS INGENS* (MIQUEL) MIQUEL IN MICE AND CHICKS" has been performed by me in the Department of Pharmacology and Therapeutics under the supervision of Professor O.A. Salawu and Professor J.A. Anuka. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any other institution

Name of student	Signature	Date

CERTIFICATION

This thesis entitled "SOME NEUROPHARMACOLOGICAL PROPERTIES OF METHANOLIC STEM BARK EXTRACT OF *FICUS INGENS* (MIQUEL) MIQUEL IN MICE AND CHICKS" by Raymond Ogbonna OFFIAH, meets the regulations governing the award of the degree of Master of Science (Pharmacology) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Professor O.A. Salawu (Chairman, Supervisory Committee)	Date
Professor J.A. Anuka (Member, Supervisory Committee)	Date
Dr. N.M. Danjuma Head of Department	Date
Professor J.A. Adebayo Dean, School of Postgraduate Studies	Date

DEDICATION

This project is dedicated to my parents- MR RAYMOND IGBOKWE OFFIAH (of blessed/happy memory) and MRS MARIA ONYEJELU OFFIAH.

ACKNOWLEDGEMENT

All glory, praise and thanks are due to God Almighty as I appreciate all the pencils He used in writing this chapter of my life.

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My prayer is that the Good LORD will remember you in all your needs. Amen.

TO GOD BE THE GLORY

ABSTRACT

Ficus ingens (Miquel) Miquel belongs to the Moraceae family. In some African countries, extracts of the bark are administered to cows with a low milk production, and to people suffering from anaemia, piles and diarrhea. The behavior of living organisms is a visible manifestation of activity of the central nervous system, and mental and behavioural disorders pose a burden to the affected individual and the society at large, and anxiety disorders are inclusive occurring as the most common mental disorder. In this study, the behavioural effects of methanolic extract of *Ficus ingens* (Miquel) Miquel stem bark were investigated in mice at doses of 75, 150 and 300 mg/kg, using diazepam-induced sleeping time, hole board test, beam walking assay, elevated plus maze, elevated zero maze and staircase test in mice. The results revealed that the extract significantly ($P<0.0005$) prolonged the duration of diazepam-induced sleep without any effect on the latency to sleep at all the doses tested. The extract slightly decreased the number of head dips in the exploratory behaviour of mice in the hole board test; it also had a slight effect on the motor coordination of the mice in the beam walking assay. The extract showed anxiolysis in the staircase test characterized by significant ($P<0.0005$) decrease in the rearing behaviour. The extract had no significant effect on the number of open arm entries nor the time spent in the open arms as compared to the control group in both the plus maze and the zero maze. The methanolic extract of *Ficus ingens* (Miquel) Miquel stem bark was screened for anticonvulsant activity at doses of 125, 250, 500, 1000 and 2000 mg/kg intraperitoneally (*i,p*.) in mice and chicks against clonic seizures induced by Pentylenetetrazole (PTZ) (85 mg/kg, *i.p.*) and tonic seizures induced by Maximal electro shock (MES) (90 mA, 100Hz, 0.8sec) respectively. The extract at all doses

did not protect the mice from convulsion and mortality from PTZ except with 1000mg/kg dose that had a 100% protection against mortality. The extract at all doses had no effect on the mean recovery time of convulsed chicks. The oral and intraperitoneal LD_{50} were both found to be greater than 5000 mg/kg. These results suggest that the extract contains biologically active principles that have sedative as well as possible anxiolytic properties. The preliminary phytochemical screening revealed the presence of saponins, triterpenoids, alkaloids, flavonoids, phlobatanins and anthraquinones.

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ABBREVIATIONS

AED(s) Anti Epileptic Drug(s)

AIDS Acquired Immune Deficiency Syndrome

AMPA Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid

CNS Central Nervous System

DA Dopamine

DZ Diazepam

EPM Elevated Plus Maze

EPSP Excitatory Postsynaptic Potential

EZM Elevated Zero Maze

GABA Gamma Aminobutyric Acid

GAD Generalised Anxiety Disorder

HLTE Hind Limb Tonic Extention

IPSP Inhibitory Postsynaptic Potential

MAOI(s) Mono Amine Oxidase Inhibitor(s)

mCPP m-chlorophenylpiperazine

MES Maximal Electroshock

NIPRD National Institute for Pharmaceutical Research and Development

NMDA N-methyl-D-aspartate

OCD Obsessive Compulsive Disorder

PD Panic Disorder

PNS Peripheral Nervous System

PTZ Pentylenetetrazole

SEM Standard Error of Mean

SSRI(s) Selective Serotonin Reuptake Inhibitor(s)

TCA Tricyclic Antidepressant

TGB Tiagabine

THLE Tonic Hind Limb Extention

TIA Transient Ischemic Attack

VGB Vigabatrin

WHO World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

The World Health organization (WHO) has recommended, especially in developing countries, the initiation of programmes designed to use medicinal plants more effectively in traditional health care systems (WHO, 1978).

Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plants, animals and mineral based medicine, spiritual therapies, manual techniques and exercises, applied singularly, or in combination to treat, diagnose and prevent illnesses or maintain well being (WHO, 2001). A number of countries are exploring the possibility of developing their own traditional health care system (with plants playing a central role) as an alternative to conventional health care systems which are usually expensive and unaffordable to the vast majority of their citizenry.

WHO (2001) also reported that in some African countries the first line of treatment for children with high fever resulting from malaria is the use of herbal medicine at home while in countries like China, traditional herbal preparations account for about 30% - 50% of the total medicine consumption.

Worldwide, more than fifty (50) million people suffer from epilepsy many of whom are refractory to treatment with standard antiepileptic drugs (AEDs). The current treatment of epilepsy with conventional AEDs is based on neuroprotection either to reduce the duration of seizures or to suppress the occurrence of seizures (Arzimanoglou *et al*., 2002).

In spite of optimal use of available AEDs by epileptic patients, adequate control of seizure has not been achieved (Stables and Kupferberg, 1997), necessitating a clear need for additional options for the physician and patient alike. In addition, AEDs possesses significant side effects such as teratogenicity and adverse effects on cognition and behaviour (Samren *et al.,* 1997; Raza *et al.,* 2001).

Furthermore, conventional antiepileptic agents, despite being very expensive and have to be taken for a very long time even for years (French and Pedley, 2008), can only suppress seizures in about 60-70% patients, the remaining epilepsies are referred to as being refractory, intractable or drug resistant (Avanzini and Franceschetti, 2003).

A number of medicinal plants have been used by traditional healers in the management of many ailments for many years (Sofowora, 1993); hence plant extracts can be important sources of natural and safer drugs for the treatment of epilepsy (Raza *et al.,* 2001).

Stress and anxiety are the most prevalent psychological problems (Norquist and Regier, 2006) and currently receiving attention due to increased incidence of the mental ailments. A number of psychological and therapeutic measures have been used to treat anxiety and stress and obtain a complete cure but none of these can be said to have satisfactory response. Almost all pharmacological treatments may produce side effects (Goldberg, 2004). According to WHO's estimation, about 450 million people worldwide suffer from a mental or behavioural disorder (WHO, 2001). Majority of such patients in the developing countries still rely on traditional healing practices and medicinal plants for treatment of these conditions. Alternative treatments are increasingly being used to alleviate affective disorders (Wildund *et al.,* 2009) and the plant kingdom can be a rich source of bioactive compounds

which can serve as lead compounds in the production of potent agents that can be used in the management of these disorders.

Ficus ingens (Miquel) Miquel which belongs to the Moraceae family (Burrows and Burrows, 2003), is one of the many medicinal plants with long historical use in traditional medical practices (Such as in the treatment of anaemia, piles and diarrhea), nevertheless, scientific research is needed to provide evidences of its safety and efficacy (WHO, 2001).

1.1 Statement of Research Problem

The behavior of living organisms is a visible manifestation of activity of the central nervous system. The nervous system is a complex sophisticated system that regulates and coordinates the body's basic function and activities. It is made up of the central nervous system (CNS) consisting of the brain and the spinal cord and the peripheral nervous system (PNS) which consists of all other neutral elements. When the brain is healthy, it functions quickly and automatically. However when problems occur, the result can be devastating. Major types of disorders of the brain include anxiety related, epileptic, neurological and psychiatric disorders, such as Parkinson's disease, Toilette's syndrome, attention-deficit and hyperactivity disorder (ADHD), schizophrenia, etc (Jansen *et al.,* 1995).

Epilepsy is the most common neurological problem worldwide. Approximately 4 million people in the European Union and the United States suffer from epilepsy. Research findings showed that approximately 3% of the general population will have epilepsy at some time in life (Duncan *et al.,* 2006).

It has been reported that more than 80% of people with epilepsy live in developing countries, where the conditions remain largely untreated due to unavailability of treatment, inadequate

heath delivery systems, lack of trained personnel, lack of essential drugs and traditional beliefs and practices that often do not consider epilepsy as treatable condition (Salih and Mustafa, 2008).

Anxiety disorders are the most common mental disorder in the world and have become a very important area of research in psychopharmacology (Rabbani *et al.,* 2007). Drugs that are the mainstay of treatment have side effects particularly of sedation, impairment of motor coordination and cognitive effects. Due to these unwanted effects, many researches are geared towards finding alternative anxiolytic agents as the current strategy is to have non sedating anxiolytics.

Medicinal plants have continued to play a dominant role since time immemorial for the protection, prevention and treatment of diseases. Approximately 170 herbal drugs were officially recognised in the United States pharmacopeia (Inamol, 2004). It has equally been reported that 80% of the world population rely on herbal medicine (Akerele, 1993). A host of helpful drugs we have today have herbal origins. A good example is the psychoactive drug, reserpine obtained from *Rawoulfia serpentina*. These plants are relatively cheap and available hence their use by majority of the population in developing countries. The World Health Organisation encourages the inclusion of herbal medicines of proven safety and efficacy in the health care programmes of developing countries because of the great potential they possess in combating various diseases (Amos *et al.,* 2001), therefore *Ficus ingens* (Miquel) Miquel with its proven ethno traditional uses will be an added tool for therapeutic use.

1.2 Justification of the Study

In recent years, there is a reawakening of interest in the utilization of medicinal plants due to the high cost and lack of access to synthetic drugs (French and Pedley, 2008), the side effects (such as teratogenicity, ataxias, nystagmus, nervousness, tremor and depression) associated with their uses (Samren *et al.,* 1997 and Duvivier and Pollack, 2009), and the belief that plant extracts can be an important source of natural and safer drugs for the treatment of many disease conditions (including CNS disorders).

The need for an alternative to conventional health care systems and the validation of the folkloric claims of these medicinal plants which will provide scientific basis for the deployment of beneficial ones as phytomedicine in the primary health care of the vast majority of the populace, especially in developing countries (WHO, 2001).

1.3 Theoretical Framework

1.3.1 Maximal electroshock (MES) induced seizure

MES induced seizure causes the spread of seizure discharge from an epileptic focus during seizure activity leading to hind limb tonic extension (HLTE) (Browning, 1992).

The MES assay in animals is used primarily as an indication for compounds which are effective in grandmal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by antiepileptic drugs (Vogel and Vogel, 1997).

Protection against hind limb tonic extension (HLTE) in the MEST predicts anticonvulsant activity of antiepileptic drugs (such as phenytoin, carbamazepine, oxcarbazepine,

lamotrigine) that prevent the spread of seizure discharge from an epileptic focus during seizure activity (Browning, 1992).

Compounds with the ability of protection against excitability and seizure susceptibility, would be important in the suppression of seizure initiation and its spread (Wickenden, 2002).

1.3.2 Pentylenetetrazole (PTZ) induced seizure

PTZ induces seizure by blocking the major inhibitory pathways mediated by GABA at all levels of the CNS. It also acts on the GABA receptor complex (Bum *et al.,* 2001). PTZ induced seizure leads to highly synchronous, large amplitude activity in the thalamic and cortical field potential traces and clonic jerking of the body and fore limbs (Costa *et al.,* 1975). Hence Anticonvulsant activity in PTZ test identifies compounds (e.g. valproic acid) that can raise the seizure threshold in the brain (White *et al.,* 1998).

Furthermore, drugs such as Felbamate that block glutamatergic excitation mediated by N-methyl-D aspartate (NMDA) receptors have shown anticonvulsant activity against PTZ induced seizure (White *et al.,* 1998).

1.3.3 Diazepam-induced sleep

This is a test for detection of sedative activity. The potentiation of diazepam induced sleep is suggestive of central depressant activity (Rakotonirina *et al.,* 2001).

1.3.4 Hole board test (test for exploratory behaviour in mice)

The Hole-Board test is frequently used to evaluate the effects of psychotropic drugs on a rodent's exploration (neophilia) and spacial learning behaviour. The Hole-Board apparatus is designed as an enclosed arena with holes in the floor into which an animal can poke its head

(head-dipping). When confronted with a new environment, the natural tendency of a mouse or rat is to explore any hole present by way of plunging its head in and out of the hole a few times and then move on to the next hole. The Hole-Board test enables assessment of the effects of psychotropic drugs on the rodent's head dipping and exploratory activity. The frequency and duration of head dipping are recorded to provide a measure of exploratory behaviour. In general, a high frequency of head dipping is reflective of normal neophilia while low levels of head dipping is usually indicative of high anxiety level that prevents the animal from exploration (Mulder and Pritchett, 2004).

1.3.5 Elevated plus maze

The plus maze consists of a perpendicular cross that is elevated above the floor, in which the sides of one axis are walled off. There are photo beams on both arms and the edges to record movements. Mice will, on one hand, prefer the safety of the enclosed, darker arms, but on the other hand like to explore the open arms and poke over the edge. Less anxious mice will venture more on to the open arms, and poke their heads more over the edges of the open arms. In this test, it is important to ensure that there are no differences in overall activity levels in the experimental groups; reduced activity could otherwise wrongfully be interpreted as increased measure of anxiety. Monitoring parameters include the total time spent in, distance moved, and entries into the open and closed arms and the number of extensions over the edges of the open arms (Mulder and Pritchett, 2004)

1.3.6 Beam walking assay

This is an assay for determination of pharmacologically-induced motor coordination deficits, and is predictive of sedative effects. This assay is more sensitive than the rotarod test

(Stanley *et al.,* 2005). The main reason is that it detects benzodiazepine induced motor coordination deficits at much lower receptor occupancy as compared to the rotarod test. The walking assays allow individual performances to be monitored more closely (Stanley *et al.,* 2005). The number of foot slips made during the beam walking assay is a more sensitive measure in the detection of motor coordination deficits than the time taken to complete the task or number of falls. Motor coordination deficit is a major side effect of the sedative hypnotic drugs used in the treatment of anxiety.

1.3.7 Staircase test

The staircase test has been proven as a relatively simple reliable and efficient experiment for the screening of anxiolytic agents (Ago *et al.,* 2007). In this test, step climbing is purported to reflect exploratory or locomotor activity while rearing behaviour is an index of anxiety state. This test though originally described for rats, was modified for rapid screening of anxiolytic activity in mice by Simiand *et al.,* (1984).

1.4 Aim and Objectives of the Study

1.4.1 Aim of the study

The aim of this study is to investigate the anticonvulsant, sedative and anxiolytic effects of the methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel.

1.4.2 Specific objectives

1 To carry out preliminary phytochemical screening of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel

2 To carry out acute toxicity studies of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel in mice via oral and intraperitoneal routes.

3 To study the sedative effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel using diazepam- induced sleep test in mice.

4 To evaluate anticonvulsant effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel chemically using Pentylenetetrazole (PTZ) in mice and electrically using Maximal Electroshock (MES) in chicks (one day old).

5 To evaluate the effect of the methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on anxiety-like behaviour in mice using Hole board test, Staircase test, Elevated Plus and Zero Maze test.

6 To evaluate the effect of the extract on motor co-ordination using Beam walking assay

1.5 Hypothesis

Methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel contain bioactive constituents that possess anticonvulsant, sedative and anxiolytic activities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Structure and Function of the Nervous System

The nervous system is divided into two functional units; the central nervous system (CNS) consisting of the brain and spinal cord, and the peripheral nervous system (PNS), which consists of all the other nerve cells in the body. A proper understanding of the physiological and anatomical organization of the nervous system is essential to correctly identify sites and mechanisms of CNS disorders and how drug can modify these disorders.

2.1.1 The organisational structure of the CNS

The central nervous system may be divided into three sections namely; the forebrain, the midbrain and the hindbrain.

The forebrain: also called Proencephalon is further divided into telencephalon, (cerebrum) and the diencephalon (thalamic nuclei). The cerebrum consists of two hemispheres that constitute the bulk of the nervous system. The cerebral hemispheres are composed of the cerebral cortex, certain limbic structures, the corpus striatum, and a multitude of nervous system pathways. The cerebral cortex is divided into four lobes; the frontal, temporal, parietal, and occipital lobes. The pathways formed by axons from neurons in the cerebral cortex transmit information throughout the CNS. Some of these pathways form the corpus callosum and the corona radiata, through which motor and sensory information pass, as well as other pathways interconnecting the four cerebral lobes (Brodal, 1992). The occipital lobe is almost exclusively devoted to the senses of vision. It is divided into a multitude of

different visual areas, of which the largest is the primary visual cortex, the major destination for visual information (Brodal, 1992).

The parietal lobe: is partially devoted to the sense of touch. It is also very important for perceiving the spatial lay out of the environment and effectively moving through it. The temporal lobes contain the primary auditory cortex, an area for hearing similar to the primary visual and somatosensory cortices, as well as secondary auditory area that further process what we hear, including in the left hemisphere, the decoding of words and sentences. The temporal lobes also contain more specialized visual areas for recognizing detailed objects such as faces. The temporal lobes are critical for memory as they contain hippocampus formation and amygdala. The frontal lobes are essential for planning and movement. The rearmost portion of the frontal lobes is the primary motor cortex, responsible for more complex movements. The rest of the frontal lobes, not directly responsible for movement, are collectively termed the prefrontal cortex. The prefrontal cortex occupies about 30 percent of the brain in humans and is indispensible for rational activity (Brodal, 1992).

The diencephalon/thalamic nuclei: Is made up of epithalamus which is composed of several small nuclei and the pineal gland, the dorsal thalamus, (the major sensory relay area to and from the cerebral cortex), the hypothalamus (maintains homeostasis) and the ventral thalamus that functions primarily with the basal ganglia. The hypothalamus is responsible for regulating the vital functions (body temperature, circadian rhythms, blood pressure and glucose level of humans) and feelings of thirst, hunger, anger, aggression and lust. The hypothalamus governs sexual, reproductive development and behavior as well as differentiates men from women, caused by early hormonal influences during nervous system

development. The thalamus is the gateway to the forebrain and all incoming sensory information except smell sense (has a direct route to cortex) go through it before reaching the cortex (Bloom, 1996).

The midbrain: also called mesencephalon, consists of the tectum, the tegmentum, the cerebral peduncles, and the associated substantia nigra. The brain stem houses the most basic programs of survival such as breathing, swallowing, vomiting, and irritation. Since the brain stem is also simply the spinal cord continuing up into the head, it performs functions for the head similar to those that the spinal cord performs for the rest of the body.

The hindbrain: also called the rhombencephalon**,** consists of reticular formation, basal ganglia, and cerebellum. Reticular formation is located in the brain stem and contain network of neurons that project up into the cerebral cortex and basal ganglia and affect general arousal. The reticular formation is also involved in inducing and terminating different stages of sleep (Bloom, 1996).

Cerebellum: is a large protuberance connected to the back of the brain stem. Its size and convoluted surface makes it look like a supplementary brain. The cerebellum is extremely important for proper motor function. Damage to the little lobes at the very bottom causes head tilt, balance problems, and a loss of smooth compensation of eye position for movement of the head. Damage to the ridge that runs up its back affect limb co-ordination (Bloom, 1996).

The basal ganglia: are a system of subcortical structures crucial for planning and producing movement. They receive input from the entire cerebral cortex and projects to the motor centers of the brain cell and, via the thalamus, back to the cortex's motor planning area.

Damage to the basal ganglia can produce symptoms ranging from the tremors and rigidity of Parkinson's disease to the uncontrollable jerky movements of Huntington's disease. The basal ganglia may also have a role in producing non verbal actions and understanding the non verbal behaviours of other people (Lieberman, 2000).

Hippocampus and amygdala: These are the two structures within the forebrain that are essential for memory and emotions respectively.

The hippocampus plays an important role in the storage of new memories, while the amygdala, which is located immediately in front of the hippocampus, serves a vital role in learning to associate things in the world with emotional responses.

Spinal cord: The spinal cord is the connection center for the reflexes as well as the afferent (sensory) and efferent (motor) pathways for most of the body below the head and neck. The spinal cord begins at the brainstem and ends at about the second lumbar vertebra. The sensory, motor, and interneuron are found in specific parts of the spinal cord and nearby structures. Sensory neurons have their cell bodies in the spinal (dorsal root) ganglion. Their axons travel through the dorsal root into the gray matter of the cord. Within the gray matter are interneurons with which the sensory neurons may connect. Also located in the gray matter are the motor neurons whose axons travel out of the cord through the ventral root. The white matter surrounds the gray matter and contains the spinal tracts which ascend and descend the spinal cord. Surrounding both the spinal cord and the brain are the meninges, a three layered covering of connective tissue. The dura mater is the tough outer layer beneath which is the arachnoid. The arachnoid has abundant space within and beneath it (the subarachnoid space) which contains cerebrospinal fluid, as does the space beneath the dura

mater (subdural space). The cerebrospinal fluid supplies buoyancy and provide cushioning effect for the spinal cord and brain. The pia mater is a very thin layer which adheres tightly to the surface of the brain and spinal cord. It follows all contours and fissures (sulci) of the brain and cord. The spinal cord is a rope of neural tissue that runs inside the hollows of the vertebrae, from just above the pelvis up into the base of the skull. In the normal adult the spinal cord ends between lumbar vertebrae LI and L2. A damage to the spinal cord at the fourth cervical vertebra (C4) result in quadriplegia, a total loss of motor and sensory functions from the level of the superior surface of the shoulders and below (Brodal, 1992).

The spinal cord is organized in segments. There are 8 cervical, 12 thoracic, 5 lumbar, 5 sacral segments and 1 coccygeal segment. This arrangement is reflected in the dermatomes of the body (Richard *et al.,* 1997) such that sensory nerves from the fourth and twelfth thoracic vertebrae (T4 and T12) sub serve a narrow band of skin at the level of the nipples and umbilicus, respectively (Lieberman, 2000).

2.1.2 Peripheral nervous system

The peripheral nervous system is composed of 31 pairs of spinal nerves and 12 pairs of cranial nerves. The peripheral nervous system can be divided into a motor nervous system and an autonomic nervous system. The motor axons originate from neurons in the ventral horn, pass through the ventral root into the spinal nerve and terminate in skeletal (somatic) muscle, cardiac muscle, smooth (visceral) muscle or glands (Brodal, 1992; Richard *et al,* 1997). The spinal cord receives (consciously perceived) sensory information from exteroceptors, proprioceptors, and interoceptors which travel from sensory organs through the spinal nerve and dorsal root before synapsing in the dorsal horn of the cord. The

exteroccptors transmit sensations of pain, touch, and temperature while the proprioceptors are responsible for muscle, joint, and tendon perceptions. These receptors are transducers that change sensory modalities into action potentials, which are generated by the receptive neurons. The parasympathetic nervous system composed of cranio-sacral outflow, that is, the fibres originate in the cranial nerves and from the sacral regions of the spinal cord (Brodal, 1992). The cranial outflow of the parasympathetic system supplies the visceral structures in the head via the oculomotor, facial, and glossopharyngeal nerves, and those in the thorax and upper abdomen are supplied via the vagus nerves.

The sacral outflow supplies the pelvic viscera via the pelvic branches of the second to fourth sacral spinal nerves. It slows the heart, lowers the blood pressure, constricts the pupils, and constricts the air passage. In addition it speeds up the digestion of food and plays an important part in defeacation and micturition. It is the 'emptying mechanism' of the body (Brodal, 1992). The sympathetic nervous system is associated with the spinal nerves in a continuous column from the first thoracic nerve to the third lumbar nerve. Although sympathetic neurons originate within the thoracic portion and part of lumbar portion of the spinal cord, they innervate effector organs throughout the body. The somatic motor neuron projects its axon out of the spinal cord and innervates a skeletal muscle. The sympathetic nervous system is involved in organizing energy mobilization resulting in selective energy expenditure, catabolic function and pulmonary adjustments for intense activity and simultaneously prepares the body for emergency in a "fight- or- flight" reaction (Jansen *et al.,* 1995).

The etiology and/or therapy of nervous system disorders (mostly neurological and psychiatric disorders), such as Parkinson's disease, Toilette's syndrome attention-deficit and hyperactivity disorder (ADHD), schizophrenia, and substance use are linked to alterations in dopaminergic transmission. Hence a major breakthrough in the management of these disorders is that they are all treated with drugs that either enhance or impede dopaminergic transmission (Jansen *et al.,* 1995).

Generally, the normal functioning of the central nervous system depends on the fine balance between the inhibitory and excitatory neurotransmitters which mediate transmission of impulses to and from the central nervous system. Dopamine (DA) is a neuro transmitter in the central and peripheral nervous systems where it regulates numerous physiological processes. Within the central nervous system (CNS), dopamine is known to regulate emotion, reward, cognition, memory, endocrine functions, and motor control (Jansen *et al.,* 1995).

2.2 Anxiety

"Anxiety" can be defined as a response to a *potential* threat. It can be conceptually distinguished from a "fear" response to an aversive stimulus that is present. A fear response would occur in response to physical exposure to a predator, while an anxiety like response might be induced by cues (odor, context) signaling the predation risk. The expression of anxiety-like behaviours in mice (e.g., reduced exploration of threatening spaces, increased vigilance) can also differ from the expression of fear behaviours (freezing, escape) (Finn *et al.,* 2003). The most common observation of anxiety is an acute stress response characterized by a state of exaggerated arousal or fear. Anxiety is both a state and a trait. Trait anxiety is

the persistent and durable feature of the individual personality that reflects the way they interact with their physical and social environment. State anxiety, on the other hand, is that seen in a given individual at a given time. It changes over time and in response to the level of stress and to the way that stress is perceived (Sandford *et al.,* 2000).

Generally, anxiety is an adaptive response to supposedly dangerous stimuli, which may distort homeostasis. However, when it becomes disproportional in intensity, chronic and/or irreversible, or not genuine, it manifest as debilitating anxious state presenting itself in form of phobia, panic attacks, post-traumatic stress disorder, social anxiety disorder or generalized anxiety disorder (Gilhotra and Dhingra, 2010).

Anxiety is a psychological, physiological, and behavioural state induced in animals and humans by a threat to wellbeing or survival either actual or potential. It is characterized by increased arousal, expectancy, autonomic and neuroendocrine activation, and specific behaviour patterns. It is also characterized by a persistent and disproportionate fear unrelated to any genuine risk. The function of these changes is to facilitate coping with an adverse or unexpected situations (Steimer, 2002). Anxiety disorders are the most common and among the most disabling of mental disorders in adults and adolescents (Oakley-Browne, 1991). Anxiety disorders affect one-eighth of the total population worldwide, and have become a very important research area in psychopharmacology (Shri, 2010). Most psychiatric patients will have two or more concurrent psychiatric diseases (co-morbidity) within their lifetime. Anxiety may be a concomitant symptom of several major psychiatric diseases. Anxiety symptoms are extremely common in patients with mood disorders, schizophrenia, delirium, dementia, and substance use disorders (Kirkwood and Melton, 2002). A genetic

predisposition to anxiety disorders is undoubted because there is increased likelihood of the disorders in siblings and children of persons with the disorder than in the relatives of someone without anxiety disorders (Goldman, 2001). The amygdala, an almond-shaped mass of nuclei located deep within the medial temporal lobes of the brain, plays a pivotal role in threat processing. It is generally regarded as fundamental for the acquisition of conditioned fear and for the expression of innate and learned fear responses. Efferent neurons emerging from the amygdala activate the sympathetic nervous system, thus driving the classic fight or-flight responses in end organs, such as increased heart rate, blood pressure, pupillary dilation and bronchodilation (Bandelow, 2004).

2.2.1 Classification of anxiety disorders

The spectrum of anxiety disorders includes generalized anxiety disorder, panic disorder and agoraphobia, obsessive-compulsive disorder, phobic disorder (including social phobia), separation anxiety disorders, substance induced anxiety disorder and post-traumatic stress disorder (Cassano *et al.,* 2002).

2.2.1.1 Generalized anxiety disorders (GAD)

Generalized Anxiety Disorders (GAD) is a state of excessive anxiety or apprehension lasting for more than six months. It is an ongoing state of excessive anxiety without any clear reason or focus but with chronic worry "as its essential feature (Gorman, 2003). It is also characterized by perversive anxiety symptoms that are not restricted to specific situations. Features include persistent and constant state of worry and anxiety. This state occurs for more than six months despite lack of an obvious or specific stressor. Symptoms include

tension, restlessness, fatigue, impaired concentration, muscle tension and insomnia. It is increasingly been recognised as an important treatable clinical disorder with an overall prevalence of about 2-5% and twice as many women as men suffers from it. (Gorman, 2003)

2.2.1.2 Panic disorders (PD)

Panic disorder (PD) is characterized by recurrent panic attacks, either unexpected or associated with particular situations. Panic attacks are sudden surges of intense fear or terror, desire of fleeing and of imminent death, going crazy or losing control. These subjective symptoms are accompanied by neurovegetative changes such as palpitation, hypertension, difficulty in breathing, sweating, urge to void the bladder and increased peristalsis. This leads to anticipatory anxiety, avoidance of places where a panic attack would be embarrassing. This may ultimately lead to agoraphobia. Panic attacks usually last for 15-30 minutes although residual effects can persist much longer. The patient is usually afraid that another attack will occur. Symptoms could be somatic or cognitive. Somatic symptoms include tachycardia, palpitations, sweating, shortness of breath, dizziness, nausea, chest pain, chills or hot flushes. The cognitive symptoms include fear of dying, fear of going insane and feelings of unreality (Pinheiro *et al*, 2007).

2.2.1.3 Phobia disorders

Phobia disorders are common and are manifested by overwhelming and irrational fear. In most cases, phobia situations can be avoided or endured, but in some cases, as with agoraphobia, the anxiety associated with the feared situation or object can be incapacitating.

Agoraphobia: The term agoraphobia was derived from the Greek word 'agora' which means 'outdoor market place'. Agoraphobia, in its most severe form, is characterized by a paralyzing terror of being in places or situation from which the patient feels there is neither escape nor accessible help in case of an attack. Consequently, agoraphobic patients avoid confronting feared situations and places.

Social phobia: Social phobia is the fear of being publicly scrutinized and humiliated and is manifested by extreme shyness and discomfort in social settings. This is also known as social anxiety disorder. This results in avoidance of social situations or gatherings. The symptoms range from mild to a full blown panic attack. They include sweating, shortness of breath, palpitations, dry mouth and tremor. Specific social phobia usually involves a phobic response to a specific event. An example is performance anxiety (stage fright) which is the most common specific social phobia and occurs when a person must perform in public. Such phobia response is absent in informal social situations.

Specific phobias: Specific phobias are an irrational fear of specific objects or situations. The most common phobias are fear of animals, flying (pterygophobia), heights (acrophobia), water (hydrophobia), confined spaces (claustrophobia), etc. The symptoms include panicky feelings, sweating, difficult breathing, rapid heartbeat (palpitations) and avoidance behaviour. Phobia situations are avoided or else endured with intense anxiety, or distress and could significantly affect the individual's daily routine (Pinheiro *et al*, 2007).

2.2.1.4 Obsessive compulsive disorder (OCD)

This disorder is characterized by either obsessions (recurrent or persistent mental images, thoughts or ideas) or compulsive behaviours (repetitive rigid self prescribed routines) or both. Although individuals recognize that the obsessive thoughts and virtualized behaviour pattern are excessive and senseless, they cannot stop them in spite of strenuous efforts to ignore or suppress the thoughts or actions. OCD is time consuming, distressing and can disrupt normal functioning of the affected individual (Pinheiro *et al*, 2007).

2.2.1.5 Post traumatic stress disorders (PTSD)

PTSD is a severe and persistent emotional reaction to a traumatic event that severely impairs the affected individual's life. The patient must have directly experienced, witnessed or learned of a life threatening or seriously injurious event and the patient's response is intense fear, helplessness or horror. Symptoms of PTSD include (i) re-experiencing the trauma either in recurrent images, thoughts, flash backs, dreams or distress in similar reminding situations (ii) Avoidance of reminders of the event by the patients which could include thoughts or people (iii) Increased arousal which is characterized by symptoms of anxiety or heightened awareness of danger (Pinheiro *et al*, 2007).

2.2.1.6 Separation anxiety disorders

This occurs mostly in children. It is characterized by extreme distress from the anticipation or actual separation from home, parents or loved ones and extreme worry (about losing or possible harm befalling a loved one or about getting lost, and being separated from loved ones). Symptoms include headache, abdominal pain and vomiting (Pinheiro *et al*, 2007).

2.2.1.7 Substance induced anxiety disorder

This occurs when symptoms of anxiety arise from the use of or withdrawal from the use of some drugs. This occurs especially with substances/drugs of abuse (Pinheiro *et al*, 2007).

2.2.2 **Drugs used in the management of anxiety**

The mechanisms by which various drugs act in the CNS have not always been clearly understood, since the causes of many of the conditions for which these drugs are used (schizophrenia, anxiety, etc) are themselves poorly understood. Nearly all drugs (with a very few exception such as alcohol and general anaesthetic which may have nonspecific actions on membranes) with CNS effects act on specific receptors that modulate synaptic transmission. Suffice it to say that most drugs that affect the CNS act by altering some step in the neurotransmission process. They may act presynaptically by influencing the production, storage, release or termination of action of neurotransmitters. Others may activate or block postsynaptic receptors (Nicolle, 2009). Drugs that alter the functions of CNS achieve this action by non-selective or selective mechanisms. Selective agents (which include the anticonvulsants, antidepressants, antipsychotics, anti-manics, analgesics etc) produce effects upon one or more CNS function via identifiable molecular mechanisms which are unique to target cells bearing receptors for such agents (Bloom, 1996). The non-selective agents affect the neural axis in such a way that the highest centres involved in intellectual disintegration are dulled first. This is followed by loss of coordination at the motor and sensory levels with the vital senses controlling respiration and cardiovascular function being most resistant. They include general anaesthetics, hypnotics, sedatives, simple aliphatic alcohols etc (Bloom, 1996).

Psychotherapies have been shown to be effective in the management of generalised anxiety disorder (GAD) when used alone, and may serve to augment the beneficial effect of anxiolytics. Although benzodiazepines have been shown to be of immense benefits, especially in severe or acute situations when more immediate anxiolytic actions are required, the anti-depressants (paroxetine and venlafaxine) are considered the first line of action (Gorman, 2003). Benzodiazepines, the protypical anxiolyitcs have been in use since 1960's and have been found to produce a rapid anxiolytic action in GAD. Their actions are mediated via the activation of Gamma aminobutyric acid (GABA) system at the $GABA_A$ receptor complex leading to reduced transmission throughout the central nervous system (Salzman *et al.,* 1993).

Buspirone (an azapirone), an anxiolytic which is structurally and pharmacologically distinct from benzodiazepines is reported to be as effective as the benzodiazepine in GAD. It acts by antagonizing serotonin, presynaptically thereby reducing the firing of serotonin-affected nerve fibres. Data have been presented demonstrating the efficacy of imipramine, a tricyclic antidepressant (TCA) in GAD, but its action is similar to buspirone in exhibiting greater benefit in cognitive dimension than the somatic symptoms of the disorder.

Panic disorder (PD) is a disabling condition which appears in late adolescence or early adulthood, affecting more frequently women than men and is frequently characterized by recurrences and sometimes by a chronic course, therefore, most patients require long-term treatments to achieve remission, to prevent relapse and to reduce the risks associated with co morbidity (Marchesi, 2008). Acute panic attacks appear to be associated with increased noradrenergic discharge in the locus ceruleus. Agents that block serotonin reuptake are

effective in preventing panic attacks. It is theorized that panic-disorder patients have a heightened sensitivity to somatic symptoms, which triggers increasing arousal, setting off the "panic attack" mechanism (Reus, 2001). Pharmacologic and metabolic probes have revealed that in panic disorder there are alterations of serotonergic, noradrenergic, GABAergic, and brain cholecystokinin function and that there is corticosteroid dysregulation as well as increased sensitivity to the anxiety provoking effects of carbon dioxide and lactate. GABA has been linked to anxiety by evidences that showed blockage of $GABA_A$ receptor produces severe anxiety both in man and animals (Reschke *et al.,* 1995). Drugs with anxiolytics and anti-panic effects inhibit locus ceruleus firing, decreasing noradrenergic sensitivity, and block the effect of anxiogenic drugs (Ninan, 2001).

Obsessive-compulsive disorder (OCD) is a chronic and severely disabling anxiety disorder with a lifetime prevalence of approximately 2-3% in the general population (Chamberlain *et al.,* 2005). Obsessive-compulsive disorder is characterised by the occurrence of either obsessions, compulsive rituals or, most commonly, both. Obsessions have four essential features which are recurrent and persistent thoughts, impulses, or images that are experienced as intrusive and cause great anxiety. Neurochemically, OCD has been most associated with the serotonergic system. Results of studies of static measures of serotonergic function in obsessive-compulsive disorder have, however, been inconsistent, and other work has focused on more informative dynamic measures. For example, administration of the serotonin (5-HT) agonist m-chlorophenylpiperazine (mCPP) has been accompanied by exacerbation of obsessive compulsive disorder symptoms and a blunted neuroendocrine

response. After treatment with a serotonin reuptake inhibitor, behavioural and neuroendocrine responses to mCPP seem to be normal.

In experimental models of anxiety, anxiogenic agents share in common the property of altering the binding of benzodiazepines to Gamma-aminobutyric acid $GABA_A$ receptor/chloride ion channel complex. Benzodiazepines are thought to bind two separate $GABA_A$ receptor sites: type I, which has a broad neuroanatomic distribution, and type II, which is concentrated in the hippocampus, striatum, and neocortex. The antianxiety effects of the various benzodiazepines and side effects such as sedation and memory impairment are influenced by their relative binding to type 1 and type II receptor sites. 5-hydroxytriptamine (5HT) also appears to have a role in anxiety. Buspirone, a partial 5HT receptor agonist, and certain $5HT_{2A}$ $5HT_{2C}$ receptor antagonists (e.g., nefazodone) may also have beneficial effects (Reus, 2001).

2.2.3 Classification of anxiolytics

2.2.3.1 Sedative/hypnotics

These are central nervous system depressants. A sedative drug (also called anxiolytic) reduces anxiety and exerts a calming effect. The degree of central nervous system depression caused by a sedative should be the minimum consistent with therapeutic efficacy. A hypnotic drug produces drowsiness and encourages the onset and maintenance of a state of sleep. Hypnotic effects involve a more pronounced depression of the CNS than sedative and this can be achieved with most drugs in this class simply by increasing the dose. Graded dose-depression of CNS function is a characteristic of the sedative hypnotics (Rang *et al.,* 2003). Example of drug in this group is the benzodiazepines (chlordiazepoxide, diazepam,

clonazepam, midiazolam, lorazepam, oxazepam, temazepam, lormetazepam, nitrazepam, etc.)

2.2.3.2 Selective serotonin reuptake inhibitors (SSRIs)

SSRIs are the first line treatment of major depression and are proving to be helpful for many anxiety disorders. SSRIs include fluoetine, sertraline, paroxetine, fluvoxamine, citaprolam, etc (Figgitt and McClellan, 2000; Nemeroff, 2003).

2.2.3.3 Tricyclic antidepressants (TCAs)

Drugs in this class include amitriptyline, imipramine, clomipramine, doxepin etc. Studies have shown TCAs to be effective in the treatment of PD, GAD and OCD (Casacalenda and Boulenger, 1998).

2.2.3.4 Mono amine oxidase inhibitors (MAOIs)

The main examples of this drug class are phenelzine, trancylpromine and iproniazid. MAOIs are used for PD or OCD that does not respond to other treatment (Stein and Matsunaga, 2002).

2.2.3.5 Azapirones

The typical example here is buspirone. Buspirone has selective anxiolytic effects, and its pharmacological characteristics are quite different from previously discussed drugs. Buspirone relieves anxiety without causing marked sedative or euphoric effects. Unlike the benzodiazepines, the drug has no hypnotic, anticonvulsant or muscle relaxant properties (Casacalenda and Boulenger, 1998).

2.2.3.6 Beta-Blockers

They are used to treat some forms of anxiety, particularly where physical symptoms such as sweating, tremor and tachycardia are present and troublesome. An example is propranolol (Rang *et al.,* 2003).

2.2.3.7 Atypical antipsychotics

In certain severe cases, these, agents may be useful. They include risperidone, olanzepine, quetiapine, ziprasidone, etc (Casacalenda and Boulenger, 1998).

2.2.4 Medicinal plants used in the treatment of anxiety

A number of medicinal plants are used as complementary and alternative medicines for the treatment of various forms of anxiety. These plants include; *Guirera senegalensis* (Amos *et al.,* 2001), *Entandropharagma angolense* (Amos *et al.,* 2002), *Dalbergia saxatiles* (Yemitan *et al.,* 2003), *Polyanthus muellerianus, Nauclea latifolia, Vertex doniana* (Mann *et al.,* 2003), *Pericopsis laxiflora* and *Sarcocephalus latifolious* (Neuwinger, 1996), Kava kava *(Piper methysticum),* St. John's wort *(Hypericum perforatum),* Passionflower *(Passiflora incarnata)*, Oatstraw *(Avena sativa)*, Lemon balm *(Melissa officinalis),* Lavender *(Lavandula angustifolia),* Valerian *(Valeriana officinalis) and* Skullcap (*Scutellaria lateriflora)* (Volz and Kieser, 1997).

2.3 An Overview of Epilepsy and its Management

An epileptic seizure, occasionally referred to as a fit, is defined as a transient symptom of “abnormal excessive or synchronous neuronal activity in the brain". Epilepsy can also be defined as a brain disorder in which a person has repeated seizures (convulsions or attacks)

over time (Fisher *et al.,* 2005). As the brain goes about its daily functions, millions of electrical and chemical signals pass from its nerve cells out to the body. These electrochemical messages are necessary for almost everything we do and feel. Seizures are episodes of disturbed brain activity that cause changes in attention or behaviour. Seizures can also be referred to as a sudden and abnormally high discharge of electrical activity among large numbers of nerve cells in the brain. This can result in involuntary and unusually large signals being sent to the muscles. The outward effect can be as dramatic as a wild thrashing movement (tonic-clonic seizure) or as mild as a brief loss of awareness. It can manifest as an alteration in mental state, tonic or clonic movements, convulsions and various other psychic symptoms. Sometimes it is not accompanied by convulsions but a full body "slump", where the person simply will lose control of their body and slump to the ground. The medical syndrome of recurrent, unprovoked seizures is termed epilepsy but seizures can occur in people who do not have epilepsy (Fisher *et al.,* 2005).

2.3.1 Classification of epilepsy

Epileptic seizures are classified according to their particular characteristics:

2.3.1.1 Partial or focal-onset seizures

Partial or focal-onset seizures is when the source of the seizure within the brain is localized. Partial or focal seizures affect a small area of the brain; the symptoms experienced by the person depend on the area of the brain that is involved (Chen *et al.,* 1995). Partial seizure is also divided into simple and complex partial seizures.

Simple partial seizures: is when consciousness is unaffected (Chen *et al.,* 1995). The person might experience a jerking arm or leg, or visual disturbance such as flashing lights and abnormal smells. Often the seizure will start in the hand or face, and then spread. A partial seizure may spread within the brain, a process known as *secondary generalization* (Chen *et al.,* 1995).

Complex partial seizures: involve some alteration or loss of consciousness. The person can lose track of where they are for a minute or two. A person in such a state may stare, make meaningless sounds, or jerk one or two limbs around automatically (Duvivier and Pollack, 2009).

2.3.1.2 Generalized seizures

Generalized seizures affect a larger area of the brain than partial ones. In essence the source of the seizure within the brain is distributed (Fisher *et al.,* 2005).

Generalized seizures are divided according to the effect on the body, but all involve loss of consciousness. These include absence, myoclonic, clonic, tonic–clonic, and atonic seizures.

Absence ("petit mal") seizures: occur primarily in children and involve a loss of consciousness lasting 10 to 30 seconds. The eyelids flutter. Children don't fall over but aren't aware of their surroundings and will have no memory of what happened during the seizure (Fisher *et al.,* 2005).

Tonic-clonic ("grand mal") seizures: involve falling, loss of consciousness, and muscle spasms throughout the body lasting 1 to 2 minutes. Bladder control is sometimes lost. On

regaining consciousness, the person may be very tired and confused. A partial seizure can develop into a tonic-clonic seizure (Fisher *et al.,* 2005).

Infantile spasms: which affect only babies, will cause the baby to suddenly bend forward at the neck or waist. Each seizure lasts just a few seconds, but they may occur in clusters. The baby often cries afterwards. A child with infantile spasms tends to have other types of seizures later in life (Duvivier and Pollack, 2009)

Myoclonic seizures: are brief body jerks that last less than a second. There is no loss of consciousness, but individuals may drop whatever they are holding (Fisher *et al.,* 2005).

Atonic or akinetic seizures: are sometimes called "drop attacks" because there is a momentary loss of muscle tone causing the person to fall to the ground without losing consciousness. Injuries are common because there is no warning (Duvivier and Pollack, 2009).

2.3.1.3 Mixed seizure

A mixed seizure is defined as the existence of both generalized and partial seizures in the same patient (Chen *et al.,* 1995).

2.3.2 Causes of epilepsy

Epilepsy that first appears in adulthood is often due to some localized damage such as poor blood flow to the brain, a brain tumour, or a previous head injury. Epilepsy in children can be brought on by meningitis during infancy or abnormalities of brain development, but it is often inherited. Common causes of epilepsy include infectious, traumatic, metabolic or

tumoral conditions or it may be idiopathic, that is unrelated to any underlying cause other than a possible hereditary predisposition (Engel, 2001).

Other common causes include; alcohol, cocaine and some other drugs, exposure to flashing or strobe lights, hormonal changes, hyperventilation, insufficient sleep, stroke or transient ischemic attack (TIA), dementia; such as Alzheimer's disease, traumatic brain injury, infections, including brain abscess, meningitis, encephalitis, and AIDS, congenital brain defect, brain injury that occurs during or near birth, metabolism disorders that a child may be born with (such as phenylketonuria), brain tumor, abnormal blood vessels in the brain and other illness that damage or destroy brain tissue (Duvivier and Pollack, 2009).

2.3.3 Symptoms and complications of epilepsy

The seizures originate in the brain and depending upon the location in the brain where the seizures originated, the symptoms may also vary. Before a seizure, many people experience a warning sign called an aura, which may involve a particular smell, feeling, or visual effect. After a seizure, a person may be confused, tired, or sleepy, experience muscle aches or soreness, and may not remember what happened. Many people believe that someone having an epileptic seizure is in danger of swallowing his or her tongue and choking. In reality, this almost never happens. If you try to prop the mouth open of someone who is experiencing a seizure, you can damage their teeth (or lose a finger). If the person is standing, you should usually do nothing; if they are unconscious, put them on their side with a headrest and loosen the top button of their shirt (Duvivier and Pollack, 2009).

An exception is in status epilepticus, when a seizure either reoccurs for more than 20 to 30 minutes or recurs many times in a short period. It may be provoked after abruptly stopping anti seizure medication (Duvivier and Pollack, 2009).

2.3.4 Prevention of epilepsy

Generally, there is no known way to prevent epilepsy. However, proper diet, sleep, staying away from illegal drugs and alcohol, may decrease the likelihood of triggering seizures in people with epilepsy. Reduce the risk of head injury by wearing helmets during risky activities; this can help lessen the chance of developing epilepsy. Persons with uncontrolled seizures should not drive. A person with uncontrolled seizures should also avoid activities where loss of awareness would cause great danger, such as climbing to high places, biking, and swimming alone (French and Pedley, 2008).

2.3.5 Drugs used in the treatment of epilepsy

Anticonvulsant medications will eliminate or reduce seizures in most people. There are several types, and not all of them work in every case. Examples of anticonvulsants include carbamazepine, lamotrigine, phenytoin, topiramate, and valproic acid. A Physician may need to try a few before finding the right one for a given person (French and Pedley, 2008).

2.3.6 Mechanism of action of AEDs

Many structures and processes are involved in the development of a seizure, including neurons, ion channels, receptors, and inhibitory and excitatory synapses. The AEDs are designed to modify these processes so as to favour inhibition over excitation and thereby

stop or prevent seizure activity. Dynamic target of seizure control in management of epilepsy is achieving balance between factors that influence excitatory postsynaptic potential (EPSP) and those that influence inhibitory postsynaptic potential (IPSP) (Ochoa, 2011).

The AEDs can be grouped according to their main mechanism of action, although many of them have several actions and others have unknown mechanisms of action. The main groups include sodium channel blockers, calcium current inhibitors, gamma-aminobutyric acid (GABA) enhancers, glutamate blockers, carbonic anhydrase inhibitors, hormones, and drugs with unknown mechanisms of action (Ochoa, 2011).

2.3.6.1 Sodium channel blockers

The firing of an action potential by an axon is accomplished through sodium channels. Each sodium channel dynamically exists in the following 3 states:

A resting state, during which the channel allows passage of sodium into the cell.

An active state, in which the channel allows increased influx of sodium into the cell.

An inactive state, in which the channel does not allow passage of sodium into the cell.

During an action potential, these channels exist in the active state and allow influx of sodium ions. Once the activation or stimulus is terminated, a percentage of these sodium channels become inactive for a period known as the refractory period. With constant stimulus or rapid firing, many of these channels exist in the inactive state, rendering the axon incapable of propagating the action potential. AEDs that target the sodium channels prevent the return of

these channels to the active state by stabilizing them in the inactive state. In doing so, they prevent repetitive firing of the axons such as phenytoin, felbamate, sodium valproate and lamotrigine (Ochoa, 2011).

2.3.6.2 Calcium channel blockers

Calcium channels exist in 3 known forms in the human brain: L, N, and T. The influx of calcium currents in the resting state produces a partial depolarization of the membrane, facilitating the development of an action potential after rapid depolarization of the cell. Calcium channels function as the pacemakers of normal rhythmic brain activity. T-calcium channels have been known to play a role in the 3 per second spike-and-wave discharges of absence seizures. AEDs such as ethosuximide and dimethadione that inhibit these T-calcium channels are particularly useful for controlling absence seizures (Ochoa, 2011).

2.3.6.3 GABA enhancers

Gamma-amino butyric acid (GABA) has 2 types of receptors, A and B. When GABA binds to a $GABA_A$ receptor, the passage of chloride, a negatively charged ion, into the cell is facilitated via chloride channels. This influx of chloride increases the negativity of the cell (i.e., a more negative resting membrane potential). This causes the cell to have greater difficulty reaching the action potential. The $GABA_B$ receptor is linked to a potassium channel. $GABA_A$ receptor mediates chloride ion (Cl^-) influx, leading to hyper-polarization of cell and inhibition. Antiepileptic drugs may act to enhance Cl^- influx or decrease GABA metabolism. The GABA system can be enhanced by binding directly to $GABA_A$ receptors,

by blocking presynaptic GABA uptake, by inhibiting the metabolism of GABA by GABA transaminase, and by increasing the synthesis of GABA. GABA is produced by decarboxylation of glutamate mediated by the enzyme glutamic acid decarboxylase (GAD). Some AEDs may act as modulators of this enzyme, enhancing the production of GABA and down-regulating glutamate. Some AEDs function as an agonist to chloride conductance, either by blocking the reuptake of GABA (e.g. tiagabine [TGB]) or by inhibiting its metabolism as mediated by GABA transaminase (e.g. vigabatrin [VGB]), resulting in increased accumulation of GABA at the postsynaptic receptors. Other examples of GABA enhancers are benzodiazepines and barbiturates (Ochoa, 2011).

2.3.6.4 Glutamate blockers

Glutamate receptors bind glutamate, an excitatory amino acid neurotransmitter. Upon binding glutamate, the receptors facilitate the flow of both sodium and calcium ions into the cell, while potassium ions flow out of the cell, resulting in excitation.

The glutamate receptor has four potential binding sites, as follows:

The alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) site,

The kainite site,

The *N* -methyl-D-aspartate (NMDA) site and

The metabotropic site, which has 7 subunits (GluR 1-7).

AEDs such as Phenobarbitone. felbamate and Topiramate that modify these receptors are antagonistic to glutamate and responses to glutamate antagonists differ, depending on the site being affected (Ochoa, 2011).

2.3.7 Medicinal plants used in the management of epilepsy

Some medicinal plants are known to possess anticonvulsant properties most especially the members of the Genus *Ficus* (Moraceae).

2.3.7.1 Ficus exasperata Vahl.

Ficus exasperata Vahl is a terrestrial afro-tropical tree that grows up to about 20 m tall and prefers evergreen and secondary forest habitats. The leaf extract has been used in African traditional medicine to treat epilepsy. A study showed that hydroalcoholic leaf extract of *Ficus exasperata* possesses anticonvulsant properties. The study demonstrated that the hydroalcoholic leaf extract of *Ficus exasperata* inhibited pentylenetetrazole-, picrotoxin- and MES-induced seizures in mice (Woode *et al.,* 2011).

2.3.7.2 *Ficus platyphylla*

Preparations of *Ficus platyphylla* have been used in Nigerian traditional medicine for the management of epilepsy for many years and their efficacy is widely acclaimed among the Hausa communities of Northern Nigeria. Results of studies by Chindo *et al.,* (2009) showed that *Ficus platyphylla* stem bark may contain psychoactive principles with potential anticonvulsant properties.

2.3.7.3 *Ficus religiosa*

Ficus religiosa is reported to have numerous therapeutic uses in folk medicine. Studies showed that methanolic extract of figs of *Ficus religiosa* had anticonvulsant activity against MES and picrotoxin induced convulsions, with no neurotoxic effect, in a dose dependent manner (Singh and Goel, 2009).

2.3.7.4 *Ficus vallis choudae*

Ficus vallis choudae is a plant widely spread in tropical Africa including Nigeria in the treatment of epilepsy. Results from studies suggested that the *Ficus vallis* extract possess biologically active compounds that have anticonvulsant properties (Malami *et al.,* 2010)

2.4 The Plant: *Ficus ingens* (Miquel) Miquel

Ficus ingens (Miquel) Miquel belong to the family: Moraceae (Plates 2.1 and 2.2).

2.4.1 Common names

Common names of *Ficus ingens* (Miquel) Miquel in some countries and languages are;

English: *red-leaved fig,*

Nigeria: Birom – *fut;* Hausa – *guluba (the ripe figs), kaawurri;* Kanuri – besege*, busuwu;* and Tiv = *non*

Other West African countries: Senegal – *a-mara, ga-maral;* Guinea – *a-mara, a-ntan;* Ghana – *galinzieha;* and Niger – *gomegi.* (Irvine, 1961)



Plate 2.1 *Ficus ingens* (Miquel) Miquel with fruits in its natural habitat



Plate 2.2 *Ficus ingens* (Miquel) Miquel stem bark

2.4.2 Description

The red-leaved fig, *Ficus ingens* (Miquel) Miquel is a semi-deciduous, shady tree or rock-splitter, and a beautiful specimen for a large rock garden, (Nichols, 2005).

It is an evergreen tree up to 10 m or higher, with a rounded or spreading crown up to 30 m wide. All the parts have milky latex when broken. The bark is grey, smooth and becomes cracked in older specimens (Burrows and Burrows, 2003).

2.4.3 Distribution and habitat

Ficus ingens (Miquel) Miquel grows in various habitats but usually outside forest and prefers rocky outcrops and cliff faces throughout the bushveld, wooded grassland and coastal regions. It occurs in the moist eastern subtropical or temperate regions of South Africa from the Albany District in the Eastern Cape northwards through Kwa Zulu-Natal, the northern provinces and further northwards into tropical Africa as far north as Ethiopia and across into West Africa. The roots spread across rock faces and penetrate almost imperceptible cracks, sometimes splitting the rock (Burrows and Burrows, 2003).

2.4.4. Uses and cultural aspects

2.4.4.1 Medicinal uses

The bark is considered a tonic. Extracts of the bark are administered to cows with a low milk production. Zulus in South Africa also give the same to people suffering from anaemia. In

Borno, Nigeria, preparations of the bark, roots and leaves are used for piles and diarrhea and as laxative and diuretic (Myburgh *et al.,* 1994).

2.4.4.2 Non-medicinal uses

The fruits are edible but not always palatable although mammals and birds eat them. In Uganda, the wood is used to make stools, tubs and milking vessels. In Malawi, stakes are used to make cattle pounds become live fences. A good quality fibre is obtained in Kenya from the bark. Latex is also present in quantity and has been used as an adulterant in better quality rubbers and as a rubber substitute in Niger. The latex is also used as a substitute disinfectant for iodine.

2.4.5 Documented works on *Ficus ingens* (Miquel) Miquel

One of the documented works on this plant is the investigation of the chemical constituents and hepatoprotective effect of *Ficus ingens* (Miquel) Miquel (Moraceae) extract against carbon tetrachloride-induced acute liver damage in male Wistar albino rats. The result revealed that the ethanol extract of *Ficus ingens* (Miquel) Miquel showed protective activity against liver injury, which might be developed into a new hepatoprotective agent (Donia *et al*., 2013).

Another study investigated the *in vitro* antiprotozoal activity of twenty-five plants [including *Ficus ingens* (Miquel) Miquel] collected from the Arabian Peninsula. The result revealed *that* antiplasmodial activity was found in the extracts of *Chrozophora oblongifolia, Ficus ingens* (Miquel) Miquel, *Lavandula dentata* and *Plectranthus barbatus* (Al-Musayeib *et al., 2012*).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Animals

Mice and chicks were used in this study. Mice were obtained from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, while one day old chicks were obtained from the National Animal Production and Research Institute (NAPRI), Zaria. The mice were maintained on feed formulated by the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Water was provided *ad libitum* for the mice from the public water supply of the A.B.U Dam. The Mice were housed in cages and transferred to the main pharmacology laboratory where they were allowed to acclimatize before experimentation. Animals of both sexes were used and the animal groupings were based on weight.

3.2 Plant Material

3.2.1 Collection of plant sample

The whole plant (stem bark, leaves and flower) of *Ficus ingens* (Miquel) Miquel was collected by Maallam Muazzam Ibrahim from Sabo Wuse Niger state of Nigeria and identified by Mrs. Jemilat Ibrahim of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu- Abuja where a voucher sample with specimen number (NIPRD/H/6590) was prepared and deposited for future references.

3.2.2 Preparation of plant extract

The stem bark was air-dried for eleven days at room temperature and crushed. About 100 g of the dry plant material was macerated in 70% methanol for 48 hours. The resulting mixture was filtered using muslin cloth followed by Whatman filter paper (No. 1). The aliquots obtained were dried on water bath at 40°C and stored at 04°C until required for use.

3.3 Drugs, Chemicals and Equipment

The following Drugs/chemicals were used in the experiment;

i. Pentylenetetrazole (Sigma-Aldrich, United States)
ii. Valproic acid (Sanofi-Synthelabo, United Kingdom)
iii. Diazepam (Roche, France)
iv. Normal saline
v. Phenytoin (BDH Chemicals, Poole, England)
vi. Ethyl alcohol (BDH Chemicals, Poole, England)

The equipments employed in this study were;

i. Electroconvulsive machine (Ugobasile electro-convulsive machine; ECT unit 7801)
ii. Mettle balance (Mettler 162)
iii. White painted wooden board (60 cm x 30 cm) with 16 evenly spaced holes (1cm diameter x 2 cm depth).
iv. Platform (a ruler 80 cm long, 3 cm wide)
v. Bench

vi. Metal support to a goal box.

vii. A goal box (Perspex glass cage with wood chippings, beddings and a small hole at the bottom).

viii. Transparent Perspex glass

ix. Wooden staircase

x. Maze, made up of two open arms (35 cm x 5 cm) and two closed arms (30 cm x 5 cm x 15 cm)

xi. Maze, made up of four quadrants (105cm in diameter and 10cm width) consisting of two open quadrants (surrounded by Perspex "lip",1cm high) and two closed quadrants (enclosed by a black Perspex wall, 27cm high)

xii. 60Watt red bulb.

xiii. Video camera

xiv. TV monitor

Sundry consumables and other materials

i. Syringes and Needles

ii. Animal cages

iii. Animal feeds (Growers mesh)

iv. Stop clock

3.4 Acute Toxicity Study

The acute toxicity of the methanolic stem bank extract of *Ficus ingens* (Miquel) Miquel was investigated in mice via oral and intraperitoneal route of drug administration. The method used was as described by Lorke, (1983).

The study was carried out in two phases. In the initial phase, three groups of three mice each were used. The first group received extract at a dose of 10mg/kg, while the second and third groups received the extract at doses of 100 and 1,000 mg/kg body weight respectively. The animals were observed for signs of toxicity and death within 24hours after which they were monitored for fourteen (14) days.

In the second phase, three groups of one mouse each were used. Extract at respective doses of (which depended on the result of the first phase) 1,600mg/kg, 2,900mg/kg and 5,000mg/kg were administered. The animals were observed for signs of toxicity and death within 24hours and monitored for 14days.

The LD_{50} value was determined by calculating the geometric mean of the highest non lethal dose (that did not cause death) and the lowest lethal dose for which the mouse died. (0/1 and 1/1).

3.5 Preliminary Phytochemical Screening of the Extract

The extract obtained was screened phytochemically for the presence or absence of secondary metabolites using standard conventional protocols (Silva *et al.,* 1998; Trease and Evans, 2002).

3.5.1 Test for alkaloids

Wagner's reagent: 600mg of the crude methanol extract was dissolved in 10 ml of methanol and evaporated to dryness. The resulting residue was mixed with 5 ml of 1% aqueous hydrochloric acid on a water bath. A 1 ml of the filtrate was treated with a few drops of Wagner's reagent. A brown precipitate was seen which indicated the presence of alkaloids in the extract.

Dragendorffs reagent: 600mg of the crude methanol extract was dissolved in 10 ml of methanol and evaporated to dryness. The resulting residue was mixed with 5 ml of 1% aqueous hydrochloric acid on a water bath. A 1 ml of the filtrate was treated with a few drops of Dragendorffs reagent. An orange brownish precipitate was seen indicating the presence of alkaloids in the extract.

3.5.2 Test for flavonoids

Shinoda test: 500 mg of the crude methanolic extract was dissolved in 10 ml of methanol and to a small portion of this solution, magnesium powder and a few drops of concentrated HCI was added. The solution turned pink colour and that indicated the presence of flavonoids.

Sulphuric acid: 500 mg of the crude methanolic extract was dissolved in 10 ml of methanol and to a small portion of this solution a few drops of concentrated H_2SO_4 was added. Orange colour was seen which further confirmed the presence of flavonoids.

3.5.3 Test for saponins

Froth test: 500mg was dissolved in 10 ml of distilled water and the resulting solution was shaken vigorously for about 30 seconds. The production of froth which was stable for up to 15 minutes indicated the presence of saponins.

3.5.4 Test for steroids/triterpenoids

Liebermann-Buchard test: 1 ml of anhydrous acetic acid was combined with 1 ml chloroform and cooled to 0^0C after which one drop of sulphuric acid is then added. A little of the crude sample was added to the above solution. Red and purple colours appeared to indicate the presence of Triterpenoids.

Salkowski reaction: 2 mg of the crude extract was dissolved in 1 ml chloroform and 1 ml of concentrated sulphuric acid was then added. There was no formation of two phases with either red or yellow colour which confirmed the absence of steroids.

3.5.5 Test for tannins

Ferric chloride test: Few drops of 5 % ferric chloride aqueous solution was added to a solution of the crude extract in methanol. There was no formation of a brown precipitate and that confirmed the absence of tannins.

3.5.6 Test for reducing sugars

To 2ml of the extract, 5ml of the mixture of Fehling's solution A and B was added. The mixture was boiled on water bath for 15 minutes. The formation of a brick-red precipitate was indicative of the presence of reducing sugar.

3.5.7 Test for anthraquinones

0.2g of the extract was taken and dissolved in 1.0ml of benzene, then shaken and filtered. To the filtrate, 5ml of 10% ammonia solution was added and then shaken. The formation of a red colour which was seen in the ammonia phase was indicative of the presence of anthraquinone.

3.6 Pharmacological Studies

3.6.1 MEST in chicks

According to the method of Swinyard and Kupferberg (1985), chicks were divided into six groups of ten chicks each. Chicks in group I were given normal saline 10ml/kg (*i.p*) and group 6 received phenytoin 20mg/kg body wieght (*i.p*). Groups 2, 3, 4 and 5 received the extract at respective doses of 125, 250, 500 and 1,000mg/kg body weight. Thirty (30) minutes later maximal electroshock were delivered to each chick to induce seizure using an ugobasile electro-convulsive machine (ECT unit 7801) connected to Claude stabilizer with corneal electrodes placed on the eyelid of each chick. The current, frequency, shock duration and pulse width were set and maintained at 90mA, 100Hz, 08s and 0.6m/s respectively. Episodes of tonic extension of the hind limbs were regarded as full convulsion, while their absence was considered as protection. (Swinyard and Kupferberg, 1985). The time taken to recover from the seizure was recorded.

3.6.2 PTZ induced seizure in mice

According to the method of Bastain *et al.,* (1979), Mice of either sex and of body weight between 18 and 22g were divided into seven groups of six mice each. Mice in group I were

given normal saline 10ml/kg (*i.p.*) and group 7 received Valproic acid 200mg/kg body wieght (*ip*). Groups 2, 3, 4, 5 and 6 received the extract at respective doses of 125, 250, 500, 1000 and 2000mg/kg body weight. Thirty (30) minutes later, PTZ (85mg/kg) was injected subcutaneously to each mouse. Each animal was placed into an individual plastic cage for observation lasting thirty (30) minutes. Episodes of clonic spasm with loss of righting reflex and mortality were recorded.

3.6.3 Diazepam-induced sleep in mice

The method described by Rakotonirina *et al.,* (2001) was adopted in this study. Swiss albino mice of either sex were randomly divided into four groups each containing 6 mice. The first group received normal saline (10 ml/kg). The second, third and fourth groups received 75, 150 and 300 mg of the extract per kg body weight. Thirty (30) minutes post-treatment: the mice were administered diazepam (Roche) at a dose of 25 mg/kg body weight. The mice were placed individually in cages. The onset and the duration of sleep were determined for each animal. Loss of righting reflex was considered as the criterion for sleep while the interval between the loss of righting reflex and the recovery of straightening was regarded as the duration of sleep (Holland *et al.,* 2000).

3.6.4 Tests for exploratory behaviour in mice

The apparatus used was a white painted wooden board (60 cm x 30 cm) with 16 evenly spaced holes (1cm diameter x 2 cm depth). Mice were randomly grouped into five each consisting of six mice. The first group received normal saline (10 ml/kg); second, third and fourth groups received 75, 150 and 300 mg of the extract per kg body weight respectively. The fifth group was treated with 0.5 mg diazepam per kg body weight. Thirty (30) minutes

post treatment, each mouse was placed at a corner of the board and the number of head dips on the hole was counted using a tally counter during a 5 minute period (Wolfman *et al.,* 1994). A head dip was considered when the mouse dipped its head into the hole to the level of the eyes.

3.6.5 Mouse beam walking assay

In this study, the method previously described by Stanley *et al.,* (2005) was adopted with some modifications by Magaji *et al.,* (2008). Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal support to a goal box. Three trials were performed for each mouse, and were designed such that the mouse tested would be aware that there was a goal box that could be reached. The goal box was a Perspex glass cage (with wood chippings/beddings) with a small hole at the side. The mice that successfully walked along the ruler were randomly grouped into five groups each containing six mice. The first group received normal saline (10 ml/kg), *i.p.* The second, third and the fourth groups received the extract at doses of *75*, 150 and 300 mg/kg body weight respectively. The fifth group received diazepam (0.5mg/kg body weight). The beam was made of wood, 8mm in diameter, 60 cm long and elevated 30 cm above the bench by metal support. Thirty (30) minutes post treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 s allowed on the beam. The number of foot slips (one or both hind limb slipping from the beam) was recorded with the aid of a tally counter. The time taken to complete the task was also recorded.

3.6.6 Elevated plus maze test in mice (EPM)

The EPM test was conducted according the method previously described by Hogg (1996) and Rodgers *et al.,* (1997). The maze comprised of two open arms (35 cm x 5 cm) and two closed arms (30 cm x 5 cm x 15 cm) which were connected by a common central area (5 cm x 5cm) and elevated to a height of 60 m above the floor. The floor and the walls of each were wooden and painted black (Rabbani *et al.,* 2007). The test was conducted in a room illuminated by a 60Watt red bulb. Thirty (30) mice were divided into five groups (n=6) and treated with normal saline (10 ml/kg), (75, 150 and 300 mg/kg) or diazepam (0.5 mg/kg). Thirty (30) minutes-post treatment, each Mouse was placed at the centre platform facing one of the open arms and its behaviour videotaped over a 5 minutes period. A mouse is said to have entered an arm when it has placed all four paws over the line separating the area and the centre. The maze was wiped with 70% ethyl alcohol and dried between trials to remove olfactory cues. The numbers of open and closed arm entries as well as the time spent in both open and closed arms were recorded.

3.6.7 Elevated zero-maze test (EZM)

The maze comprised of black Perspex annular platform (105cm in diameter, 10cm width) elevated to 65cm above the ground level and divided equally into four quadrants. The two opposite quadrants were enclosed by a black Perspex wall (27cm high) on both the inner and outer edges of the platform, while the remaining two opposite quadrants were surrounded by Perspex “lip” (1cm high) which served as a tactile guide to animals on these open areas. The apparatus was illuminated by dim white light arranged in such a manner as to provide similar lux levels in open and enclosed quadrants for 5min test period. Thirty (30) mice were divided

into five groups (n=6) and treated with normal saline (10 ml/kg), (75, 150 and 300 mg/kg) or diazepam (0.25 mg/kg). Thirty (30) minutes post treatment, each mouse was placed on one of the enclosed quadrants and its behaviour videotaped over a 5 minutes period. The maze was cleaned with 5% ethanol/water solution and dried thoroughly between test sessions. Animals were scored as being in the open area when all four paws were in the open quadrants and in the enclosed area only when all four paws had passed the open-closed divide .The numbers of open and closed quadrants entries as well as the time spent in both open and closed quadrants were recorded (Pellow and File, 1986).

3.6.8 Staircase test in mice

The device used in this study consists of a wooden staircase similar to the one described by Simiand *et al.,* (1984). The staircase was enclosed in transparent Perspex vertical walls (45 cm x 12 cm x 25 cm) and had 5 identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. Thirty (30) minutes before placing each mouse individually on the floor of the Perspex box (with its back to the staircase), five groups each consisting of six mice were treated with normal saline (10 ml/kg), the extract (75, 150 and 300 mg/kg) and diazepam (0.5 mg/kg) *i.p.*, respectively. The behaviour of each mouse videotaped and number of upward steps climbed and rearings were recorded over a 5 minutes period. A step was considered climbed if a mouse placed all its four paws on it. Rearing was counted when a mouse rose on its hind limb both against the wall and on a step. The staircase was wiped with 70% ethyl alcohol and allowed to dry between tests to remove any olfactory cue

3.7 Statistical Analysis

Results were expressed as Mean ± Standard Error of Mean (SEM), quantal effect or percentages, where applicable. Statistical analysis was conducted using SPSS version 17.0. Student's t-test was applied. $P<0.05$ was considered to be statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 LD_{50} Value

The median lethal dose (LD_{50}) value of methanolic extract of *ficus ingens* (Miquel) Miquel stem bark in mice was found to be greater than (>) 5,000 mg/kg body weight, orally and intraperitoneally (Table 4.1).

Table 4.1 Median lethal dose, LD_{50}

Route of administration	LD_{50} values mg/kg body weight
Intraperitoneal	>5,000
Oral	>5,000

4.2 Preliminary Phytochemical Constituents of *Ficus ingens* (Miquel) Miquel Methanolic Stem Bark Extract

The result of preliminary phytochemical screening of the extract revealed the presence of alkaloids, triterpenoids, flavonoids, saponins, phlobatanins, anthraquinones and reducing sugars, while steroids and tannins were absent (Table 4.2).

Table 4.2 Phytochemical constituents of *Ficus ingens* (Miquel) Miquel methanolic stem bark extract

Constituents	Inference
Alkaloids	++
Triterpenoids	++
Flavonoids	+
Saponins	++
Phlobatannins	++
Steroids	-
Tannins	-
Anthraquinolones	++
Reducing sugars	++

++ = Present

+ = little trace

- = Absent

4.3 Effect of Methanolic Stem Bark Extract of *Ficus ingens* (Miquel) Miquel on Maximal Electroshock (MES) Test in Chicks

The extract neither protected the chicks from convulsion nor affected their mean recovery time. Phenytoin (20mg/kg body weight) used as a positive control produced 90% protection of the Chicks against HLTE (Table 4.3).

Table 4.3 Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on Maximal Electroshock (MES) test in chick

Treatment	Quantal protection	% protection	Mean recovery time (mins) against unprotected animals ± SEM
N/saline 10m/kg	0/10	0	6.00 ± 0.68
Extract 125 mg/kg	0/10	0	6.10 ± 0.75
Extract 250mg/kg	0/10	0	5.00 ± 0.82
Extract 500mg/kg	0/10	0	5.00 ± 0.33
Extract 1,000mg/kg	0/10	0	6.40 ± 0.40
Phenytoin 20mg/kg	9/10	90%	9.00 ± 0.0

Values are Mean ± SEM

n = 10

SEM = Standard Error of Mean

Mins = Minutes

4.4 Effects of Methanolic Stem Bark Extract of *Ficus ingens* (Miquel) Miquel on Pentylenetetrazole (PTZ) Induced Seizure in Mice

The extract at all dose levels tested (125mg/kg - 2,000mg/kg) offered no significant protection on mice against PTZ induced convulsion when compared to normal saline treated group. Valproic acid (200mg/kg) used as a positive control protected 83.3% (5/6) of the mice against PTZ induced convulsion (Table 4.4).

The extract at all doses produced no significant difference in protection in mice against mortality from PTZ induced convulsion when compared to normal saline treated except with 1,000mg/kg dose (the second to the highest dose) that had a 100% protection.

Valproic acid (200mg/kg) used as a positive control protected the mice (100%) against mortality from PTZ induced convulsion (Table 4.4).

Table 4.4 Effects of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on Pentylenetetrazole (PTZ) induced seizure in mice

Treatment	Quantal protection against seizure	% protection against seizure	Mean onset of seizure ± SEM	Quantal protection against mortality	% protection against mortality
N/saline 10mg/kg	1/6	16.7	8.60 ± 1.24	3/6	50.0
Extract 125mg/kg	2/6	33.3	8.00 ± 1.87	4/6	66.7
Extract 250mg/kg	1/6	16.7	10.20 ± 1.88	2/6	33.3
Extract 500mg/kg	2/6	33.3	15.75 ± 4.03	2/6	33.3
Extract 1,000mg/kg	2/6	33.3	11.00 ± 2.04	6/6	100
Extract 2,000mg/kg	1/6	16.7	9.00 ± 1.90	4/6	66.7
Valproic Acid 200mg/kg	5/6	83.3	8.30 ± 0.0	6/6	100

Values are Mean ± SEM

n = 6

SEM = Standard Error of Mean

4.5 Effects of Methanolic Stem Bark Extract of *Ficus ingens* (Miquel) Miquel on Diazepam Induced Sleep in Mice

The extract did not significantly affect the onset of sleep at the doses tested but significantly ($P< 0.0005$) prolonged the duration of diazepam induced sleep at the doses tested (Table 4.5). The sleep time increased from 169.67 ± 34.504 min in the control group to about 335.17 ± 64.65 min, 336.83 ± 63.13 min and 364.50 ± 39.59 min at doses of 75, 150 and 300 mg/kg body weight *i.p.*, respectively (Table 4.5).

Table 4.5 Effects of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on diazepam induced sleep in mice.

Treatment/Dosage	Mean onset of sleep (mins) ± SEM	Mean duration of sleep (mins) ± SEM
N/Saline 10 ml/kg	3.17 ± 0.40	169.67 ± 34.50
Extract 75 mg/kg	3.17 ± 0.60	335.17 ± 64.65*
Extract 150 mg/kg	4.16 ± 0.91	336.83 ± 63.13*
Extract 300 mg/kg	3.67 ± 0.21	364.50 ± 39.59*

Values are Mean ± SEM

n = 6

*P< 0.0005, student's t-test

SEM = Standard Error of Mean

4.6 Effects of Methanolic Stem Bark Extract of *Ficus ingens* (Miquel) Miquel on Exploratory Behavioural Patterns in Mice

The extract at the doses tested showed a no significant decrease in the number of head dips in the hole-board test compared with the control group (Table 4.6). The standard drug, diazepam 0.5 mg/kg caused a significant ($P<0.0005$) increase in the exploratory behaviour (Table 4.6).

Table 4.6 Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on exploratory behaviour (head dip test) in mice.

Treatment/Dosage	Mean number of head dips in 5 min ±SEM
N/Saline 10 ml/kg	19.83 ± 1.99
Extract 75 mg/kg	16.50 ± 1.59
Extract 150 mg/kg	16.83 ± 4.09
Extract 300 mg/kg	16.80 ± 4.07
Diazepam 0.5 mg/kg	58.50 ± 4.93*

Values are Mean ± SEM

n = 6

*P<0.0005, student's t-test

SEM = Standard Error of Mean

4.7 Effects of Methanolic Stem Bark Extract of *Ficus ingens* (Miquel) Miquel on Beam Walking Assay (Motor Coordination) in Mice

Diazepam at 0.5 mg/kg significantly ($P<0.0005$) impaired motor coordination while the extract showed no significant difference in the number of foot slips compared to the control group. The extract exhibited a non significant reduction in the number of foot slips in a non dose dependent manner relative to the diazepam treated group (Table 4.7).

Table 4.7 Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on beam walking assay (motor coordination) in mice

Treatment/Dosage	Mean duration of beam walk (s) ± SEM	Mean number of foot Slips ± SEM
N/Saline 10 ml/kg	49.17 ± 6.57	0.43 ± 0.48
Extract 75 mg/kg	43.50 ± 8.87	0.39 ± 0.22
Extract 150 mg/kg	34.17 ± 9.59	0.36 ± 0.48
Extract 300 mg/kg	44.50 ± 9.95	0.38 ± 0.33
Diazepam 0.5 mg/kg	50.17 ± 7.38	6.17 ± 1.42*

Values are Mean ± SEM

n = 6

*P<0.0005, student's t-test

SEM = Standard Error of Mean

4.8 Effect of Methanolic Stem Bark Extract of *Ficus ingens* (Miquel) Miquel on Staircase Test in Mice

The extract significantly ($P<0.0005$) decreased the number of rearing and the number of upward steps climbed when compared to the control group (Table 4.8).

Diazepam when compared to the control group, exhibited a slight reduction in the number of rearing but significantly ($P<0.025$) increased the upward steps climbed (Table 4.8).

Table 4.8 Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel on staircase test in mice

Treatment/Dosage	Mean no. of upward steps climbed ± SEM	Mean no. of rearings ± SEM
N/Saline 10 ml/kg	39.17 ± 4.12	37.33 ± 2.47
Extract 75 mg/kg	27.67 ± 3.54**	24.17 ± 4.86**
Extract 150 mg/kg	19.33 ± 2.86**	19.50 ± 3.93**
Extract 300 mg/kg	17.16 ± 2.56**	14.33 ± 2.62**
Diazepam 0.5mg/kg	46.83 ± 6.19*	33.83 ± 3.26

Values are Mean ± SEM

n = 6

*P<0.025; **P<0.0005, student's t-test

SEM = Standard Error of Mean

4.9 Effect of Methanolic Stem Bark Extract of *Ficus ingens* (Miquel) Miquel on Elevated Plus Maze and Elevated Zero Maze in Mice

In the elevated plus and zero mazes, the extract showed no significant difference in both the number of entries in the open arm/quadrant and the time spent in the open arm/quadrant as compared to the control group (Tables 4.9 and 4.10).

However, with the zero maze, the extract at 150mg/kg showed a statistically significant increase ($P<0.0005$) in the amount of time spent in the open quadrant (Table 4.10).

In the elevated plus and zero mazes, Diazepam showed a statistically significant ($P<0.0005$) increase in both the amount of time spent in the open arm/quadrant and the number of entries in the open arm/quadrant (Table 4.9 and 4.10).

Table 4.9 Effect of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel *on* elevated plus maze test in mice

Treatment/Dosage	Mean no. of open arm entries ± SEM	Mean time spent in open arm± SEM (sec)	Mean no. of closed Arm entries ± SEM	Mean time spent in closed arm± SEM (sec)
N/Saline 10 ml/kg	5.83 ± 1.80	82.33 ± 27.03	6.83 ± 0.87	148.50 ± 29.99
Extract 75 mg/kg	1.67 ± 0.76	24.83 ± 11.91	3.83 ± 0.91	206.000 ± 22.12
Extract 150 mg/kg	1.67 ± 1.09	25.00 ± 19.15	4.67 ± 1.28	243.50 ± 23.97
Extract 300 mg/kg	3.50 ± 0.76	38.83 ± 13.01	6.67 ± 1.33	228.83 ± 12.45
Diazepam 0.5 mg/kg	13.69 ± 0.75*	239.83±10.86*	1.83 ± 1.91	25.50 ± 10.31

Values are Mean ± SEM

n = 6

*P ≤ 0.0005, student's t-test

SEM = Standard Error of Mean

Sec = Seconds

Table 4.10 Effect of methonolic stem bark extract of *Ficus ingens* (Miquel) Miquel on elevated zero maze tests in mice

Treatment/dosage	Mean no. of open arm entries ±SEM	Mean no. of time spent in open arm ± SEM (sec)	Mean no. of closed arm entries ± SEM	Mean no. of time spent in close arm± SEM (sec)
N/Saline 10ml/kg	1 ± 0.28	5 ± 0.85	4.4 ± 0.46	295 ± 0.85
Extract 75mg/kg	1.6 ± 0.64	25.6 ± 1.15	3.2 ± 0.33	274 ± 1.15
Extract 150mg/kg	1.02 ± 0.46	156 ± 0.63*	1.17 ± 0.52	144 ± 0.63
Extract 300mg/kg	0.8 ± 0.19	5.2 ± 0.91	4.6 ± 0.54	294.8 ± 0.91
Diazepam 0.5mg/kg	6.6 ± 0.53*	286.4± 1.89*	1.4 ± 0.13	13.6 ± 1.89

Values are Mean ± SEM

n = 6

*P ≤ 0.0005, student's t-test

SEM = Standard Error of Mean

Sec = Seconds

CHAPTER FIVE

5.0 DISCUSSION

Survival of mice after oral and intraperitoneal administration of the extract up to 5,000mg/kg body weight, within observation period of 14 days implies that the estimated oral and intraperitoneal median lethal dose (LD_{50}) of the extract at 5,000mg/kg is non-toxic (Lorke, 1983).

The preliminary phytochemical screening of the extract revealed the presence of saponins, triterpenoids, alkaloids, flavonoids, phlobatannins, anthraquinones. Several plants have been reported to have CNS depressant and anxiolytic activities due to the presence of triterpenoids, saponins and flavonoids (Dubois *et al.,* 1986). Triterpenoids and saponins are reported to have agonistic/facilitatory activities at $GABA_A$ receptor complex (Chakraborty *et al.,* 2010; Barua *et al.,* 2009) and led to the hypothesis that they act in similar way to benzodiazepine-like molecules. This is supported by their behavioural effects in animal models of CNS depression and anxiety (Dubois *et al.,* 1986)

Prevention of seizures induced by PTZ and MES in laboratory animals is the most commonly used preliminary screening test to characterize potential anticonvulsant drugs. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures whereas the PTZ test represents a valid model for human generalized myoclonic and also absence seizures (Loscher and Schmidt, 1988). There is no false negative in MES test and all currently available AEDs that are clinically effective in the management of generalised clonic-tonic and partial seizures such as phenytoin, carbamazepine and lamotrigine all suppresses tonic hind limb extention (THLE) in MES test

(Browning, 1992). The behavioural and electrographic seizures generated in the MES are consistent with the human disorder (Swinyard *et al.,* 1999). MES induced tonic seizures can be prevented by agents that either inhibits sodium channel such as phenytoin, valproate, felbamate and lamotrigine (Sayyah *et al.,* 2004) or agents that block glutamatergic excitation mediated by N-methyl-D-Aspartate (NMDA) receptors such as phenytoin (Sayyah *et al.,* 2004). Pentylenetetrazole (PTZ) is a known convulsant and anticonvulsant effect against PTZ test identifies compounds that can raise seizure threshold in the brain (White *et al.,* 1998). The PTZ-induced seizures are similar to the symptoms observed in the absence seizures and drugs useful in treatment of absence seizures suppress PTZ-induced seizures (McNamara, 2006). Drugs that block T-type calcium ion (Ca^{2+}) currents, such as ethosuximide can prevent seizures induced by PTZ. Similarly, PTZ has been shown to interact with GABA neurotransmitters and the GABA receptor complex (Loscher and Schmidt, 1998). Therefore, agents that enhance $GABA_A$ receptor mediated inhibitory neurotransmission such as benzodiazepines and barbiturates prevent PTZ-induced seizure. Dopamine has been found to reduce the threshold of PTZ convulsions in mice and specific receptor antagonists of dopamine such as pimozide protected experimental animals against PTZ-induced seizures (Amabeoku, 1989). Drugs such as felbamate, which block glutamatergic excitation mediated by NMDA receptors, have demonstrated activity against PTZ-induced seizures suggesting the involvement of N-methyl-D-aspartate (NMDA) system in the initiation and propagation of PTZ-induced seizures (MacDonald and Kelly, 1993).

The inability of the methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel to prevent convulsion induced by both MES and PTZ in chicks and mice respectively, and to protect the mice from mortality suggests that the extract seems not to posses' anticonvulsant potential.

Potentiation of sleep induced by CNS depressant such as the barbiturates and benzodiazepines has been used as an effective model in screening CNS active agents (Danjuma, 2007). The extract prolonged the duration of sleep induced by diazepam. Agents that prolong duration of sleep are used to maintain sleep. The prolongation of diazepam-induced sleeping time may be attributed to an action on the central mechanism involved in the regulation of sleep (N'Gouemo *et al.,* 1994), thus suggests *Ficus ingens* (Miquel) Miquel as a neurosedative drug (Capasso *et al.,* 1996).

The determination of exploratory behaviour in animals is measured by the hole board experiment (File and Wardil, 1975) and is an accepted parameter for evaluating anxiety conditions in animals (Crawley, 1985). The behavioural head dipping in hole-board assay is sensitive to changes in the emotional state of the animals and increase in head dipping behaviour is a reflection of anxiolytic activity whereas a decrease in the parameter reveals a sedative behaviour (File and Pellow, 1985). The extract produced a non significant decrease in exploratory behaviour as shown by the decrease in the number of head dips in the hole-board test. A decrease in number of head dips reveals sedative behaviour (File and Pellow, 1985) and is thus a measure of CNS depressant activity (Adzu *et al.,* 2002; Viswanatha *et al.,* 2006).

Stanley *et al.,* (2005) reported that the number of foot slips made by mice in the motor coordination test has been found to be a sensitive measure at determining benzodiazepine

induced motor coordination deficits and is a good predicator of doses producing clinical sedation. The extract had no observable effect on motor coordination when compared with the negative control suggesting that the inhibitory effect observed in the other tests might be elicited centrally and not due to a peripheral neuromuscular blockade (Perez *et al.,* 1998). That is to say that the sedative properties of the extract are likely to be produced centrally. The non observation of motor coordination deficit in the extract is a beneficial effect which indicates that the extracts at these doses are not likely to have marked motor deficits as adverse effects as some CNS depressants used in the treatment of anxiety do e.g. benzodiazepines (Rang *et al.,* 2003). From our results, Diazepam produced motor coordination deficits even at a low dose. It also suggests that the extract is not likely to cause clinical sedation at the doses tested (Stanley *et al.,* 2005).

The staircase test is a simple, quick and differential test based on the inherent tendency of rodents to explore a novel environment which gives a general idea on the level of emotivity of the rodent (Lepicard *et al.*, 2000). In the staircase test, the number of steps climbed and rearing is measured as behavioural parameters of exploratory/locomotor activity and anxiety, respectively (Simiand *et al.,* 1984; Weizman *et al.,* 2001; Abid *et al.,* 2006; Milman *et al.,* 2006). Some studies have shown that anxiolytic drugs decrease the number of rearing without any effect on step climbing behavior (Simiand *et al.,* 1984; Weizman *et al.,* 2001; Abid *et al.,* 2006; Milman *et al.,* 2006). The extract exhibited dose dependent decrease in the number of rearing and this is suggestive of an anxiolytic effect. Diazepam at the dose tested in this study also exhibited this effect in reducing the number of rearing. This is supported by the report of Emmanouil and Quock (1990) that midazolam, a benzodiazepine reduced the

number of rearing which was blocked by flumazenil, a benzodiazepine receptor antagonist. In another study by Pock and Vali (2001), flumazenil was found to exert an anxiogenic effect demonstrated by an increase in the number of rearing. Similarly, an anxiogenic agent, FG-7142 has been reported to produce a dose dependent increase in the number of rearing in the staircase test (Ago *et al.,* 2007).

From our study, Diazepam was also found to increase the step climbing behaviour; a parameter which the extract tested had no effect. Ago *et al.,* (2007) has suggested that selective increase in step climbing behaviour reflects exploratory behaviour with anxiolytic state in addition to the fact that selective increase in rearing behaviour reflects the anxiety state.

This variation could be due to strain differences as Ago *et al.,* (2007) used ddY mice while the current study employed Swiss albino mice. The inability of the extract to increase step climbing behaviour may reflect anxiolytic action via a different mechanism compared to that of the benzodiazepines.

The important parameters in the elevated plus and elevated zero mazes tests are the number of entry and time spent in the open arms/quadrants. Both diazepam and the 150mg extract/kg body weight in the zero maze significantly increased the open arm/quadrant entries and open arm/quadrant duration. It also increased the total number of entries. This is in agreement with other studies (Cha *et al.,* 2004; Rabbani *et al.,* 2007). The extract did not increase the number of open arm/quadrant entries nor increase the duration in the open arms/quadrants. It is known that some clinically used anxiolytic drugs show no effect or even anxiogenic effect on the elevated plus maze. For instance, some serotonergic agents such as fluoxetine was found

to show no effects and anxiogenic effects after chronic and single administration respectively (Silva and Brandao, 2000; Pinheiro *et al.,* 2007). Behavioural and pharmacological validation of the elevated plus maze has shown that the increase in avoidance latency is a function of the aversive character of the open arm, and suggests that the pharmacological profile of the avoidance task is similar to that of GAD (Graeff *et al.,* 1998, Zangrossi and Graeff, 1997) hence, consistent with literature data, serotonergic agents are less reliable as compared to the benzodiazepines. The extract may possess bioactive compounds that act via a different mechanism from diazepam.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Acute toxicity studies carried out on the methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel showed that the extract was non toxic. Using oral and intraperitoneal routes, median LD_{50} value was >5,000 mg/kg with both routes.

The anticonvulsant potential of methanolic stem bark extract of *Ficus ingens* (Miquel) Miquel was carried out electrically using MES in chicks and chemically using PTZ in mice. In the MES test, the extract did not protect the chicks against MES seizure; neither did it shorten the mean recovery time of the convulsed animals when compared with normal saline treated chicks. In the PTZ test, the extract did not protect the mice from PTZ induced convulsion when compared to normal saline; neither did it protect the mice against mortality from PTZ induced convulsion. By these result, stem bark extract of *Ficus ingens* (Miquel) Miquel seems not to posses any anticonvulsant potential.

Behavioural studies carried out on the methanolic stem bark extracts of *Ficus ingens* (Miquel) Miquel using animal models include diazepam-induced sleep, hole board assay, the beam walk assay, the staircase test, the elevated plus maze and the elevated zero maze.

The extract significantly ($P<0.0005$) prolonged the duration of diazepam induced sleep. This is suggestive of sedative property. The extract slightly decreased exploratory behaviour on the hole board assay. This may reflect a sedative potential of the extract.

There was no motor coordination deficit observed with the extract at the doses tested. In essence, the extract did not increase the mean number of foot slips, neither did they increase

the total time taken to complete the task. This finding suggests that the activities of the extract may be centrally mediated.

In the staircase model, the extract showed a statistically significant ($P<0.0005$) decrease in the rearing behavior which suggested anti anxiety effects.

In the elevated plus maze and zero maze models, the extract showed no statistically significant effect on the number of open arm/quadrant entries nor the duration spent on the open arms/quadrants except with the zero maze at 150mg/kg. These findings further corroborate CNS depressant properties of the extract.

The results obtained from the elevated plus maze and the elevated zero maze revealed that the zero maze is an improvement over elevated plus maze and has several advantages over it. The zero maze has no centre square which can produce ambiguous measures as was observed in this study; and, it has a circular design that allows for uninterrupted exploration.

The extract does not have anticonvulsant potential but contain bioactive compound that possess CNS depressant properties and possible anxiolytic properties

Further research should involve isolation of the bioactive constituents responsible for the observed pharmacological activities and elucidation of the mechanism of action.

REFERENCES

Abid, M., Hrishikeshavan, H.J. and Assad, M. (2006). Pharmacological evaluation of *pachyrrhizus enosus* (L) seeds for central nervous systems depressant activity. *Indian Journal of Physiology and Pharmacology,* 50 (2): 143-151.

Adzu, S., Amos, S., Dzarma, C.W. and Gamaniel, K. (2002). Effect of *Zizypus spinchristi* wild aqueous extract on the central nervous system in mice. *Journal of Ethnopharmacology,* 79: 13-16.

Ago, Y., Takahashi k., Nakamura, S. Hashimoto, H., Baba, A. and Matsuda, T. (2007). Anxiety-like and exploratory behaviours of isolation-reared mice in the staircase test. *Journal of Phamacology real Sciences,* 104:153-158.

Akerele, O. (1993). Nature's medicinal bounty: Don't throw it away. *World health forum,* 14:390-395.

Al-Musayeib, N.M., Mothana, R.A., Matheeussen, A., Cos, P. and Maes, L. (2012). In vitro antiplasmodial, antileishmanial and antitrypanosomal activities of selected medicinal plants used in the traditional Arabian Peninsular region. *BMC Complement Alternative Medicine* 12 (49): 12-49.

Amabeoku, J.G. (1989). Neuropharmacological profile of Quinine: An unpublished PhD (Pharmacology) Dissertation, Ahmadu Bello University, Zaria.

Amos, S., Kolawole, E., Akah, P., Wambebe, C. and Gamaniel, K. (2001). Behavioural effects of the aqueous extract of Guiera senegalensis in mice and rats. *Phytomedicine,* 8(5): 356-361.

Amos, S., Orisadipe, A., Binda, L., Emeje, M., Adesomoju, A., Okogun, J., Akah, P., Wambebe, C. and Gamaniel, K. (2002). Behavioural effects in rodents of Methyl angolensate: a triterpenoid isolated from *Entadrophragma angolense*. *Pharmacology and Toxicology,* 91: 71-76.

Arzimanoglou, A., Hirsch, E., Nehlig. A., Catelnau, P., Gressens, P., Vasconcelos, A. (2002). Epilepsy and Neuroprotection. *Epileptic Disorder,* 3: 173 -182.

Avanzini, G. and Franceschetti, S. (2003). Prospect for novel antiepileptic drugs. Current opinion in investigational drugs. *Epilepsia,* 4(7): 805 – 814.

Bandelow, B. (2004). Future perspectives: New compounds with putative anxiolytic effects. In: Program and abstracts of the International Congress of Biological Psychiatry, Sydney, Australia. Workshop 56.

Barua, C.C., Roy, J.D., Buragohain, B., Barua, A.G., Borah, P. and Lahkar, M. (2009). Anxiolytic activity of hydroethanolic extract of *Drymaria cordata* Willd. *Indian Journal of Experimental Biology,* 47:969–973.

Bastian J.W., Krause W.E., Ridlon S.A., Ercolin I.V. (1979). CNS drug specificity as determined by the mouse intravenous pentylenetetrazole. *Journal of Pharmacology and therapeutics,* 127: 75-80.

Bloom, F.C. (1996). Neurotransmission and the central nervous system. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. and Gilman, A.G. (Eds) Goodman and Gilman's The pharmacological Basis of Therapeutics, 9th Edition. Mc Graw-Hill Co. Inc. New York, USA. PP. 267-293.

Brodal, P. +(1992). The somatosensory system. In the central nervous system, structure and function. Oxford University Press, New York, PP 113-115.

Browning, R. (1992). The electroshock model. Neuronal network and antiepileptic drugs In: Faingold, L.L. and Form, G.H, (Eds) *Drugs for control of Epilepsy: Action on neuronal networks in seizure Disorders,* CRC press, Boca Raton, FL, PP 195-211

Bum, E.N., Schmutz, M., Meyer, C., Rakotonirina, A., Bopelet, M., Portet, C., Jeker, A., Rakotonirina, S.V., Olpe, H.R and Herrling, P. (2001). Anticonvulsant properties of the methanolic extract of *Cyperus articulatus* (Cyperaceae). *Journal of Ethnopharmacology,* 76(2): 145-150.

Burkill, H.M. (1995). The useful plants of West Africa. *Royal Botanical gardens,* Kew, England, 2 (1) pp. 14-16, 239.

Burrows, J. and Burrows, S. (2003). Figs of southern and south-central Africa. Umdaus Press, Hatfield. PP. 19-67

Capasso, A., Aquino, R., DeSimone, F. and Sorrentino, L. (1996). Neoropharmacological effects of extracts from *Sickingia Williamsii. Journal of pharmacy and pharmacology,* 48(6): 592-595

Casacalenda, N. and Boulenger, J.P. (1998). Pharmacologic treatments effective in both generalised anxiety disorder and major depressive disorder; Clinical and theoretical implications. *Canadian Journal of Psychiatry,* 43 (7); 722-30.

Cassano, G.B., Rossi, N.B. and Pini, S. (2002). Psychopharmacology of anxiety disorders. *Dialogues in Clinical Neuroscience,* 4: 271-285.

Cha, Hwa-Young, Seo, Jeong-Ju, Park, Jeong-Hill, Choi, Kang-Ju, Hong, Jin-Tae and Oh, Ki-Wan (2004). Anxiolytic Effects of Total Saponin Fraction from *Ginseng Radix Rubra* on the Elevated Plus Maze Model in Mice. *Journal of GinSeng Research,* 28 (3): 132-135.

Chakraborty, A., Amudha, P., Geetha, M. and Surjit Singh, N. (2010). Evaluation of anxiolytic activity of methanolic extract of *Sapindus mukorossi* Gaertn. in mice. *International Journal of Pharmacy and Biological Science,* 1:1-8.

Chamberlain S.R, Blackwell, A.D., Fineberg, N.A., robbins T.W. and Sahakian, B.J. (2005). The neuropsychology of obsessive compulsive disorder: the importance of failures in cognitive and behavioural inhibition as canditate endophenotypic markers. *Neuroscience and Biobehavioral Reviews*, 29: 399-419.

Chen, L.S., Mitchell, W.G., Horton, E.J. and Snead, O.C. (1995). "Clinical utility of video-EEG monitoring".*Pediatric. Neurology,* 12 (3): 220–4.

Chindo, B.A., Anuka, J.A., McNeil, L., Yaro, A.H., Adamu, S.S., Amos, S., Connelly, W.K., Lees, G. and Gamaniel, K.S. (2009). *Brain Research Bulletin*, 78 (6): 276-282.

Costa, E., Guidotti, A. and Moa, C.L. (1975). New concepts in the mechanism of action of benzodiazepines. *Life Science,* 17: 167-186.

Cragg, G.M., Newman, D.J. and Snader, K.M. (1997). Natural products in drug discovery and development. *Journal of Natural products,* 60: 52-60.

Crawley, J.N. (1985). Exploratory behavior models of anxiety in mice. *Neuroscience and Behavioural Reviews,* 9: 37-44.

Danjuma, N.M. (2007). Some neuropharmacological studies on the stem bark of *Randia nilotica* Stapf. (Rubiaceae). An unpublished PhD (Pharmacology) Dissertation, Ahmadu Bello University, Zaria-Nigeria.

Donia, A.M., Soliman, G.M., Zaghloul, A.M., Alqasoumi, S.I., Awaad, A.S., Radwan, A.M. and Basodan, O.A. (2013) Chemical constituents and protective effect of *Ficus ingens* (Miq.) Miq. on carbon tetrachloride-induced acute liver damage in male Wistar albino rats. *Journal of Saudi Chemical Society,* 17(1):125-133

Dubois, M. A., Ilyas, M. and Wagner H. (1986). Cussonosides A and B, two Triterpene-saponins from *Cussonia barteri. Planta Medica,* 52(2): 80-83

Duncan, J.S., Sander, J.W., Sisodiya, S.M. and Walker, M.C. (2006). Adult epilepsy. Lancet, 367: 1087-1100

Duvivier, E.H. and Pollack Jr, C.V. (2009). Seizures. In: Marx, J.A., ed. Rosen's Emergency Medicine: Concepts and Conical Practice. 7th ed. Philadelphia, Pa: Mosby Elsevier

Emmanouil, D.E. and Quock, R.M. (1990). Effects of benzodiazepine agonist, inverse agonist and antagonist drugs in the mouse staircase test. *Psychopharmacology,* 102:95-97

Engel, J. Jr. (2001). A proposed diagnostic Scheme for people with epileptic seizures and with epilepsy. Report of the ILAE Task Force on classification and terminology. *Epilepsia*, 42(6): 796-803.

Farnsworth, N.R. (1994). Ethnopharmacology and Drug Development. In: *Ethnobotany and the search for new drugs,* Ciba foundation symposium 185, Prance, G.T. and Marsh, J. (Eds). John Wiley and Sons, Chichester, pp. 42-59.

Farwell, J.R., Lee, Y.J. and Hirtz, D.G. (1990). Phenobarbital for febrile seizures effects on intelligence and on seizure recurrence. *New England Journal of Medicine,* 322: 364-369

Figgitt, D.F. and McClellan, K.J. (2000). Fluvoxamine: An updated review of its use in the management of adults with anxiety disorders. Drugs, 60 (4): 925-54.

File, S. and Pellow, S. (1985). The effect of triazolobenzodiazepines in two animal tests of anxiety and in the hole board. *British .Journal Pharmacology,* 86: 729-735.

File, S.E.and Wardill, A.G. (1975). Validity of head dipping as a measure of exploration in a modified hole-board. *Psychopharmacology,* 44: 53-59.

Finn, D.A., Rutledge-Gorman, M.T. and.Crabbe, J.C. (2003). Management of anxiety. *Neurogenetics,* 4: 109.

Fisher, R., Van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P. and Engel, J. (2005). "Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE) ". *Epilepsia,* 46 (4): 470–2.

French, J.A. and Pedley, T.A. (2008). Clinical practice. Initial management of epilepsy. *New England Journal of Medicine,* 359: 166-76

Gilhotra, N. and Dhingra, D. (2010). GABA ergic and nitriergic modulation by *Curcumin* for its antianxiety-like activity in mice. *Brain Research*, 1352: 167-175.

Goldberg, H.L. (2004). Stress and anxiety. *Psychopathology,* 17: 45-55

Goldman, W.T. (2001). Childhood and Adolescent Anxiety Disorders. Retrieved on April 16, 2011 from the http://www.keepkids healthy.com

Gorman, J.M. (2003). New molecular targets for anti-anxiety interventions. *Journals of Clinical Psychiatry,* 64:28-35.

Graeff, F.G., Netto, C.F and Zangrossi-Jr, H. (1998). The elevated T maze as an expiremental model of anxiety. *Neuroscience and Biobehavioural Reviews,* 23:237-246.

Guillemain, J. and M. Tetau, M. (1980). Contribution a 'I'etude d'un "tranquillisant vegetal" Tilia Tomentosa Bourgeons. Cahirers de Biotherapie, 68:1-8.

Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacology, Biochemistry and Behaviour,* 54: 21-30

Holland, E.C., Celestino, J., dai, C., Schaefer, L., Sawaya, R.E., and Fuller, G.N. (2000). Combined activation of RAS and kT in neural progenitors induces glioblastoma formation in mice. *Genetic,* 25:55-57.

Howland, R.D., Mycek., M.J., Harvey, R.A. and Champe, P.C. (2006). Drug affecting the central nervous system. In: Lippincott's Illustrated Reviews: Pharmacology, 3rd Edition, Lippincott Williams and Wilkins, PP. 181-270.

Husain, G.M., Mishra, D., Singh, P.N., Rao, C. and Kumar, V. (2007). Ethnopharmaeological review of native traditional medicinal plants for brain disorders. *PharmacognosyReviews,* 1:19-27.

Inamol, J. (2004). Safety of medicinal plants (NAPRALERT Review article). *Pakistan journal of medical research,* 43:4

Irvine, F.R. (1961). Woody Plants of Ghana with special reference to their uses. London: Oxford University Press. PP. 41-79

Jansen, A.S.P., Nguyen, X.V., Karpitsky, V., Mettenlieter, T.C. Leowy, A.D. (1995). Central command neurons of the sympathetic nervous system: Basis of the fight or flight response. *Science*, 270: 644-646.

Kirkwood, C.K. and Melton, S.t. (2002). Anxiety disorders. In: J.T. Dipiro, R.L. Talbert, G.c. Yee, G.R. Matzke, B.G. Wells, L.M. Posey (Eds). Pharmacotheraphy: A pathophysiologic approach (5th Edition), McGraw-Hill, New York. NY. PP. 65-112

Kumar, S. and Sharma, A. (2005). Anti anxiety activity studies on homeopathic formulations of *Turnera aphrodisiaca* Ward. *Evidence-based Complementary and Alternative Medicine, 2* (1): 117-119.

Lanhers, M., Fleurentin, J., Cabation, P., Rolland, A., Dorfman, P., Misslin, R. and Pelt, J. (1990). Behavioural effects of *Euphorbia hirta L*. Sedative and anxiolytic properties. *Journal of ethnopharmacology,* 29: 189-198.

Lieberman, M.D. (2000). Intuition: A social cognitive neuroscience approach. *Psychological Bulletin,* 126: 109-137

Lepicard, E.M., Joubert, C., Hagneau, I., Perz-Diaz, F. and Chapouthier, G. (2000). Differences in anxiety-related behavior and response to diazepam in BALB/cByJ and C57BL/6J strains of mice. *Pharmacology, Biochemistry and Behaviour,* 67(4):739-748

Loscher, W. and Schmidt, D. (1998). Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Research*, 2: 145-181.

Lorke, D. (1983). A new approach to practical acute toxicity. *Archives of Toxicology*, 54:275-287

Macdonald, R.L., and Kelly, L.M. (1993). Antiepileptic drug mechanisms of action. *Epilepsia*, 34 (supplement 5): 51-58

Magaji, M.G., Anuka, J.A., Abdu-Aguye, I., Yaro, A.H. and Hussaini, I.M. (2008). Behavioural Effects of the methanolic root bark extract of *Securinega Virosa* in rodents. *African Journal of Complementary and Alternative medicines,* 5(2): 147-153

Mann, A. Gbate, M. and Nda Umar, A. (2003). Medicinal and Economic plants of Nupeland. Jubes Evans Books and publication, Bida, Nigeria. PP. 1-276.

Marchesi, C. (2008). Pharmacological management of panic disorder. *Journal of Neuropsychiatric Disease and Treatment,* 4(1): 93-106.

McNamara, J.O. (2006). Pharmacotherapy of Epilepsies. In: Brunton, L.L., Lazo, J.S., and Parker, K.L. (Eds) Goodman and Gillman's, *The Pharmacological Basic of Therapeutics,* 11th Edition. McGraw-Hill Co. Inc. New York, USA. PP.501-525

Milman, A., Weizman, R., Rigai, T., Rice, K.C. and Piuck, C.G. (2006). Behavioural effects of opiod subtypes compared with benzodiazepines in the stairecase test paradigm. *Behavioural Brain Research,* 170:141-147

Mulder, G. B. and Pritchett, K. (2004). The elevated plus-maze. *Contemporary Top Laboratory Animal Science,* 43, 39–40

Myburgh, J.G., Fourie, N., Van der Lugt, J.J., Kellerman, T.S., Cornelius, S.T. and Ward, C. (1994). A nervous disorder in cattle, caused by the plants *Ficus ingens Var. ingens and Ficus cordata subsp. Salicifolia. Journal of Veterinary Research,* 61: 171-176.

Nemeroff, C.B. (2003). The role of GABA in the pathophysiology and treatment of anxiety disorders. *Pschopharmacology Bulletin,* 37 (4): 133-146.

Neuwinger ,J.E. (translated from the German by Porter, A.) (1996). African ethnobotany,poisons and drugs. Chapman and Hall, Weinheim, Germany. PP. 495-499.

N'Gouemo, P., Nquemby-Bina, C. and Baldy-Moulinier, M. (1994). Some neuro pharmacological effects of an ethanol extract of *Maprounea africana* in rodents. *Journal of Ethnopharmacology, 43*: 161-166.

Nicolle, R.A. (2009). Introduction to the pharmacology of CNS drugs. In: Katzung, B.G., Masters, S.B. and Trevor, A.J. (Eds) Basic and Clinical phamacology. 11th edition McGraw-Hill Companies Inc.m New York, USA. PP. 3573-370.

Nichols, G. (2005). The indigenous garden. Draping green over rocks. *Farmers Weekly* 30 September 2005.

Ninan, P.T. (2001). Dissolving the burden of generalized anxiety disorder. *Journal of clinical psychiatry,* 62 (Suppl 19): 5-10.

Norquist, G.S. and Regier, D.A. (2006). Anxiety disorders. *Annual reviews in medicine,* 47:473-479

Oakley-Browne, M.A. (1991). The epidemiology of anxiety disorders. *International Review in Psychiatry,* 3: 243-252.

Ochoa, J.G. (2011). Antiepileptic Drugs. *New York Springer,* 53: 7-15.

Pellow, S., and File, S.E. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacology, Biochemistry and Behaviour,* 24: 524-529.

Perez, G.R.M., Perez, I.J.A., Garcia, D. and Sossa, M.H. (1998). Neuropharmacological activity of *Solanum nigrum* fruit. *Journal of Ethnopharmacology*, 62:43-48.

Pinheiro, S.H., Zangrossi-Jr, H.L., Del-Ben, C.M. and Graeff, F.G. (2007). Elevated mazes as animal models of anxiety: effects of serotonergic agents. *Annals of the Brazilian Academy of Science,* 79 (1): 71-85.

Pock, P. and Vali, M. (2001). The effects of flumazenil, Ro 15-4513 and B-CCM on the behavior of control and stressed mice in the staircase test. *Journal of Psychopharmacology,* 15 (3): 155-159.

Rabbani, M., Sajjadi, S.E. and Mohammadi, A. (2007). Evaluation of the anxiolytic effect of *Napeta persica Boiss* in mice. *Evidence-based Complementrary and Alternative Medicine Advance Access,* pp. 1-6.

Rakotonirina, S.V., Ngo Bum, E., Rakotonirina, A. and Bopelet, M (2001). Sedative Properties of thedecoction of the rhizome of Cyperus articularis. *Fitoterapia,* 72: 22-29.

Rang, H.P., Dale, M.M., Ritter, J.M. and Moore, P.K. (2003). Antiepileptic drugs. In Rang, H.P., Dale, M.M., Ritter, J.M. and Moore, P.K. (Eds) *Pharmacology*, Churchill Livingstone, Elsevier science limited, pp. 515-547.

Raza., M., Shaheen, F., Choudhary, M.L., Sulria, A., Altaur-Rahman, Sombuti, S. and De loranzo, R.J. (2001). Anticonvulsant activities of the FS-I sub fraction isolated from roots at delphinium denudatum. *Phytotheropy research,* 15:426-420.

Reschke, A.H., Mannuzza, S., Champman, T., Lipsitz, J.D., Liebowitz, M.r., Gorman, J.M., Klein D.F. and Fyer, A.J. (1995). Sodium lactate response and familial risk for panic disorder. *American Journal of Psychiatry,* 152: 277-279.

Reus, V.I. (2001). Neurological disorders In : Brayunwald, E., Fauci, A.S., Kasper, D.L., Hauser, S.L., Longo, D.L., and Jameson, J.L. (Eds) Harison's principles of internal medicine 15th edition. McGraw-Hill medical publishing division part 14, section 4, 385.

Richard, A., Sugerman, F. and Cleveland, K. (1997). Neuroanatomy in Psychotropic Drugs: 2nd edition. *Mosby,* PP. 10-26

Rodgers, R.J., Cao, B.J., Dalvi, A. and Holmes, A. (1997). Animal models of anxiety: an ethological perspective. *Brazilian journal of medical and biological research,* 30 (3): 289-304

Salih, M.A. and Mustafa, M.M. (2008). A substance in broad beans (*Visiafafa*) is protective against experimentally induced convulsions in mice. *Epilepsy Behaviour,* 12: 25-29

Salzman C., Miyawaki, E.K., Bars, P. and Kerrihard, T.N. (1993). Neurobiologic basis of anxiety and its treatment. *Harvard Review of Psychiatry*, 14: 197-206.

Sanford, J.J., Spilios V. Arguropoulos, S.V. and Nutt, D.J. (2000). The psychobiology of anxiolytic drugs Part 1: Basic neurobiology. *Journal of Pharmacology and Therapeutics,* 88: 197-212.

Samren, E.B., Van Dulijin, C.M. and Koch, S. (1997). Maternal use of antiepileptic drugs and the risk of major congenital malformations: A joint European prospective study of human teratogeneiss associated with maternal epilepsy. *Epilepsia,* 38:98-990.

Sayyah, M., Nadjafnia, L. and Kamalinejad, M. (2004). Anticonvulsant activity and chemical composition of *Artemisia dracunculus L*. essential oil. *Journal of Ethnopharmacology,* 94:283-287

Shri, R.L. (2010). Anxiety: Causes and Management. *The Journal of Behavioural Science*, 5(1): 100-118.

Silva, G.L., Lee, I. and Kinghorn, A.D (1998). Special problems with extraction of plants. In: Carnell, R.J.P. (Ed). *Methods in Biotechnology*. Human Press, New Jersy, Vol 4 pp. 343-360

Silva, R.C. and Brandao, M.L (2000). Acute and chronic effects of Gepirone and Fluoxetine in rats tested in the elevated plus maze: An ethological analysis. *Pharmacology, Biochemistry and Behaviour, 65(2):209-16*

Simiand, J., Keane, P.E. and Moore, M. (1984). The staircase test in mice: A simple and effective procedure for primary screening of anxiolytic agents. *Psychopharmacology, (Berl)*, 84: 48-53

Singh, D. and Goel, R.K. (2009). Nervous disorders. *Journal of Ethnopharmacology* , 123 (2) : 330-334.

Sofowora, A.A., (1993). Medicinal plants in Africa. Spectrum Book Ltd., Ibadan, Nigeria. PP. 31-41

Soulimani, R., Younos, C., Jarmouni, S., Bousta, D., Misslin, R. and Mortier, F. (1997). Behavioural effects of *Passifiora incarnata L*. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. *Journal of Ethnopharmacology,* 57 (1): 11-20.

Stables, J.P. and Kupferberg, H.J. (1997). The NIH Anticonvulsant Drug Development (ADD) program: preclinical anticonvulsant screening project. In: Avanzini, G., Regesta, G., Tanganelli, P., and Avoli, M. (Ed). *Molecular and Cellular Targets for Antiepileptic drugs,* John Libbey and company Ltd. Pp 191-198.

Stanley, J.L., Lincoln, R.J., Brown, T.A., McDonald, L.M., Dawson, G.R. and Reynolds, D.S. (2005). The mouse beam walking assay offers more sensitivity over the rotarod in determining motor coordination deficits induced by benzodiazepines. *Psychopharmacology,* 19: 221-227.

Steimer, T (2002). The biology of fear and anxiety related bahaviour. *Dialogues in clinical Neuroscience,* 4: 231-249.

Stein, D.J. and Matsunaga, H. (2002). Cross-cultural aspects of social anxiety disorders. *Psychiatric Clinics of North America,* 24 (4): 773-82.

Swinyard, E.A. and Kupferberg, H.J. (1985). Antiepileptic drugs: Detection, Quantification and Evaluation. *Fed Proc,* 44:39-45.

Swinyard E.A., Woodhead, J.A., White, H.S. and Franklin, M.R. (1999). General Principles: Experimental selection, quantification and evaluation of anticonvulsants. In: Levy, R.H., Matsson, B., Melrum,J.K. and Dreifus, F.E. (Eds) *Antiepileptic Drugs,* 3rd edition. Raven Press. New York. USA. PP. 85-103

Trease. G.E. and Evans, M.C. (2002). Textbook of Pharmacognosy. 14th edition. Balliere Tindal London, 81-90, 269-275,300

Viswanatha, S.A.H.M., Thippeswamy, A.H.M., Manjala, D.V. and Meahendra Kumar, C.B. (2006). Some neuropharmacological effects of the methanolic root extract of *Cissus quadrangularis* in mice. *African Journal of Biomedical Research,* 9: 69-75.

Vogel, H.G. and Vogel, W.H. (1997). Psychotropic and Neurotropic activity in: Vogel, H.G. and Vogel, W.H. (ed) Drug Discovery and Evaluation: Pharmacological Assays New York. Springerverlag, Berlin Heidelberg. PP 1-5.

Volz, H.P. and Kieser, M. (1997). Kava-kava extract WSI490 versus placebo in anxiety disorders: A randomized placebo-controlled 25-weeks outpatient trial. *Pharmacopsychiatry,* 30: 1-5.

Weizman, R., Paz, L., Backer, M.M., Amiri, Z., Modai, I. and Pick, C.G. (1999). Mouse strains differ in their sensitivity to alprazolam effect in the straircase test. *Brain Research,* 839:58-65.

Weizman, R., Paz, L., Peter, Y. and Pick, C.G. (2001). Mice performance on the staircase test following acute ethanol administration. *Pharmacology, Biochemistry and Behaviour,* 68:491-495

White, H.S., Wolf, H.H., Woodhead, J.H. and Kupferberg, H.J. (1998). The national institute of Health anticonvulsant drug development programme. Screening for efficacy. In: French, J., Leppik, I.E. and Dichter, M. A. (Eds). Antiepileptic drug development: *Advances in Neurology* vol. 76, Lippincott – Raven Publishers. Philadelphia, pp. 29-39.

WHO-World Health Organization (1978). Resolution on drug politics and management of medicinal plants, WHO Document WHA, pp. 31-33.

WHO (2001). The World Health Report. Mental health: new understanding new hope. *Geneva.* PP. 1-15.

WHO/EDM/TRM/2000.1 General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. PP. 1-88

Wickenden, A.D. (2002). Potassium channels as antiepileptic drug targets. *Neuropharmacology,* 43: 1055-1060.

Wildund, I.K.; Mattson, L.A.; Lingren, R and Limon SC. (2009): *International Journal of clinical phamaceutical research,* 19:89-99.

Wolfman, C., Viola, H., Paladini, A.C., Dajas, D. Medina, J.H.(1994). Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coeruiea. Pharmacology, Biochemistry and Behaviour,* 47: 1-4

Woode, E., Poku, R.A. and Abotsi, W. K. M. (2011). *International Journal of Pharmacology,* 7 (3): 405-409.

Yemitan, O.K. and Adeyemi, O.O. (2003). Anxiolytic and muscle – relaxant activities o*f Dalbergia saxatiilis. West African Journal of Pharmacology and Drug Research*, 19 (Nos 1 & 2): 42-4

Zangrossi-Jr, H. and Graeff, I. (1997). Behavioural validation of the elevated T maze, a new animal model of anxiety. *Brain Reserch Bulletin*, 44:1-5.

Sofowora, A.A., (1993). Medicinal plants in Africa. Spectrum Book Ltd., Ibadan, Nigeria. PP. 31-41

Soulimani, R., Younos, C., Jarmouni, S., Bousta, D., Misslin, R. and Mortier, F. (1997). Behavioural effects of *Passifiora incarnata L*. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. *Journal of Ethnopharmacology,* 57 (1): 11-20.

Stables, J.P. and Kupferberg, H.J. (1997). The NIH Anticonvulsant Drug Development (ADD) program: preclinical anticonvulsant screening project. In: Avanzini, G., Regesta, G., Tanganelli, P., and Avoli, M. (Ed). *Molecular and Cellular Targets for Antiepileptic drugs,* John Libbey and company Ltd. Pp 191-198.

Stanley, J.L., Lincoln, R.J., Brown, T.A., McDonald, L.M., Dawson, G.R. and Reynolds, D.S. (2005). The mouse beam walking assay offers more sensitivity over the rotarod in determining motor coordination deficits induced by benzodiazepines. *Psychopharmacology,* 19: 221-227.

Steimer, T (2002). The biology of fear and anxiety related bahaviour. *Dialogues in clinical Neuroscience,* 4: 231-249.

Stein, D.J. and Matsunaga, H. (2002). Cross-cultural aspects of social anxiety disorders. *Psychiatric Clinics of North America,* 24 (4): 773-82.

Swinyard, E.A. and Kupferberg, H.J. (1985). Antiepileptic drugs: Detection, Quantification and Evaluation. *Fed Proc,* 44:39-45.

Swinyard E.A., Woodhead, J.A., White, H.S. and Franklin, M.R. (1999). General Principles: Experimental selection, quantification and evaluation of anticonvulsants. In: Levy, R.H., Matsson, B., Melrum,J.K. and Dreifus, F.E. (Eds) *Antiepileptic Drugs,* 3rd edition. Raven Press. New York. USA. PP. 85-103

Trease. G.E. and Evans, M.C. (2002). Textbook of Pharmacognosy. 14th edition. Balliere Tindal London, 81-90, 269-275,300

Viswanatha, S.A.H.M., Thippeswamy, A.H.M., Manjala, D.V. and Meahendra Kumar, C.B. (2006). Some neuropharmacological effects of the methanolic root extract of *Cissus quadrangularis* in mice. *African Journal of Biomedical Research,* 9: 69-75.

Vogel, H.G. and Vogel, W.H. (1997). Psychotropic and Neurotropic activity in: Vogel, H.G. and Vogel, W.H. (ed) Drug Discovery and Evaluation: Pharmacological Assays New York. Springerverlag, Berlin Heidelberg. PP 1-5.

Volz, H.P. and Kieser, M. (1997). Kava-kava extract WSI490 versus placebo in anxiety disorders: A randomized placebo-controlled 25-weeks outpatient trial. *Pharmacopsychiatry,* 30: 1-5.

Weizman, R., Paz, L., Backer, M.M., Amiri, Z., Modai, I. and Pick, C.G. (1999). Mouse strains differ in their sensitivity to alprazolam effect in the straircase test. *Brain Research,* 839:58-65.

Weizman, R., Paz, L., Peter, Y. and Pick, C.G. (2001). Mice performance on the staircase test following acute ethanol administration. *Pharmacology, Biochemistry and Behaviour,* 68:491-495

White, H.S., Wolf, H.H., Woodhead, J.H. and Kupferberg, H.J. (1998). The national institute of Health anticonvulsant drug development programme. Screening for efficacy. In: French, J., Leppik, I.E. and Dichter, M. A. (Eds). Antiepileptic drug development: *Advances in Neurology* vol. 76, Lippincott – Raven Publishers. Philadelphia, pp. 29-39.

WHO-World Health Organization (1978). Resolution on drug politics and management of medicinal plants, WHO Document WHA, pp. 31-33.

WHO (2001). The World Health Report. Mental health: new understanding new hope. *Geneva.* PP. 1-15.

WHO/EDM/TRM/2000.1 General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. PP. 1-88

Wickenden, A.D. (2002). Potassium channels as antiepileptic drug targets. *Neuropharmacology,* 43: 1055-1060.

Wildund, I.K.; Mattson, L.A.; Lingren, R and Limon SC. (2009): *International Journal of clinical phamaceutical research,* 19:89-99.

Wolfman, C., Viola, H., Paladini, A.C., Dajas, D. Medina, J.H.(1994). Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coeruiea. Pharmacology, Biochemistry and Behaviour,* 47: 1-4

Woode, E., Poku, R.A. and Abotsi, W. K. M. (2011). *International Journal of Pharmacology,* 7 (3): 405-409.

Yemitan, O.K. and Adeyemi, O.O. (2003). Anxiolytic and muscle – relaxant activities o*f Dalbergia saxatiilis. West African Journal of Pharmacology and Drug Research*, 19 (Nos 1 & 2): 42-4

Zangrossi-Jr, H. and Graeff, I. (1997). Behavioural validation of the elevated T maze, a new animal model of anxiety. *Brain Reserch Bulletin*, 44:1-5.