ANTICONVULSANT EFFECT OF METHANOL LEAF EXTRACT OF *CASSIA MIMOSOIDES* LINN IN LABORATORY ANIMALS

BY

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DECLARATION

I hereby declare that the work in this dissertation entitled "Anticonvulsant Effect of *Cassia mimosoides*Linn in Laboratory Animals" has been carried out by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for the award of another degree or diploma at this or any other Institution.

Abdulkadir Muhammad KABIRU _____ _____
Name of Student Signature Date

CERTIFICATION

This dissertation entitled "ANTICONVULSANT EFFECT OF *CASSIA MIMOSOIDES* LINN IN LABORATORY ANIMALS" by Abdulkadir Muhammad KABIRU, meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dr. M.G. Magaji Head of Department	_____ Signature	_____ Date
Prof. S. A. Abdullahi Dean, School of Postgraduate Studies	_____ Signature	_____ Date

DEDICATION

This Dissertation is dedicated to my beloved parents, Hon. Alkali Kabiru Idris and Hajiya Rabiatu Muhammad Sani and all people suffering from epilepsy.

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All praises and gratitude are due to Almighty Allah, the One and Only, The Self-Sufficient Master, Whom all creatures need, He neither eats nor drinks, He begets not, nor was He begotten, and there is none co-equal or comparable unto Him for making this dissertation possible. May the peace and blessings of Allah be upon His final Messenger, Muhammad (S.A.W), his progeny and all those who follow the right path till the last day. My sincere appreciation goes to my supervisory team; Dr. Ya'u Jamilu and Dr. Yerima Musa for their inspirational guidance, support, rational judgment and technical input throughout the course of this study, I eternally remain grateful.

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ABSTRACT

*Cassia mimosoides*Linn. belongs to the family of Caesalpinioideae. It is an annual herb which is found growing as weeds in Kudingi forest,Zaria LGAof Kaduna State, Nigeria and spread throughout the sub-Saharan Africa. In Nigeria, *Cassia mimosoides*is used as a remedy for ulcer, diarrheal disease and in the management of epilepsy in Zaria, Kaduna State, Nigeria. The study was conducted to evaluate the anticonvulsant potential of the methanol leaf extract of *Cassia mimosoides* using pentylenetetrazole (PTZ) induced seizure in mice, maximal electroshock (MES) induced seizure in chicks, picrotoxin induced seizure in mice and strychnine induced seizure in mice. Chronic model of epilepsy involving pentylenetetrazole induced kindling in rats as well as mechanistic studies were conducted using cyproheptadine, flumazenil, naloxone and sildenafil. Extraction of 1000g of the powdered leaves afforded a 14.17% $^{w}/_{w}$ yield by cold maceration using 70% methanol. The preliminary phytochemical screening of the methanol leaf extract of *Cassia mimosoides*revealed the presence of alkaloids, flavonoids, saponins, tannins and steroids/terpenoids. The oral median lethal dose (LD_{50}) values for rats, chicks and mice using Organization for Economic Cooperation and Development 425 (OECD 425) method was found to be greater than 5000 mg/kg suggesting a non-toxic profile of the extract. In the maximal electroshock induced seizure model, the extract did protect the animals against tonic hind limb extension (THLE). It also decreases significantly (p<0.05) the mean recovery time of the convulsed chicks. The extract protected the mice against PTZ-induced seizure by delaying the mean onset of seizure at all doses tested significantly (p<0.05) in a dose depended manner. The extract produced protection against both strychnine and picrotoxin induced seizure in mice by delaying the mean onset of seizure significantly (p<0.05) in the convulsed mice at all doses and there was increase quantal protection against seizure.

Single oral dose administration of the extract (1500 mg/kg), phenytoin (20 mg/kg) and cyproheptadine (4 mg/kg) offered 40%, 100% and 0% protection against tonic hind limb extension respectively, while co-administration of cyproheptadine (4 mg/kg) and extract (1500 mg/kg) as well as cyproheptadine (4 mg/kg) and phenytoin (20 mg/kg) offered a reduced protection of 20% and 30% protection against seizure respectively. This indicate the involvement of serotonergic and histerminergic pathways in the anticonvulsant effect of the extract. The effect of flumazenil was studied on the anticonvulsant activity of the plant extract to determine $GABA_A$ receptor involvement. Mean onset of seizure in extract group (1500 mg/kg) with flumazenil (10 mg/kg) and diazepam group (1.5 mg/kg) with flumazenil (10mg/kg) was significantly ($p<0.05$) reduced as compared with extract (1500mg/kg) and Diazepam (1.5 mg/kg) group respectively. There was increase percentage mortality of 16.67% in the diazepam with flumazenil and extract with flumazenil groups. This shows the likely involvement of $GABA_{-A}$ as mechanism of anticonvulsant action. Effect of naloxone also was studied on the anticonvulsant activity of the plant extract. Mean onset of seizure in extract group (1500 mg/kg) with naloxone (0.3 mg/kg) and diazepam group (1.5 mg/kg) with naloxone (0.3 mg/kg) was significantly ($p<0.05$) reduced as compared with extract (1500 mg/kg) and diazepam (1.5 mg/kg) groups. There was decrease quantal protection and increase percentage mortality of 16.67%. This shows the likely involvement of opioidergic pathway. The extract at all doses tested reduced the severity of seizure episodes induced by kindling with reduced seizure score significantly ($p<0.05$) after eleven injections. The results suggest that the methanol leaf extract of*Cassia mimosoides*Linn.*p*ossess significant anticonvulsant and antiepileptogenic properties.

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LIST OF ABBREVIATIONS

5 –HT: Hydroxytryptamine

5HIAA: Hydroxyindole Acetic Acid

ADC: Adenylate Cyclase

AEDS: Antiepileptic Drugs

AIS: Axon Initial Segment

AMP: Adenosine Monophosphate

AMPA: Alpha – amino- 3- hydroxyl – 5- methylisoxazole – 4 – Propionic Acid.

Ca^{+}: Calcium Ion

Cl^{-}: Chloride Ion

CNS: Central Nervous System

CSF: Cerebrospinal Fluid

CSF: Cerebrospinal Fluid

CT: Computed Tomography

CYP: Cyproheptadine

DAG: Diacyl Glycerol

DC: Decarboxylase

DZP: Diazepam

EEG: Electroencephalography

FMH: Alpha flouromethyl - histidine

GABA: Gamma AminoButyric Acid

GAD: Glutamic Acid Decarboxylase

HTR: Hydroxy Tryptophan Hydroxylase

IBE: International Bureau for Epilepsy

ILAE: International League against Epilepsy

IP3: Inositol Triphosphate K+ Potassium Ion

CRE: *Cassia mimosoides* linn Methanol Leaf Extract

LD_{50}: Median Lethal Dose

MAO-A: Monoamine Oxidase A

MES: Maximal Electroshock Test

MRI: Magnetic Resonance Imaging

N/S: Normal Saline

Na^{+}: Sodium Ion

NINDS: National Institute of Neurological Disorders and Stroke

NMDA: N- Methyl –D - Aspartate

PHB: Phenobarbitone

PTY: Phenytoin

PL_C: Phospholipase C

PTZ: Pentylenetetrazole

ROS: Reactive Oxygen Species

SUDEP: Sudden unexpected death in Epilepsy

TLE: Temporal Lobe Epilepsy

TM: Traditional Medicine

TMN: Tuberomammillary nucleus

TPH: Tryptophan Hydroxylase

TRP: Tryptophan

VA: Valproic Acid

VGCCS: Voltage Gated Calcium Channels

W.H.O: World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

The use of plants as medicine is an ancient practice common to all societies especially the African society and this practice continues to exist in the developing nations. It is on this basis that researchers keep searching for medicinal plants in order to produce the best for physiological uses as medicines (Usman and Osuji, 2007). The medicinal value of plants lies in some chemical substances that produce a definite physiological action in the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al.,* 2005). The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries (Sandhu and Heinrich, 2005). Traditional healers claim that their medicine is cheaper, more effective and impart least side effects as compared to synthetic medicines. In developing countries, low-income people such as peasant farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections (Rojas *et al.,* 2006). World Health Organization (WHO) encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries (Amos *et al.,* 2001). The degree of sensitization and mobilization by the WHO has encouraged some African countries to commence serious development on Traditional African medicine (Elujoba *et al.,* 2005).

Epilepsy is a chronic disorder affecting both sexes (Blume *et al.,* 2001). It is the second most common chronic neurological condition seen by neurologist worldwide (Sridharan, 2002). It has no age, racial, social, sexual or geographical boundaries. Common causes 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, 2003). It affects

approximately 50 million people worldwide and accounts for 0.5% of the global burden of diseases (WHO, 2019). Among brain disorders, epilepsy stands out not only because of its high prevalence and incidence rates, but in particular because of the myths and beliefs attached to the condition in various cultures and the resulting impacts on the individual, the family and the community as a whole (Jamison *et al.,* 2006). Medicinal plants used for the therapy of epilepsy in traditional medicine (TM) have been shown to possess promising anticonvulsant activities and can be valuable source of new antiepileptic drugs (Kabir *et al*., 2005).

1.1 Statement of Research Problem

Epilepsy is one of the most common serious neurological disorders (Hirts *et al.,* 2007). Close to 80% of people with epilepsy live in low- and middle-income countries (WHO, 2019).The estimated proportion of the general population with active epilepsy (i.e. continuing seizures or with the need for treatment) at a given time is between 4 and 10 per 1000 people (WHO, 2019).

Globally, an estimated five million people are diagnosed with epilepsy each year. In high-income countries, there are estimated to be 49 per 100 000 people diagnosed with epilepsy each year. In low- and middle-income countries, this figure can be as high as 139 per 100 000. This is likely due to the increased risk of endemic conditions such as malaria or neurocysticercosis; the higher incidence of road traffic injuries; birth-related injuries; and variations in medical infrastructure, the availability of preventive health programmes and accessible care (WHO, 2019). About 10 million people from Africa (Coleman *et al*., 2002). The prevalence of epilepsy in Nigeria is estimated to be around 37 to 41 per 1000 persons (Banerjee *et al*., 2009) which is alarming. The prevalence of active epilepsy in Nigeria is 3-14 per 1,000 populations (WHO, 2004). In another study in south-eastern Nigeria the point prevalence of active epilepsy was 4.3/1,000 for the total

population, 4.9/1,000 for males and 3.7/1,000 for females and the age-adjusted prevalence for the total population was 4.1/1,000 (Nwani *et al* 2015). The world health organization (WHO) estimates that of the 10 million people living with epilepsy in Africa, 7 million (70%) are not receiving adequate treatment (WHO, 2019). The incidence and prevalence of epilepsy is varying among countries. However, recently it has been shown that the estimated prevalence of the general population is 10 per 1000 people. It accounts for about 0.5% of global burden of disease and up to 2.4 million new cases are diagnosed each year (WHO, 2019). Although, standard therapy permits control of seizures in 70% of these patients, about three fourths of people with epilepsy living in low and middle income countries do not get the treatment they need (WHO, 2019).

Around thirty thousand develop epilepsy every year globally and the condition will affect about twenty at some times in their lives (Dhanasekaran and Palayan, 2010). The incidence of epilepsy varies greatly with age, with high rates in early childhood, low levels in early adult life and a second peak people over 65 years old from as high as 560 cases per 100,000 of the population per year for infants, to as low as 20.3 cases per 100,000 per year for ages 15 to 30 years (Noebels and Avoli, 2012).

Epilepsy can have adverse effects on social and psychological well-being (Baker, 2002). These effects may include social isolation, stigmatization, or disability, which may result in lower educational achievement and worse employment outcomes. Learning difficulties are common in those with the condition, and especially among children with epilepsy. The stigma of epilepsy can also affect the families of those with the disease (WHO, 2019). People with epilepsy are at an increased risk of death and this increase is between 1.6 and 4.1 fold greater than that of the general population and is often related to the underlying cause of the seizures, *epilepsy*, suicide,

trauma, and sudden unexpected death in epilepsy (SUDEP) (Hitiris *et al.,* 2007). Death from *status epilepticus* is primarily due to an underlying problem rather than missing doses of medications (Hitiris*et al.,* 2007). The risk of suicide is increased between two and six times in those with epilepsy (Mula *et al.,* 2013).

SUDEP appears to be partly related to the frequency of generalized tonic-clonic seizures (Ryvlin *et al.,* 2013), and accounts for about 15% of epilepsy related deaths (Kwan, 2012). Patients with epilepsy fail to experience adequate control of their seizures despite optimal use of available antiepileptic drugs-AEDs (Stables and Kupferberg, 1997). Synthetic AEDs are effective only in approximately 50% of patients and many refractory cases of epilepsy still remain highly resistant to their treatment (Danjuma *et al*., 2009). Furthermore, AEDs are associated with side effects, including teratogenicity and adverse effects on cognition and behaviour (Raza *et al.,* 2001).

1.2 Justification for the Study

Antiepileptic drugs (AEDs) are the mainstay in management of epilepsy and have great impact on the quality of life of epileptic patients. Despite the continued development and release of new antiepileptic drugs, many patients have seizures that do not respond to drug therapy or have related side effects that preclude continued use (Perucca *et al*., 2007). Even in patients in whom pharmacotherapy is efficacious, current AEDs do not affect the progression of epilepsy (Loscherand Schmidt, 2006). These factors and more therefore, call for development and search for new AEDs especially from medicinal plant sources which may have fewer side effects and greater efficacy.

There are many medicinal plants employed locally in the management of epilepsy but with limited scientific evidences for their safety and effectiveness (WHO, 2008). This necessitates the

need to scientifically evaluate the anticonvulsant profile of *Cassia mimosoides* linn. in order to validate its use for treatment of convulsions by traditional medical practitioners (TMPs) around Zaria, Kaduna state of Nigeria.

1.3 Theoretical Framework

Development of new drugs often starts with discovery and followed by screening. This involves determination of toxicity and potency as well as pharmacological activity of the test material.

In this study, toxicity study was first carried out to investigate the acute toxicity of the methanol leaf extract of *Cassia mimosoides*. The index of acute toxicity, median lethal dose (LD_{50}) of the plant leaf was determined using OECD Method limit test (2001). Using this method, it is possible to obtain adequate information on the acute toxicity and the median lethal dose (LD_{50}) of a compound with fewer experimental animals. It is also useful for oral route of administration applicable to drugs, industrial and agricultural chemicals.

Detailed studies are difficult to carry out on epileptic patients, therefore many different animal models of epilepsy have been devised for the evaluation of anticonvulsant activity of the test compounds. These models for testing antiepileptic drugs have also shed light on the etiopathogenesis of epilepsy (Tripathi, 2008). Electrical and chemical method of seizure induction were employed in this study.

1.3.1 Electrically induced seizures

The maximal electroshock seizure test (MES) is a standard AEDs test that evaluate the testing material' ability to protect against hind limb tonic extension (HLTE)phase of the test (DeLorenzo *et al.,* 2001). The MES model induces seizure by sending an electrical signal that

hyper excites focus itself (Tripathi, 2008). Examples of AEDs effective against generalized tonic-clonic seizures are phenytoin, carbamazepine and phenobarbitone (Loscher *et al.,* 1991).

1.3.2 Chemically induced seizures

1.3.2.1 Pentylenetetrazole (Metrazole) induced seizure

This assay has been used primarily to evaluate antiepileptic drugs. However, it has been shown that most anxiolytic agents are also able to prevent or antagonize metrazole induced convulsion. Stimulants, anti-depressants, neuroleptics and some antiepileptic drugs do not show metrazol-antagonism at tolerable doses (Lippa *et al.,* 1979). This model primarily identifies compounds that raise seizure threshold. Absece of clonic spasm in the observed time period indicates a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold (Swinyard *et al.,* 1989).

According to DeSarro *et al.,* (1999), pentylenetetrazole (PTZ) may exert its convulsive effect by inhibiting the activity of gamma amino butyric acid (GABA) at $GABA_A$ receptors; the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhanced convulsion respectively (Gale, 1992). AEDs effective against this type of seizure (e.g phenobarbitone, sodium valproate, ethosuximide and benzodiazepines) are effective in the treatment of myoclonic and absence seizures (Loscher *et al.,* 1991).

1.3.2.2 Strychnine induced seizure

The convulsing action of strychnine is due to interference with postsynaptic inhibition mediated by glycine. Glycine is an important inhibitory transmitter to motor neurons and inter-neurones in the spinal cord, and strychnine acts at all glycine receptors. Strychnine-sensitive postsynaptic

inhibition in higher centers of the CNS is also mediated by glycine.Compounds which reverse the action of strychnine have been shown to have anxiolytic properties (Costa *et al.,* 1975) and anti-epileptic effects (Larson, 1969). Drugs effective against strychnine induced convulsion include phenobarbitone, valproate and diazepam.

1.3.2.3 Picrotoxin induced seizure

Picrotoxin induced convulsions are used to further evaluate CNS-active compounds. Picrotoxin is regarded as a $GABA_A$ antagonist modifying the function of the chloride ion channel of the $GABA_A$ complex (Vogel, 2008). Drugs effective against picrotoxins induced convulsion include phenobarbitone and diazepam.

1.3.3 Chronic seizure models

These models are used to investigate epilepsy mechanisms. They lead to animals that are truly epileptic and exhibit spontaneous seizures and they are in addition used to investigate temporal lobe epilepsy (TLE). Two most commonly used animal models of chronic seizure development are; *kindling and status epilepticus*. Several reviews have considered kindling and/ or status epilepticus as models of clinical epilepsy (Coulter *et al.*, 2002; McIntyer and Poulter, 2001; Sutula, 2001).

1.3.3.1 The kindling model

Kindling is widely accepted as a functional epilepsy model in which the altered neuronal response develops in the absence of gross morphological damage, such as those seen in other epilepsy models (Cavazos *et al.* 1994). The kindling models employ either electrical or chemical stimulation to produce a progressive, highly reliable, permanent increase in epileptic response to inducing agent (Goddard *et al.* 1969). PTZ-kindlingis a well-known model for epilepsy and is

described as a protocol in which repeated administration of the convulsant causes gradual seizure development culminating into generalized tonic-clonic seizures (Becker *et al*., 1997).

1.4 Aims and Objectives

1.4.1 Aim of the study

The aim of this study was to provide some pharmacological rationale for the ethnomedicinal use of *Cassia mimosoides*Linn. in the management of epilepsy.

1.4.2 Objectives of the study

The specific objectives of this study are;

i. To establish the acute toxicity profile of *Cassia mimosoides*Linn. in mice, chicks and rats.

ii. To determine the phytochemical constituents present in the *Cassia mimosoides*Linn.

iii. To evaluate anticonvulsant activity of methanol leaf extract of *Cassia mimosoides* in acute and chronic models of epilepsy

iv. To establish the possible mechanism(s) of anticonvulsant activity of the methanol leaf extract of *Cassia mimosoides*

1.5 Statement of Research Hypothesis

The methanol leaf extract of *Cassia mimosoides*Linnpossesses a significant anticonvulsant activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epilepsy

Epilepsy is defined as a chronic disorder of the central nervous system of various etiologies characterized by recurrent seizures due to excessive discharge of cerebral neurons (Olubunmi, 2006). Seizure can be defined as a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (Malvi *et al.,* 2011). International League Against Epilepsy (ILAE) and International Bureau for Epilepsy (IBE) in 2005 defined Epilepsy as a brain disorder characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychologic, and social consequences of this condition (Fisher *et al.,* 2005). In epilepsy, the normal pattern of neuronal activity becomes disturbed, causing strange sensations, emotions, and behavior, sometimes convulsions, muscle spasm, and loss of consciousness. During a seizure, neurons may fire as many as 500 times a second, much faster than normal. In some people, this happens occasionally; for others, it may happen up to hundreds of times a day (NINDS, 2004). The clinical signs and symptoms of seizures depend on the location of the epileptic discharges in the cortex and the extent and pattern of the propagation of the epileptic discharge in the brain (Daniel and Steven, 2011).

2.2 Etiology of Epilepsy

Epileptic conditions are multifactorial disorders in which the action of more than one gene together with environmental factors contributes to the disease phenotype (Todorova *et al.,* 1999).

Epilepsy is characterized by abnormal synchronized discharge of neurons leading to alterations in electroencephalograph activity and behavior, it may result from long lasting phasic changes in the brain affecting neurotransmitter release and transport, the properties of receptors and channels, synaptic reorganization and astrocyte activity (Sierra *et al.,* 2007). Three important factors have been implicated in the etiology of epilepsy. The first factor is predisposition, or threshold. The ease with which a seizure can be provoked, or an epileptic condition can be induced, is referred to as a threshold. Individual differences in threshold are largely attributable to genetic variations but could also be acquired via different means e.g. certain types of perinatal injuries which can alter threshold. Threshold is a dynamic phenomenon which varies throughout the day, and it also changes in relation to hormonal influences during the menstrual cycle in women (Omer*et al.,* 2011). Patients with a high seizure threshold can experience severe epileptogenic brain injuries and precipitating factors but never have seizures, while those with low seizure thresholds can develop epilepsy with minimal insults and, in many, from precipitating factors alone (provoked seizures). Stimulant drugs lower seizure threshold and sedative drugs increase it but withdrawal from sedative drugs can lower threshold and provoke seizures. However, antiepileptic drugs work by increasing seizure threshold (Fisher *et al.,* 2005).

The second important factor for epilepsy is the epileptogenic abnormality itself which is attributable to identifiable brain defects including brain malformations, infections, vascular disturbances, neoplasms, scars from trauma, including strokes, and disorders of cerebral metabolism. Treatment for this abnormality is most effective if it is directed at the underlying cause and the most common type of epilepsy related is temporal lobe epilepsy (TLE), usually associated with a characteristic lesion called "hippocampal sclerosis". Hippocampal sclerosis appears to be caused by cerebral injury within the first few years of life in individuals with a

genetic predisposition to this condition and it is relatively found among elderly people over the age of 85 years (Nelson *et al.,* 2011). Some forms of epilepsy are unassociated with identifiable structural lesions or diseases and are usually unassociated with other neurological or mental deficits. These are genetically inherited, generally easily treated with medications without sequelae, and referred to as idiopathic epilepsies (Sheth and Hermann, 2007).

The third important factor is the precipitating condition, which determines when seizures occur. Common precipitating factors include fever for children with febrile seizures, alcohol and sedative drug withdrawal, hypoglycemia, anoxia sleep deprivation, stimulant drugs and in some patients' stress. Reflex seizures are precipitated by specific sensory stimuli. The most common are photosensitive seizures induced by flickering light, but some patients have very specific reflex epilepsy with seizures precipitated by such stimuli as being startled, particular types of music, certain visual patterns, reading (NINDS, 2003). Identification of precipitating factors is helpful if they can be avoided, but in most patients specific precipitating factors are not apparent, and may not exist at all.

2.3 Types of Epilepsy

The International Classification of Epilepsies and Epileptic Syndromes have distinguished various types of epilepsy based on the accompanying symptoms or the region of the brain where they occur. The most common form is temporal lobe epilepsy. Other forms include the following:

I. Absence epilepsy with recurring episodes of absence seizures with brief lapses of conscious state.

II. Frontal lobe epilepsy involving a bundle of brief seizures with abrupt onset and ending

III. Occipital lobe epilepsy resembling temporal or frontal lobe epilepsy andOccipital lobe epilepsy resembling temporal or frontal lobe epilepsy and commencing with some visual hallucinations or rapid eye movements

IV. Psychomotor epilepsy characterized by recurring partial seizures.

Other types of epilepsy developed during childhood are:

I. Lennox-Gastaut syndrome

II. West's syndrome (infantile spasms)

III. Juvenile myoclonic epilepsy (impulsive petit mal) (NINDS, 2003).

2.4 Classification of Seizures

Seizures can be classified into three broad groups according to commission on classification and terminology of the International League against Epilepsy (ILAE, 2017). The seizures can either be focal onset seizure, generalized onset seizure or a seizure of unknown onset; which is based upon the nature of the seizure rather than presence or absence of an underlying cause.

2.4.1 Focal onset seizures

Focal onset seizures are defined as "originating from within networks limited to one hemisphere''. They may be discretely localized or more widely distributed. Focal seizure may originate in subcortical structures e.g partial seizures (ILAE, 2017).

2.4.2 Generalized onset seizures

Generalizedonset seizures are defined as “originating at some point within and, and rapidly engaging, bilaterally distributed networks” e.g tonic clonic seizure, absence seizure (ILAE, 2017).

2.4.3 A seizure of unknown onset

A seizure of unknown onset may still evidence certain defining motor (e.g tonic-clonic) or non-motor (e.g behavior arrest) characteristics. With further information or future observed seizures, a reclassification of unknown-onset seizures into focal or generalized-onset categories may become possible e.g febrile convulsion(ILAE, 2017).

2.5 Ion Channels and their Roles in Epilepsy

It is known that every heartbeat, every nerve impulse, every movement and thought is critically dependent on the tightly controlled and precised timed flow of ions across cell membranes (Nestler *et al.,* 2009). Ion channels are important in cellular functions and are altered in many pathological conditions either directly or indirectly, as in the channelopathies (Camerino *et al.,* 2007). Their role is most obvious in the membrane of electrically excitable cells, such as the neuron, the cardiac myocyte, and the skeletal muscle fiber.

Voltage-gated K^+ channels are essential in the repolarisation and hyperpolarisation that follows paroxysmal depolarisation shifts (PDSs), and their mutations are the substrate for neonatal epilepsy and they are new targets for AEDs such as retigabine (Armijo *et al.,* 2005). Voltage-gated Ca^{2+} channels are involved in neurotransmitter release, in the sustained depolarization

phase of PDSs, and in the generation of absence seizures; their mutations are a substrate for juvenile myoclonic epilepsy and the absence seizure. Other drugs like phenytoin and carbamazepine, are potent antiepileptic agents which act by altering Na+ channel kinetics (Nestler*et al.*, 2009).

2.6 Action Potential and Seizure Development

An action potential is a rapidly propagating depolarization of the axonal membrane that can lead to the release of neurotransmitter from axon terminals (Nestler *et al.,* 2009). Neurons, cardiac muscle, smooth muscle, skeletal muscle, and many endocrine cells have an excitable character, and thus, capable of generating and propagating electrical action potentials (Dekker *et al.*, 2008). It is this signal that is responsible for the transfer of information from one part of neuron to another. The threshold is important to ensure that small, random depolarization of the membrane do not generate action potentials. Only stimuli of sufficient importance result in information transfer via action potential in the axon. Another essential feature of action potentials is that they are all-or-none events. The all-or-none law demonstrates that any stimulus large enough to produce an action potential produces the same size action potential, regardless of stimulus strength. In other words, once the stimulus is above threshold, the amplitude of the response no longer reflects the amplitude of the stimulus. However, the latency, the time delay from the onset of the stimulus to the peak of action potential, is a function of stimulus strength (Levitan and Kaczmarek, 2002).

Dendrites are the recipients of incoming synaptic activity and are said to be electrically active. They contain voltage-dependent Na^+, Ca^{2+} and K^+ channels and are capable of generating action potential and thus amplify incoming synaptic signals so that they can be propagated to the soma. Due to the presence of Na^+ channels along the length of axon, the action potential propagates down the axon and invades the presynaptic nerve terminals, where it triggers the influx of Ca^{2+} by activating voltage-dependent Ca^{2+} channels and subsequently leads to the Ca^{2+}-dependent release of neurotransmitter (Nestler *et al.,* 2009). Voltage-gated (delayed rectifier) K^+ channels contribute to the rapid repolarisation phase of the action potential. Although membrane depolarization opens these channels, they open and close more slowly than do Na^+ channels in response to depolarization. Therefore, inward Na^+ current dominates the early (depolarization) phase of action potential, and outward K^+ current dominates the later (repolarisation) phase. Thus, action potential is characterized by an initial depolarization as a result of fast inward Na^+ current, followed by a prolonged repolarization caused by slower and more sustained outward K^+ current (Dekker *et al.,* 2008).

2.7 Epileptogenesis and Ictogenesis

Epileptogenesis refers to the multiphase process in which a normal brain undergoes alterations to support the generation of spontaneous seizures. It may be initiated by brain damage produced by events such as head trauma, stroke, infection, or status epilepticus. Following such an initial insult, a latency phase without seizures follows and may last for weeks to years. During these initial stages, progressive brain alterations result in lowered seizure thresholds which eventually cause spontaneous seizures. Once seizures occur, the epileptic disease state probably continues to progress, with each seizure having the potential to induce additional neuronal alterations that may further lower seizure thresholds (Klitgaard and Pitkanen, 2003). Epileptogenesis also refers

to the process by which the previously normal brain is functionally altered and biased towards the generation of abnormal increase in electrical activity that subserves chronic seizures (Goldberg and Douglas, 2013). Drugs with antiepileptogenic properties, would act by blocking the initial epileptogenic process or by altering the epileptic disease state after the seizure onset. This would be by the ability of such drugs to reduce alterations in molecular, cellular, and network properties that occur during the epileptogenic process (Klitgaard and Pitkanen, 2003).

Ictogenesis is the initiation of paroxysmal activity or the process of seizure generation that is caused by many factors which could lead to sprouting wave-like changes in the electroencephalograph (EEG) (Silva and Francisco, 2008). Drug treatment options for epilepsy predominantly combat ictogenesis, and traditional AEDs have their effects by reducing the expression of epileptic seizures; nevertheless, their function invariably elicits some impairment of the normal neuronal excitability underlying cognitive function. The fact that ictogenesis and cognition are both mediated by neuronal excitability, it may not be possible to discover optimal non-impairing AEDs using traditional screens. This may be improved by performing drug screens in animal models of chronic epilepsy. Thus, by applying genetically modified or kindled animals it may be possible to discover new AEDs that inhibit the neuronal hypersynchronization leading to an ictal event, without interfering with normal neuronal excitability (Klitgaard and Pitkanen, 2003).

2.8 Neurotransmitters and Epilepsy

2.8.1 Serotonin and epilepsy

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that influences multiple processes, including autonomic function, motor activity, hormone secretion, cognition, and complex

processes associated with affection, emotion, and reward (Zhou *et al.,* 2007). In the terminal axon of the serotonergic neuron, free tryptophan (TRP) is converted to 5-HT (Struder *et al*., 2001). 5-HT synthesis is a two-step process catalyzed by tryptophan hydroxylase (TPH) and aromatic decarboxylase (DDC). TPH is the rate-limiting enzyme and exists in two isoforms TPH1 and TPH2. The TPH2 isoform is the predominant form in neuronal tissue (Sakowski *et al.,* 2006).

Serotonin uptake into presynaptic storage vesicles is mediated by the vesicular monoamine transporter (SLC18A2). The transporter accumulates serotonin into synaptic vesicles using a proton gradient across the vesicular membrane (Hoffman *et al.,* 2008). The 5-HT that is not stored in vesicles is degraded by monoamine oxidase A (MAOA) to 5-hydroxyindoleacetic acid (5-HIAA).An action potential stimulates a calcium-dependent exocytotic release of serotonin from presynaptic vesicles into the synaptic cleft, where it interacts with both post- and presynaptic receptors. At the presynaptic side, 5-HT activates 5-hydroxytryptamine (serotonin) receptor ($5HT1_A$), ($5HT1_B$), and ($5HT1_D$), which results in an attenuation of the 5-HT exocytosis (Struder *et al.,* 2001). This feedback loop regulates the 5-HT concentration in the synaptic cleft and therefore, the extent of stimulation of various 5HT receptor subclasses at the postsynaptic membrane (Boadle-Biber, 2003).

The $5HT_1$ receptors ($5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{1E}$, $5HT_{1F}$) work together with $5HT_2$ receptor subtypes ($5HT_{2A}$, $5HT_{2C}$) in mediating effector signals via activation of second messenger cascades (Struder *et al.*, 2001).

The main signaling pathway for 5HT1 receptor subtypes is via coupling of G protein alpha subunit. This interaction decreases cyclic AMP formation by inhibiting adenylate cyclases (ADC) (Bockaert *et al.,* 2006). After interaction with 5-HT, the main signaling linkage for the

5HT2 receptor sub-population is to activate phospholipase C (PL_C) through coupling of Gq/11 protein alpha (GNAQ) (Bockaert *et al.,* 2006). PLC catalyzes the formation of myoinositol-1, 4, 5-trisphosphate (IP3) and diacylglycerol (DAG) (Raymond *et al.,* 2001). The ionotropic 5HT3 receptor is a cation-specific ligand-gated ion channel, which does not activate a second messenger system (Niesler *et al.,* 2008).

The binding of 5HT to this receptor depolarizes the postsynaptic membrane by sodium influx and potassium efflux, which is assumed to influence the activation of 5HT2 receptors. 5HT4, 5HT6, and 5HT7 primarily couple G protein alpha, which results in an activation of adenylate cyclase, and consequently in an increase of cyclic AMP levels (Raymond *et al.,* 2001).

The amplification of all these second messenger signals in further downstream reactions which leads to the mediation of neurotransmitter release from central serotonergic, noradrenergic, and dopaminergic neurons in the brain by regulating potassium channels, several protein kinases, and other calcium dependent signals. Epilepsy can be caused by either abnormal ionic conductance or other alteration of neuronal membranes, or an imbalance between excitatory and inhibitory influences.

Several different types of neurons express serotonin (5HT) receptors in the CNS, e.g. at least $5HT_{1A}$, $5HT_{1B}$, $5HT_{2A}$, $5HT_{2C}$ and $5HT_7$ receptors are present on cortical and/or hippocampal glutamatergic or GABAergic neurons or terminals (Barnes and Sharp, 1999). G-protein-coupled 5HT receptors and the ligand gated ion channel 5HT3 receptor may directly or indirectly change ionic conductance and/or concentration. within the cells, resulting in de or hyperpolarization of neurons (Barnes and Sharp, 1999).

2.8.2 Histamine and epilepsy

In the CNS, the synthesis of histamine [2-(4-imidazolyl)-ethylamine] from 1-histidine by the catalytic activity of the rate-limiting enzyme histidine decarboxylase (Moya-Garcia *et al*., 2005). this takes place in a restricted population of neurons located in the tuberomammillary nucleus (TMN) of the posterior hypothalamus (Bhowmik *et al.*, 2012). They give rise to widespread and diffuse projections extending through the basal forebrain virtually to the entire brain including the cortex, striatum, thalamus, hippocampus, hypothalamus, locus coeruleus and spinal cord (Bhowmik *et al.*, 2012).

This morphology renders histamine to be able to act as a neurotransmitter and neuromodulator of a wide spectrum of physiological functions and behaviours of the CNS such as the circadian rhythms, catalepsy, energy homeostasis, thermoregulation, neuroendocrine and cardiovascular control, drinking and feeding, learning and memory, locomotion, sexual behaviour, analgesia and emotion (Haas *et al*., 2008).Histamine has been one of the most studied substances in medicine for a century, regulating a wide spectrum of activities, including its function in neurotransmission (Brown *et al*., 2001). The association between the histaminergic system with the pathogenesis of epilepsy is subject of extensive evaluation owing to the complex brain neurophysiology of histamine (Haas*et al*., 2008) and pleiotropic receptor ligand pharmacology (Bhowmik *et al.*,2012).The understanding of the pathophysiology of epilepsy is mostly confined to the conventional theory of deranged inhibitory GABAergic and protracted excitatory glutamatergic neurotransmission in excitotoxic neuronal death (Rowley *et al*., 2012). The imbalance could be modulated by various other neurotransmitter systems including the histaminergic system. The latter, through H_3 heteroreceptors, modulates the release of a wide spectrum of vital neurotransmitters, for example, GABA, glutamate, dopamine, 5-HT, noradrenaline and acetylcholine, in a pathway-dependent manner (Haas *et al*., 2008). Histamine

release is not only regulated by its own H_3 autoreceptor system but also by GABA via $GABA_A$ and $GABA_B$ receptors and by glutamate via NMDA receptors. Brain histamine plays an important role in protection against myoclonic jerks and generalized tonic-clonic seizures and its action is via H3 receptor (Bhowmik *et al.,* 2012). L-Histidine decreases the duration of clonic convulsion in electrically-induced seizure, but do not affect tonic convulsion. This effect of l-histidine is antagonized by α-flouromethyl histidine (FMH), indicating that it is due to histamine formed by decarboxylation of L-histidine in the central nervous system.

2.9 Basic Mechanisms of Focal Seizure Initiation and Propagation

The hypersynchronous discharges that occur during a seizure may begin in a very discrete region of the cortex and then spread to neighbouring regions. Seizure initiation is characterized by two concurrent events namely:

I. High-frequency bursts of action potentials

II. Hypersynchronization of a neuronal population (Bromfield *et al.,* 2006).

The synchronized bursts from a sufficient number of neurons result in a so-called spike discharge on the EEG. At the level of single neurons, epileptiform activity consists of sustained neuronal depolarization resulting in a burst of action potentials, a plateau-like depolarization associated with completion of the action potential burst, and then a rapid repolarization followed by hyperpolarization. This sequence is called the paroxysmal depolarizing shift. The bursting activity resulting from the relatively prolonged depolarization of the neuronal membrane is due

to influx of extracellular $Ca2^{+}$, which leads to the opening of voltage-dependent Na^{+} channels, influx of Na^{+}, and generation of repetitive action potentials. The subsequent hyperpolarizing after-potential is mediated by GABA receptors and Cl^{-} influx, or by K^{+} efflux, depending on the cell type (Lee *et al.*, 2011). Seizure propagation is the process by which a partial seizure spreads within the brain. It occurs when there is sufficient activation to recruit surrounding neurons. This leads to a loss of surrounding inhibition and spread of seizure activity into contiguous areas via local cortical connections, and to more distant areas via long association pathways such as the corpus callosum. Epileptogenesis is promoted by both non-synaptic and synaptic mechanisms that affect synchronicity as well as signal amplification by cerebral neurons (Engelborghs *et al.*, 2000). Acquired epilepsy may involve a wide range of mechanisms including alterations in neurotransmitter receptors (e.g. $GABA_A$ receptors) and/or voltage dependent currents e.g. sodium channels (Dyhrfield *et al.*, 2010).

2.9.1 Non-synaptic mechanisms of seizure initiation and propagation

Nonsynaptic mechanisms exert a powerful influence on seizure threshold. The regulation of intracellular and extracellular ions is necessary for establishing ionic gradients required for the operation of neuronal ion channels (Payne *et al.*, 2003). It is well established that non-synaptic epileptiform activity can be induced in hippocampal slices by reducing extracellular Ca2+ concentration (Marom *et al.*, 2001). Changes in ionic concentrations observed during hyperexcitation such as increased extracellular K^{+} or decreased extracellularCa2+may be caused by decreases in extracellular size or volume. Failure of Na^{+}-Kpump due to hypoxia or ischemia is known to promote epileptogenesis in animal models, and interference with Cl^{-}-K^{+} transport, which controls intracellular Cl^{-} and regulates GABA-activated inhibitory Cl^{-} currents, may lead to enhanced excitation. Excitability of synaptic terminals depends on the extent of depolarization

and the amount of neurotransmitter released. Synchronization following abnormal bursts of spikes in the axonal branching of thalamocortical relay cells plays a key role in epileptogenesis. Epileptic interactions that occur between neighboring neurons separated by small extracellular spaces also contribute to increased synchronization (Jefferys, 1995).

2.9.2 Synaptic mechanisms

Synaptic pathophysiology of epilepsy and epileptic disorders primarily involves reduced GABAergic inhibition or enhanced glutamatergic excitation (Bromfield *et al.,* 2006).

2.9.2.1 Gamma aminobutyric acid (GABA) - receptor inhibition

Occurrence of Gamma aminobutyric acid (GABA) in the central nervous system was demonstrated in 1950 and in the same decade GABA was shown to inhibit seizure activity after its direct cerebral application in dogs (Meldrum, 1978).The major inhibitory neurotransmitter GABA interacts with 2 major subtypes of receptor: $GABA_A$ (ionotropic) and $GABA_B$ (metabotropic) receptors. $GABA_A$ receptors are found postsynaptically while $GABA_B$ receptors are found presynaptically, and can thereby modulate synaptic release (Jefferys, 1995). In the adult brain, $GABA_A$ receptors are permeable to Cl^- ions; upon activation, Cl^- influx hyperpolarizes the membrane and inhibits action potentials. Neuronal circuits that are epileptic are known for being hyperexcitable and for lacking the normal balance of glutamatergic neurons (those that usually increase excitation) and GABAergic ones (those that decrease it) (Aroniadou *et al.,* 2008).

The action of GABA in the mammalian brain is mediated via the $GABA_A$ and $GABA_B$ receptors, these receptors differ in terms of their distribution in the brain, pharmacological profile and their

mechanisms of signal transduction (De Sarro *et al.,* 2000). The functional role of $GABA_{-B}$ receptors is to regulate the release of excitatory and inhibitory neurotransmitters. Both GABAA and $GABA_B$ are involved in the control of neuronal excitability in the brain and invariably play a major role in epileptogenesis (Kaila *et al.,* 2014). In addition, the levels of GABA and the sensitivity of $GABA_A$ receptors to the neurotransmitter may decrease, resulting in less inhibition (Armijo *et al.,* 2002).

Therefore, substances which are $GABA_A$ receptor agonists, such as barbiturates and benzodiazepines, are well known to suppress seizure activity (Bromfield *et al.,* 2006).

GABA levels have been shown to be reduced in the cerebrospinal fluid (CSF) of patients with certain kinds of epilepsy, such as infantile spasms, untreated generalized tonic-clonic seizures, and in excised epileptic tissue from patients with drug-resistant epilepsy, suggesting that these patients have decreased inhibition (Loscher and Siemes, 1985). Dogs with epilepsy have been shown to have low CSF levels of GABA and mice genetically susceptible to audiogenic seizures have a lower number of GABA receptors than non- seizure prone animals. Reduced GABA binding to GABA decarboxylase levels have been shown in kindled rats and in excised human epileptic tissue, suggestive of decreased GABAergic inhibition (Engelborghs *et al.,* 2000).

2.9.2.2 Glutamate receptors activation

Glutamate is the predominant excitatory neurotransmitter in the motor and sensory systems of the central nervous system. Glutamate interacts with a range of specific receptor and transporter systems to produce fast and sustained synaptic excitation. It initiates various calcium dependent processes in target cells including the production of nitric oxide (Bienvenu*et al.,* 2002). Three main glutamate receptor subtypes are N-Methyl-D-aspartate (NMDA), non-NMDA (Alpha-

amino-3- hydroxyl-5- methylisoxazole-4-propionic Acid) and kainate receptors. Epilepsy may result from excessive release of glutamate from central nerve terminals (Leonard, 2003). Research has shown that excessive stimulation of glutamate receptors causes excitotoxicity, a phenomenon implicated in both acute and chronic neurodegenerative diseases (ischemia, Huntington's disease and amyotrophic lateral sclerosis). Several lines of evidence indicate that excessive stimulation of glutamate receptors, perhaps due to impairment of the glutamate-transport system could lead to Ca^{2+} overload in mitochondria, resulting in overproduction of reactive oxygen species (ROS) and oxidative stress-mediated motor-neuron damage (Kong and Xu, 1998).

Hippocampal recordings from conscious human brains have shown sustained increases in the levels of extracellular glutamate during and preceding seizures. GABA levels remain low in the epileptogenic hippocampus, but during seizures, GABA concentrations increase, although mostly in the non-epileptogenic hippocampus. This leads to a toxic increase in extracellular glutamate due to reduced inhibition in the epileptogenic areas (During and Spencer, 1993).

2.10 Diagnosis of Epilepsy

Seizures can be confused with the symptoms of a number of other conditions. For this reason, four distinct methods are relied upon to properly diagnose epilepsy. These methods are; history, examination, electroencephalography (EEG) and magnetic resonance imaging (MRI).

2.10.1 Neurological history

Neurological history is the first method used in the diagnosis of epilepsy and this is preferable when the physician is given a clear description of any past seizure activity. Most seizures have a clear start and finish, last from seconds to a few minutes, occur at seemingly random times and

comprise certain sensations and behaviours that clinicians can recognize. Patients may not remember their behaviour during seizures, so descriptions from observers are very important.

2.10.2 Physical examination

The second method used to diagnose epilepsy is the physical examination. A physical examination cannot uncover epilepsy, but it can show problems indicating that a part of the brain isn't working properly and therefore may be generating seizures (www.epilepsy com).

2.10.3 Electroencephalography (EEG)

The third method is electroencephalograph (EEG) supply supportive evidence for the diagnosis of epilepsy and also provide critical clues to the classification of epileptic seizures and syndromes. In addition, it may help in anatomical localization of an underlying cerebral pathology, but neuroimaging techniques provide more useful information concerning structural abnormalities. EEG indirectly aids in the selection of appropriate antiepileptic drug(s) and in certain circumstances, also helps in formulating a prognosis, since it is extremely valuable in the determination of seizure type. Most routine EEGs in epileptic patients are obtained in the interictal state and the diagnostically useful finding is the epileptiform patterns (EPs) which suggests the presence of real epileptogenic process (Lodder *et al.,* 2014).

2.10.4 Neuroimaging

The two most commonly used neuroimaging tests are a brain computed tomography (CT) scan and a brain magnetic resonance imaging (MRI). Neuroimaging cannot show abnormal electrical activity or a seizure itself, but may show physical changes in the brain which may suggest a

reason for seizure. Diagnostic imaging by CT scan and MRI is recommended after a first non-febrile seizure to detect structural problems in and around the brain. MRI is generally a better imaging test except when bleeding is suspected, for which CT is more sensitive and more easily available (Wilden and Cohen, 2012). If a patient attends the emergency room with a seizure but returns to normal quickly, imaging tests may be done at a later point, but if a patient has a previous diagnosis of epilepsy with previous imaging, repeating the imaging is usually not needed even if there are subsequent seizures (Wilden and Cohen, 2012).

2.11 Treatment of Epilepsy

Anticonvulsants, more accurately called antiepileptic drugs (AEDs) are a diverse group of drugs used in the treatment of epileptic seizures. They are sometimes referred to as anti-seizure drugs. For effective treatment of epileptic seizures, it is very important to choose appropriately anticonvulsant of maximal benefit with minimal adverse effects. Many factors must be considered when prescribing an AED for a particular patient including the patient's seizure type, epilepsy syndrome, history of allergies, medical and psychiatric co morbidities, potential drug-drug interactions, renal function, hepatic function, protein binding, possibility of pregnancy, dosing schedule, availability of liquid, parenteral and extended release formulations, pharmacogenetics, and cost. When AEDs are similar in efficacy, differences in tolerability often guide medication selection (Andrew, 2011). Available antiepileptic drugs control seizures in about two-thirds of patients (Chandradhar, 2001).

2.12 Classification of Antiepileptic Drugs

Three major mechanisms are recognized (Graeme, 2005): modulation of voltage-gated ion channels; enhancement of gamma-aminobutyric acid (GABA)-mediated inhibitory neurotransmission; and attenuation of glutamate-mediated excitatory neurotransmission. The principal pharmacological targets of currently available AEDs are as follow:

2.12.1 Voltage-gated sodium channels blockade

The primary function of voltage-gated sodium channels is to allow the propagation of action potentials. Voltage-gated sodium channels play key roles in determining neuronal excitability. They are involved in the generation of the neuronal action potential, as they mediate the initial inward current during depolarization. Similarly, they are responsible for this same process in cardiac tissue and other excitable cells. They represent the molecular site of action of various neurotoxins, local anesthetics, anticonvulsants, and anti-arrhythmics (Benjamin *et al.*, 2006).

Sodium channel blockade is the most common and best-characterized mechanism of available antiepileptic drugs (Taylor, 1995). They are expressed throughout the neuronal membrane, on dendrites, soma, axons, and nerve terminals. Density of expression is higher in the axon initial segment (AIS) where action potentials are generated. Each sodium channel dynamically exists in the following 3 states: A resting state, during which the channel allows passage of sodium into the cell which is followed by active state in which the channel allows increased influx of sodium into the cell then an inactive state, in which the channel does not allow passage of sodium into the cell. With constant stimulus or rapid firing, many of these channels exist in the inactive state, rendering the axon incapable of propagating the action potential. Some antiepileptic drugs stabilize the inactive configuration of sodium (Na) channel, preventing high-frequency neuronal firing. 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. Antiepileptic drugs like phenytoin, carbamazepine and valproate which appear to be effective in both partial and generalized seizures act via this mechanism (Nolan *et al.,* 2013).

2.12.2 Voltage-gated calcium channels (VGCCs) blockade

Calcium ion is an important signaling molecule that is present in low concentration in extracellular fluid and in minute concentration in most cell interiors. The opening of $Ca2^{+}$ channels is the critical link between cell depolarization and $Ca2^{+}$ entry which can result in its high concentration. The subsequent binding of $Ca2^{+}$ to intracellular molecules can lead to; muscle contraction, the triggering of neurotransmitter release from nerve terminals, the activation of second messenger system that cause many changes, including alteration in gene expression and in extreme cases, neuronal self-destruction. Some $Ca2^{+}$ channels also impart electrical properties to the cells in which they are expressed, thus, may show action potentials in which the depolarizing current is carried predominantly by $Ca2^{+}$ (Nestler *et al.,* 2009). VGCCs are key regulators of Ca^{2+} entry into neurons, and are known to control a variety of cellular processes that regulate neuronal excitability. Voltage-gated calcium channels can be divided into two groups; high-voltage activated and low-voltage activated. High voltage activated, also known as L-type (large current or long open time) controls the release of neurotransmitters such as the excitatory neurotransmitter glutamate. Whereas, low voltage activated, also known as T-type (tiny current or transient) controls membrane potential that lead to low threshold stimulation in thalamic neurons, which may underlie the synchronizing discharges characteristic of epilepsy.

These channels have been shown to be blocked by known antiepileptic drugs such as ethosuximide, gabapentin and levetiracetam (Nicholas *et al.*, 2002). Sodium valproate is also known to inhibit T-type Ca^{2+}channels in thalamic neurons (Lowestein *et al.*, 2001).

Similarly, zonisamide inhibits T-type Ca^{2+} currents and also inhibits the sustained repetitive firing of spinal cord neurons presumably by prolonging inactivation of voltage-gated Na^{+} channels in manners similar to actions of phenytoin and carbamazepine (McNamara, 2006). A low threshold calcium current (T-type) governs oscillatory responses in thalamic neurons. Reduction of this current by antiseizure drugs *(*e.g.ethosuximide, dimethadione, valproate) explains their mechanism of action against absence seizures.

2.12.3 Reduction of excitatory glutaminergic neurotransmission

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 5 potential binding sites of glutamate receptor are as follows: The alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) site, the kainate site, the N -Methyl-D-aspartate (NMDA) site and the glycine site which are ionotrpic receptors (Dingledine *et al.*, 2009).

The fifth binding site is the metabotropic receptor site, which has 8 subunits: mGluR1, mGluR2, mGluR3, mGluR4, mGluR5, mGluR6, mGlu7and mGluR8 (Con and Pin 1997).Some anti-seizure drugs (e.g. phenobarbitone, topiramate) block the AMPA receptor and some (Felbamate, remacemide) block NMDA receptors. The understanding of these basic mechanisms has resulted

in the development of many new antiseizure drugs (Chandradhar, 2001). AEDs that act via these receptors are antagonistic to glutamate. Responses to glutamate antagonists differ, depending on the site being affected (Chapmann, 1998).

2.12.4 Enhancement of GABA-mediated inhibition

GABA is the predominant inhibitory neurotransmitter in the brain, and the expression and function of GABA receptors also are developmentally regulated. Three types of GABA receptors, GABA-A, $GABA_B$ and GABAc are found in the mature central nervous system. $GABA_A$ and $GABA_C$ are ionotropic receptors whereas, $GABA_B$ is ametabotropic receptor. Most fast synaptic inhibition in the mature brain is mediated by $GABA\text{-}_A$ receptors whereas slow inhibition is mediated by $GABA_B$ receptors (Kayal- Brooks *et al.,* 2009). When GABA binds to a $GABA\text{-}_A$ 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) and causes the cell to have greater difficulty reaching the action potential. The $GABA_B$ receptor is linked to a potassium channel (Porter and Meldrum, 1995). Antiepileptic drugs may act to enhance CI- 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 such as valproate act as modulators of this enzyme enhancing the production of GABA and down-regulating glutamate. Some AEDs (e.g, Tiagabine) function as an agonist to chloride conductance, either by blocking the reuptake of GABA or by inhibiting its metabolism as mediated by GABA transaminase (Vigabatrin), resulting in increased accumulation of GABA at the postsynaptic

receptors. Drug(s) may act directly on the GABA-receptor-chloride channel complex (benzodiazepines, barbiturates), and inhibit the metabolism of GABA (Vigabatrin, valproate) or increase the release of GABA (gabapentin). This mechanism provides protection against generalized and focal seizures (Kayal- Brooks *et al.,* 2009).

2.13 Taxonomic Classification of *Cassia mimosoides* Linn.

Kingdom Plantae

Phylum Magnoliophyta

Class Magnoliopsida

Order Asterales

Family Caesalpinioideae / Fabaceae

Genus *Cassia*

Specie *mimosoides*

Name *Cassia mimosoides*

Local Names: Hausa; *Gabaruwan kasa,* Yoruba; *Eru-taba,* Fulani; *Lallami,* Hindi; *Patwa ghas,* Konkani;*Lajri, Marati*; *Chinchini* and *Pivala lajalu,* Telugu; *Nela ponna*.

2.13.1 Medicinal plants with anticonvulsant properties

Several medicinal plants have been reported to possess anticonvulsant properties and some of these plants have been reviewed in literature: *Carissa edulis (Yau et al., 2008), Randia nilotica* (Danjuma *et al.,* 2009), *Olax subscorpiodea* (Nazifi *et al.,* 2015), *Cissus corniforlia* (Yaro *et al.,* 2015), *Spondias mombin* (Ayoka *et al.,* 2006), *Abelmoschus angulosus, Allium*

sativum,Artemisia spp, Cannabis sativa, Cinchona officinalis, Egletes viscosa, Icacinatrichantha, Magnolia grandiflora, Plumbago zeylanica and others. A study with Brazilian Northeastern plants showed anticonvulsant activity for species of *Bauhinia outimouta, Rauvolfia ligustrina* and *Ximenia americana* (Quintans-Júnior *et al.*, 2008),

2.13.2 Description

Cassia mimosoides linn. is a low diffuse shrub, 15 to 30 centimeters or more in height Leaves are 7 to 10 centimeters long, with 40 to 60 pairs of narrow leaflets, and a solitary, sessile gland on the rachis below the leaflets. Flowers grow one or two together in the axils of the leaves, shining, small and yellow. Pods are strap-shaped, flat, and about 5 centimeters long, containing rhomboid, dark-brown seeds (Hemen and Lalita, 2012).

2.13.1 Reported Phytochemical Constituents of the Plant Ethanol extract yielded

2.13.3 Phytochemical constituents present in *Cassia mimosoides*

Emodin, luteolin, 1, 3-benzenediol, oleanolic acid, (R)-artabotriol, α-L-rhamnose, β-sitosterol and daucosterol reported from root. Anthraquinones reported from seeds (physcion, physcion-9-antrhone, emodine-9-anthrone, and physcion 10, 10-bianthrone from aerial part (Hemen et. al., 2012).

2.13.4 Ethnomedicinal uses

In Japan, young stems and leaves are dried and used as a substitute for tea. An aqueous extract from leaves, stems and pods called "hama-cha" is a conventional beverage in the San-in district of Japan (Hemen and Lalita, 2012). In tropical Africa roots are used as a cure for diarrhea.Decoction of the root is used in dysentery.Entire plant used as remedy for facial eruptions. Roots given for stomach spasms, in northwestern Tanzania, aerial parts of *Cassia*

mimosoides linn. are pounded and mixed with animal fat, applied topically or taken orally for fractures, cleaning of the uterus by pregnant women, as well as antibacterial agent. Also, aerial parts pounded with the leaves of Cassia polytricha and the paste tied around a fracture to promote healing. In Japan, raw material used as diuretic or antidote in folk remedy (Hemen and Lalita, 2012). The plant has also acclaimed to have anticonvulsant activity Malam Usman Wanzami (by personal contact)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Material, Collection and Authentication

The leaves of plant*Cassia mimosoides*Linn. We0re collected from Kudingi Village forest, Zaria, Kaduna State Nigeria, in the month of October, 2016. The plant was identified and authenticated by Malam Namadi Sunusi of the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria-Nigeria, where a voucher specimen (No. 557) was collected for future reference.

3.1.1 Equipment

Animal cages, cotton wool, Claude Lyon voltage stabiliser, crucible, evaporating dish, funnel, Metler balance P165, measuring cylinder, pestle and mortar, separating funnel, syringes (1ml, 2ml, 5ml and 10ml), TLC precoated plate, Ugo Basile current electroshock machine (Model 7800 with corneal electrodes), Whatman filter paper, Wire mesh cage.

3.1.2 Drugs and chemicals

Diazepam (Roche Product Ltd Welwyn Garden City) , Dragendorff reagent (BDH Ltd Poole, England), methanol (Sigma, St. Louis U.S.A), silica gel, ethyl acetate, diethyl ether, ferric chloride (BDH Ltd Poole, England), glacial acetic acid (Searle Essex, England), N-Butanol, meyer reagent (BDH Ltd Poole, England), pentylenetetrazole (Sigma Chemical Co.USA), strychnine (Sigma-Aldrich, St.Louis U.S.A.), phenorbarbitone (Sigma-Aldrich, St.Louis U.S.A.), picrotoxin (Sigma-Aldrich, St.Louis U.S.A.), phenytoin sodium capsules (Parke-Davis and Co. Ltd), vanillin, sulphuric acid (BDH Ltd Poole, England), sodium valproate (Sanofi Synthelabo, One Onslow St.Surrey, Canada), cyproheptadine (PT Kalbe Pharma, Bakesi, Indonesia), flumazenil and naloxone (Plymouth Meeting, PA, USA).

3.1.3 Experimental animals

The pharmacological experiments were conducted using adult Swiss Albino mice of both sexes (20-24 g) and Wistar rats of both sexes (160-200g) obtained from Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria. Day old Ranger cockerels (28 – 40 g), were obtained from the National Animal Production Research Institute (NAPRI). The animals were fed on a Standard Animal Feeds obtained from Excel Feeds Plc (Kaduna, Nigeria) and allowed food and water *ad libitum*. The animals were housed in a standard cage at room temperature and then allowed to acclimatize with the laboratory

environment for at least five days prior to the commencement of the experiments. All experiments performed on laboratory animals were in accordance with the Ahmadu Bello University Research Policy Guidelines. Ethical approval of the ABUCAUC with number ABUCAUC/2018/074 was obtained.

3.2 Plant Extraction

The leaves of*Cassia mimosoides* Linn. were air dried under shade until constant weight was obtained. The leaves were then crushed into a coarse powder with the aid of a mortar and pestle. A portion 1000g (1kg) of the powdered plant was extracted with 2 Litres of absolute methanol for 72 hours using Soxhlet method of extraction. The solvent was collected in a round bottom flask where it was decanted into an evaporating dish and evaporated to dryness over water bath maintained at about 50˚C. The dried methanol leaf extract of *Cassia mimosoides* was stored in an airtight container. Fresh solution of the extract wasprepared for each study by dissolution of the appropriate amount required in deionized water under standard laboratory conditions.

3.3 Thin Layer Chromatography (TLC) Analysis

Solutions of samples were spotted on silica gel precoated thin layer chromatographic (TLC) plates (4cm × 8cm). The plates were developed in a solvent system (Butanol Acetic acid: Water, 6: 3: 0.5). Each plate was sprayed with a different visualizing reagent (Wagner and Bladt, 1996; FAO/IAEA, 2000; Pascual, 2002).

3.3.1 Alkaloids: Dragendoff's reagent was used as visualizing reagent. Orange red spots was observed indicating presence of alkaloids.

3.3.2 Anthraquinones: Bontrager's reagent (5% ethanolic potassium hydroxide) was used as visualizing reagent. Red color spots were observed indicating presence of Anthraquinones.

3.3.3 Cardiac Glycosides: Kedde's reagent (3, 5-dinitrobenzoic acid + 2M NaOH) was used test the presence of cardenolides. Pink – violet spots was observed indicating presence of cardenolides. **3.3.4 Tannins**: Vanillin/HCl reagent was used as visualizing reagent. Red spots were observed indicating presence of tannins.

3.3.5 Triterpenes: 1% ethanolic Vanillin spray followed by 10% ethanolic sulphuric acid was used as visualizing reagent. Red spots were observed indicating presence of triterpenes.

3.3.6 Flavonoids: Ferric chloride solution was used as visualizing reagent. Blue spots were observed indicating presence of flavonoids.

3.4 Acute Toxicity Study (LD_{50} Determination)

Median lethal dose (LD_{50}) was investigated in rats, mice and chicks using the Limit test of OECD 425 method (2001). One animal (i.e. one mice, one rat and one chick) received 5000 mg/kg of methanol leaf extract of *Cassia mimosoides*, it was observed for signs and symptoms of toxicity including death within 24 hours after treatment. Then, the remaining animals two each received the same dose of the extract and all the dosed animals were observed for signs and symptoms of toxicity including death for 14 days.

3.5 Anticonvulsant Studies

3.5.1 Maximal electroshock-induced seizure (MES) test in chicks

The methods previously described by Swinyard and Kupferberg (1985) and Browning (1992) was employed. Fifty (50) one day old cockerels were randomly divided into 5 groups of 10chicks

each. The first group received normal saline (10 ml/kg) *i.p*, while groups 2, 3 and 4 received extract (375, 750 and 1500 mg/kg, per oralrespectively). The fifth group received phenytoin 20 mg/kg (*i.p.).* Thirty minutes later for group 1 and 5 and 60 minutes later for group 2, 3 and 4, maximal electroshock was delivered to induce seizure in the chicks using Ugo basile electroconvulsive machine (model 7801) with corneal electrodes placed on the upper eye lid of the chick after dipping them in normal saline.

The current, shock duration, frequency and pulse width were set and maintained at 90 mA, 1.0 sec, 200 Hz and 1.0 ms^{-1} respectively. An episode of tonic extension of the hind limbs of the chicks was considered as convulsion while lack of it was regarded as protection. The recovery time was also recorded for the unprotected animals.

3.5.2 Pentylenetetrazole-induced seizure (Sc-PTZ) test in mice

The method of Swinyard *et al.,* (1952) was employed. Thirty mice were randomly divided into 5 groups of six mice each. The first group which served as negative control was treated with normal saline at a dose of 10 ml/kg (*i.p.*), while groups 2, 3 and 4 received graded doses of the methanol extract reconstituted in water (375, 750 and 1500 mg/kg, per oral respectively). Group 5 which served as positive control was treated with 200 mg/kg *i.p.* sodium valproate. Thirty minutes later, 90 mg/kg of freshly prepared solution of pentylenetetrazole was administered subcutaneously to group 1 and 5, while 60 minutes later to group 2, 3 and 4 mice. The mice were observed for 30 minutes for onset and incidence of seizures. An episode of clonic spasm of at least 5 seconds duration was considered as seizure. Lack of clonic spasm during 30 minutes of observation was regarded as protection. The number of mice protected was noted and the anticonvulsant activity of the extract expressed as percentage protection.

3.5.3 Strychnine – induced seizure test in mice

The method described by Krall *et al.,* (1978) was adopted. Thirty mice were randomly divided into 5 groups of six mice each. Group 1 served as a negative control and received normal saline (10 ml/kg *i.p.*), while groups 2 – 4 received the extract at a dose of 375, 750 and 1500 mg/kg (per oral*)* respectively. Group 5 which served as the positive control received phenobarbitone (30 mg/kg *i.p*). Thirty minutes later, 1.0 mg/kg of freshly prepared solution of strychnine was administered subcutaneously to group 1 and 5 mice and 60 minutes later for groups 2, 3 and 4. The mice were observed for presence or absence of tonic convulsion and latency to death within a 30-minutes period.

3.5.4 Picrotoxin – induced seizure test in mice

The method described by Swinyard *et al., (*1989) was adopted. Thirty mice were randomly divided into 5 groups of six mice each. Group 1 served as a negative control and received normal saline (10 ml/kg*i.p.*), while groups 2 – 4 received the extract at a dose of 375, 750 and 1500 mg/kg (p*e*r oral*)* respectively. Group 5 which served as the positive control received diazepam (10 mg/kg, *i.p).* Thirty minutes later, for group 1 and 5 mice and 60 minutes later for groups 2, 3 and 4, 4 mg/kg of freshly prepared solution of picrotoxin was administered subcutaneousl. The mice were observed for presence or absence of tonic hind limb extension within a 30-minutes period. Prolongation of the latency of tonic hind limb extension was also considered as indication of anticonvulsant activity.

3.5.5 Pentylenentetrazole – induced kindling model in rats

The method described by Gupta *et al* (2001) and Dhir *et al* (2007) was employed. A sub – convulsive dose of 35 mg/kg of PTZ was injected *i.p.* every 48 hours (Zhang *et al.*, 2003), for 22

days (11 injections). Forty rats were divided into five groups of eight rats each. Group 1 served as a negative control and received normal saline 1 ml/kg *i.p*, Groups (2, 3 and 4) received the extract at a dose of (375, 750 and 1500 mg/kg peroral respectively. Group 5 received sodium valproate (100 mg/kg, *i.p.).* Thirty minutes post treatment, groups 1 and 5 were administered pentylenetetrazole (PTZ), while sixty minutes post treatment, groups 2, 3 and 4 were administered pentylenetetrazole (PTZ). Drug and Extract were administered every other day before the injection of PTZ. Rats were observed for seizure intensities within a 30-minutes period and classified as follows:

Stage 0: no response

Stage 1: facial automatism with twitching of ears and wrinckles

Stage 2: convulsive waves throughout the body

Stage3: myoclonic jerks, rearing

Stage 4: tonic-clonic convulsions with loss of posture

Stage 5: fully generalized tonic-clonic seizures with falling (Wu *et al.,* 2006).

3.6 Mechanistic Study

3.6.1 Evaluation of the effect of cyproheptadine on methanol extract of *Cassia mimosoides* in chicks

The chicks were divided into six groups of ten chicks each. Group 1 served as negative control and was given normal saline (10 ml/kg *i.p*), group 2 received the extract at doses 1500 mg/kg per oral, Group 3 received phenytoin 20 mg/kg and group 4 received cyproheptadine at a dose of 4 mg/kg *i.p*. Groups 5 and 6 received cyproheptadine at the dose of 4mg/kg each. Fifteen minutes

later, group 5 received the extract (1500 mg/kg) and 6 received Phenytoin (20 mg/kg *i.p.)* and allowed for 30 minutes. Seizure was then induced to these groups using MES as previously described by Swinyard and Kupferberg (1985).

3.6.2 Evaluation of the effects of flumazenil on anticonvulsant activity of methanol extracts of *Cassia mimosoides in mice*

The effects of selective $GABA_A$-BZD receptor antagonist;flumazenil were also studied (File *et al*. 1982; File and Pellow, 1986), on the anticonvulsant activity of extracts in order to investigate the probable involvement of $GABA_A$-BZD receptors. Forty-two mice were randomly selected and grouped in seven groups of six mice each. Group 1 served as negative control and was given normal saline (10 ml/kg *i.p*), group 2 received the extract at doses 1500 mg per oral, Group 3 received diazepam at a dose 1.5 mg/kg i.p, group 4 received flumazenil at a dose of 10 mg/kg *i.p*. Thirty minutes post treatment for group 1, 3, 4 and sixty minutes for group 2, PTZ at a dose of 90 mg/kg i.p was administered and the animals were observed for 30 minutes. In group 5, mice were given flumazenil (10 mg/kg) 15 min before the administration of *Cassia mimosoides*(1500 mg/kg) extracts (45 min before the injection of PTZ). In group 6, the animals received, diazepam at a dose of 1.5mg/kg ip and *Cassia mimosoides* at a dose of 1500 mg/kg per oral (45 min before the injection of PTZ). Group 7 received flumazenil 10 mg/kg i.p 15minutes before administration of diazepam at a dose of 1.5 mg/kg i.p and 45minutes before administration of PTZ. The anticonvulsant activity of the extract and diazepam in mice pretreated with flumazenil was assessed for 30 minutes and compared with controls and extract treated animals as well as flumazenil treated animals (Yau et al., 2008)

3.6.3 Evaluation of the effects of naloxone on anticonvulsant activity of extracts of *Cassia mimosoides*in mice

Naloxone was used as an opioid receptor antagonist (Mannino and Wolf,1974; Cowan *et al*. 1979; Lauretti et *al*. 1994) at the doses of 0.3 mg/kg. Forty-two mice were randomly selected and grouped in seven groups of six mice each. Group 1 served as negative control and was given normal saline (10 ml/kg *i.p*), group 2 received the extract at dose of 1500 mg/kg per oral, Group 3 received diazepam 1.5 mg/kg i.p, group 4 received naloxone at a dose of 0.3 mg/kg *i.p*. Thirty minutes post treatment for group 1, 3 and 4 but sixty minutes for group 2 PTZ at a dose of 90 mg/kg i.p was administered and the animals were observed for 30 minutes. In group 5, mice were given naloxone (0.3 mg/kg i.p) 15 min before the administration of *Cassia mimosoides*(1500 mg/kg) extracts (60 min before the injection of PTZ). In group 6, the animals received, diazepam at a dose of 1.5mg/kg i.p and *Cassia mimosoides* at a dose of 1500mg/kg per oral (45 min before the injection of PTZ). Group 7 received naloxone 0.3mg/kg i.p 15minutes before administration of diazepam 1.5mg/kg i.p and 45minutes before administration of PTZ. The anticonvulsant activity of the extract and diazepam in mice pretreated with naloxone was assessed for 30 minutes and compared with controls and extract treated animals as well as naloxone treated animals (Yau, et al. 2008)

3.6.4 Evaluation of the effects of sildernafil on anticonvulsant activity of extracts of Cassia mimosoide in mice

Further investigation was carried out on the probable modulatoryproperties of a phosphodiestrase receptors antagonist on the anticonvulsant activity of the extracts in PTZ-inducedseizure. Here, Sildernafil was used as a phosphodiestrase enzyme antagonist (Mannino and Wolf,1974; Cowan *et al*. 1979; Lauretti et *al*. 1994). Forty-two mice were randomly selected and grouped in seven groups of six mice each. Group 1 served as negative control and was given normal saline (10 ml/kg *i.p*), group 2 received the extract at doses 1500 mg/kg o.p, Group 3 received diazepam

1.5mg/kg i.p, group 4 received sildernafil at a dose of 5 mg/kg *i.p*. Thirty minutes post treatment for group 1, 3 and 4 but sixty minutes for group 2 PTZ at a dose of 90 mg/kg i.p was administered and the animals were observed for 30 minutes. In group 5, mice were given sildernafil (5 mg/kg i.p) 15 min before the administration of *Cassia mimosoides*(1500 mg/kg) extract (45 min before the injection of PTZ). In group 6, the animals received, diazepam at a dose of 1.5 mg/kg i.p and *Cassia mimosoides* at a dose of 1500 mg/kg o.p (45 min before the injection of PTZ). Group 7 received sildernafil 5 mg/kg i.p 15 minutes before administration of diazepam 1.5 mg/kg i.p and 45 minutes before administration of PTZ. The anticonvulsant activity of the extract and diazepam in mice pretreated with sildernafil was assessed for 30 minutes and compared with controls and extract treated animals as well as sildernafil treated animals.

3.7 Statistical Analysis

Statistical analysis was carried out using SPSS (Version 20) and data obtained were expressed as Mean ± SEM. All analysis was done using One Way Analysis of Variance (ANOVA), when a statistically significant result was obtained with ANOVA, a post hoc Dunnets or Turkey test was performed for multiple comparisons. Values of $P \leq 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of the Plant Extract of *Cassia mimosoides*

The extraction of 1000g (1kg) of powdered leaf of *Cassia mimosoides*with absolute methanol afforded a yield of 14.17% $^{w}/_{w.}$The extract wasdark brown in colour and sticky in nature.

4.2 Phytochemical Constituents of the Methanol Leaf Extract of *Cassia mimosoides*

Preliminary phytochemical screening of the methanol extract of *Cassia mimosoides*revealed the presence of glycosides, cardiac glycosides, saponins, steroid and triterpene, tannins, alkaloids, flavonoids(Table 1).

Table 1: Phytochemical Constituents of the Methanol Extract of *Cassia mimosoides*

Phytoconstituents	Result
Flavonoids	+

Alkaloids	+
Tannins	+
Saponins	+
Cardiac glycosides	+
Steroids and triterpenes	+
Anthraquinones	-

Key: + = Present, - = absent

4.3 Acute Toxicity Study of the Methanol Leaf Extract of *Cassia mimosoides*

Median lethal dose (LD_{50}) in rats, mice and chicks was found to be greater than 5000mg/kg in rats, mice and chicks.

4.4 Effect of the Methanol Extract of *Cassia mimosoides*on Maximal Electroshock-Induced Seizures in Chicks

The methanol leaf extract of *Cassia mimosoides*offered protection to the chicks against hind limb tonic extension (HLTE) induced by maximal electroshock and reduce the mean recovery time of the chicks after electroshock treatment compared to the control group. The 750 and 1500 mg/kg extract dose produced a significant decrease ($P< 0.05$) in the mean recovery time of the chicks

after electroshock treatment compared to the values obtained for the control group. Similarly, it also offered a 10%, 20%, 40% protection against hind limb tonic extension (HLTE) induced by maximal electroshock at 375, 750 and 1500mg/kg dose respectively. The standard anticonvulsant drug used, phenytoin (20 mg/kg) protected all the chicks (100%) from hind limb tonic extension induced by maximal electroshock (Table 2).

Table 2: Effect of Methanol Leaf Extract of *Cassia mimossoides* against MaximalElectroshock Induced Convulsion in Chicks

Treatment (mg/kg)		Mean Recovery Period (Min.)	Quantal Protection	% Protection against seizure
NS	10 ml/kg	13.30 ± 0.37	0/10	0.00
CRE	375	11.7 ± 0.52	1/10	10.00

CRE 750	11.14± 0.67*	2/10	20.00
CRE 1500	9.80 ± 0.79**	4/10	40.00
PTY 20	-	10/10	100.00

Values are presented as Mean ± SEM, *= $p<0.05$, ** = $p< 0.01$, compared to normal saline control group - One-way ANOVA, n=10, NS = Normal saline, CRE = Methanol Extract of *Cassia mimosoides*, PTY = Phenytoin

4.5 Effect of the Methanol Leaf Extract of *Cassia mimosoides*on Pentylenetetrazole-Induced Seizures in Mice

The methanol leaf extract of *Cassia mimosoides*at the tested doses (375, 750 and 1500 mg/kg) offered protection against seizures induced by pentylenetetrazole (90 mg/kg) 60 minutes after administration of the extract. The extract at doses of 375, 750 and 1500 mg/kg produced a significant increase ($P< 0.05$) in the mean latency to onset of seizure compared to the control

group. The extract at a dose 1500 mg/kg protected the mice (50.00%) against mortality. The 750 mg/kg and 375 mg/kg dose of the extract offered 33.3% protection against mortality (Table 3).

Table 3: Effect of Methanol Leaf Extract of *Caessia mimossoides*against Pentelentetrazole-induced Convulsion in Mice

Treatment (mg/kg)	Mean Onset of Seizures (min.)	Quantal protection	% Protection against seizure	% Mortality
NS 10 mls/kg	3.67 ± 0.49	0/6	0.00	100.00
CRE 375	6.60 ± 0.75*	1/6	16.67	66.67
CRE 750	9.50 ± 1.08**	2/6	33.33	66.66

CRE	1500	15.67 ± 0.88***	3/6	50.00	50.00
SV	200	-	6/6	100.00	0.00

Onset of seizures presented as Mean ± SEM,*= $p<0.05$, ** = $p< 0.01$, *** = $p< 0.001$ compared to normal saline group - One way ANOVA followed by Dunnett tests, n=6, NS - Normal Saline, CRE = CRE = Methanol leaf Extract of *Cassia mimosoides*, SV = Sodium Valproate

4.6 Effect of the Methanol Leaf Plant Extract of *Cassia mimosoides*against Strychnine Induced Seizures in Mice

The methanol leaf extract of *Cassia mimosoides* at all the doses tested (375, 750 and 1500 mg/kg) did protect the animals against strychnine induced seizure with 16.67%, 16.67% and 33.33% protection respectively. There was significant difference between the mean onsets of seizure at doses 750 and 1500 mg/kg mice as compared to the control group. Phenobarbitone (20 mg/kg), the standard drug used, afforded an 83.33% protection of the mice against strychnine-induced seizure. Protection against mortality of the extract at a dose of 375 mg/kg, 750 mg/kg and 1500 mg/kg in mice was 33.33%, 33.33% and 50.00% respectively. However, the standard drug Phenorbabitone (20 mg/kg) offered 83.33% protection against mortality (Table 4).

Table 4: Effect of Methanol Leaf Extract of *Cassia mimosoides* on Strychnine Induced Seizure in Mice

Treatment (mg/kg)	Mean Onset of Seizures (min.)	Quantal protection	% Protection against seizure	% Mortality
NS 10 mls/kg	3.50 ± 0.43	0/6	0.00	100.00
CRE 375	5.20 ± 0.37	1/6	16.67	66.66
CRE 750	5.80 ± 0.66*	1/6	16.67	66.66
CRE 1500	8.50 ± 0.65***	2/6	33.33	50.00
PHB 20	11.50 ±0.50***	5/6	83.33	16.67

Onset of seizures presented as Mean ± SEM, *= $p<0.05$ **=$p<0.01$ *** = $p< 0.001$ compared to normal saline control group - One-way ANOVA followed by Dunnett tests, n=6, NS - Normal Saline, CRE = Methanol Extract of *Cassia mimosoides*, PHB = Phenobarbitone

4.7 Effect of the Methanol Leaf Extract of *Cassia mimosoides*on Picrotoxin-Induced Seizure in Mice

The methanol leaf extract of *Cassia mimosoides* at all the doses tested (375, 750 and 1500 mg/kg) protected the animals against picrotoxin-induced seizure with 16.67%, 33.33% and 50.00% protection respectively. There was significant difference between the mean onsets of seizure at doses 750 and 1500 mg/kg mice as compared to the control group. Phenobarbitone (20 mg/kg), the standard drug used, afforded 83.33% protection of the mice against picrotoxin-induced seizure. Protection against mortality of the extract at a dose of 375 mg/kg, 750 mg/kg and 1500 mg/kg in mice were 16.67%, 33.33% and 50.00% respectively. However, the standard drug phenorbabitone (20 mg/kg) offered 100% protection against mortality (Table 5).

Table 5: Effect of Methanol Leaf Extract of *Caessia mimossoides* against Picrotoxin-Induced Seizure in Mice

Treatment (mg/kg)		Mean onset of seizures (min.)	Quantal protection	% Protection against seizure	% Mortality
NS	10 ml/kg	8.33 ± 0.56	0/6	0.00	83.33
CRE	375	9.80 ± 0.49	1/6	16.67	66.66
CRE	750	10.75 ± 0.75*	2/6	33.33	50.00
CRE	1500	15.00 ± 0.58**	3/6	50.00	50.00
PHB	20	10.00 ± 0.00*	5/6	83.33	0.00

Onset of seizures presented as Mean ± SEM, * = $p < 0.05$, ** = $p < 0.001$ compared to normal saline control group - One-way ANOVA followed by Dunnett tests, n=6, NS - Normal Saline, CRE = Methanol Extract of *Cassia mimosoides*, PHB = Phenobarbitone

4.8 Effect of Methanol Leaf Extract of *Cassia mimosoides*against PTZ-Induced Kindling in Rats

Alternate day administration of sub convulsive dose of PTZ (35 mg/kg) up to eleven injections to rats in 375 mg/kg, 750 mg/kg, 1500 mg/kg and diazepam 2 mg/kg groups with the drug administered every day before the injection of PTZ (35 mg/kg). There was gradual increase in mean seizure score as the days goes by. There was also significant difference in mean seizure score between the negative control group (N/S 1 ml/kg) as compared with extract groups and the positive control group (diazepam 2 mg/kg) as in (Figure 1).

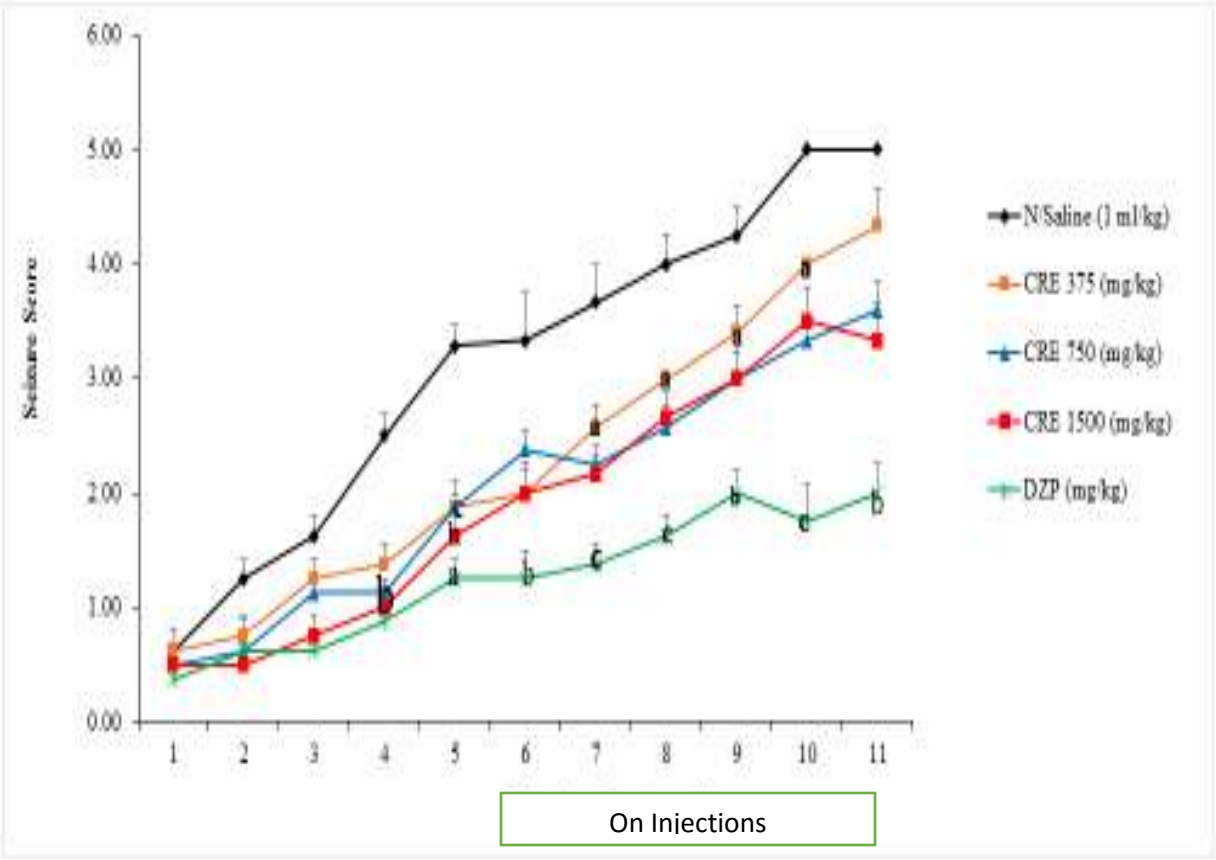

Figure 1: Effect of Methanol Leaf Extract of *Cassia mimosoides*against PTZ-Induced Kindling in Rats

Seizure score presented as Mean ± SEM, the superscripts a, b and c represent $p< 0.05$, $p< 0.01$ and $p< 0.001$ compared to normal saline control group respectively – Non parametric Kruskal-Wallis test followed by Dunn's post hoc test, n=8, NS = Normal Saline, CRE = Methanol Leaf Extract of *Caessia mimossoides*, DZP = Diazepam 2mg/kg

4.9 Effect of Cyproheptadine on the Anticonvulsant Activity of Methanol Leaf Extract of *Cassia mimosoides* and Phenytoin against Maximal Electroshock- Induced Seizure Induced

Administration of cyproheptatine 4 mg/kg at 15 minutes prior to the administration of the methanol leaf extract of *Cassia mimosoides* (1500 mg/kg) reduced the quantal protection 30% of the extract to 10%. There was a significant difference ($p<0.05$) increase in mean recovery period of extract group pretreated with cyproheptadine as compared with extract alone group. The phenytoin (20 mg/kg) offered 100% quantal protection against seizure but when pretreated with cyproheptadine there was 30% reduction in quantal protection. There was a significant difference ($p<0.05$) increase in mean recovery period of extract group pretreated with cyproheptadine as compared with extract alone group. However, cyproheptadine alone did not offer any protection against seizure or significant difference in mean recovery period as compare to normal saline group. (Table 6)

Table 6: Effect of Cyproheptadine on the Anticonvulsant Activity of Methanol Leaf Extract of*Cassia mimosoides* and Phenytoin against Maximal Electroshock-Induced Seizurein Chicks

Treatment (mg/kg)	Mean Recovery Period (Min.)	Quantal Protection	% Protection against seizure
NS 10 mls/kg	12.60 ± 0.48	0/10	0.00
CRE 1500	8.29± 0.64*	3/10	30.00
PHT 20	-	10/10	100.00
CYP 4 + CRE 1500	11.00 ± 0.65[a]	1/10	10.00
CYP 4 + PTY 20	9.40± 0.51 [#]	7/10	70.00
CYP 4	13.10 ± 0.84	0/10	0.00

Data presented as Mean ± SEM and percentage, n=10, $P<0.005$ One-way ANOVA) followed by post hoc test (Turkey) for multiple comparison; N/S= Normal Saline, PTY=Phenytoin, CYP=Ciproheptadine and CRE= *Cassia mimosoides* Methanol Extract. Data presented as Mean ± SEM and percentage, n=10, $P<0.005$ One-way ANOVA) followed by post hoc test (Turkey) for multiple comparison. *=$P<$ 0.05 compared to control group N/S, [a]= $P<0.05$ compared to CRE 1500,[#] = P< 0.01 compared to PHT 20mg/kg group.

4.9.1 Effect of Flumazenil on the Anticonvulsant Activity of Methanol Extract of *Cassia mimosoides* and Diazepam in Pentylentetrazole-Induced Seizure Model in Mice

Administration of flumazenil (10 mg/kg) 15 minutes prior to the administration of the methanol leaf extract of *Cassia mimosoides* (1500 mg/kg) increase protection against mortality 66.66% of the extract to 83.33%. There was significant decrease ($p<0.05$) in mean onset of seizure of extract group pretreated with flumazenil as compared with extract alone group. Diazepam (1.5 mg/kg) offered 66.66% protection against seizure but when pretreated with flumazenil the protection against seizure was reduced to 33.33%. There was significant increase ($p<0.05$) in mean onset of seizure of extract group pretreated with flumazenil (10 mg/kg) as compared with extract alone group. There was an enhanced protection against seizure when diazepam and extract were administered (83.33%) together as compared with diazepam (1.5 mg/kg) group (66.66) alone. However, flumazenil alone did not offer any protection against seizure or significant difference ($p<0.05$) in mean onset of seizure as compare to normal saline (10 ml/kg) group (Table 7).

Table 7: Effect of Flumazenil on the Anticonvulsant Activity of Methanol Extract of *Cassia mimosoides* and Diazepam against Pentylentetrazole Induced Seizure Model in Mice

Treatment (mg/kg)	Mean Onset of Seizures (min.)	Quantal protection	% Protection against seizure	% Mortality
NS 10 mls/kg	5.50± 0.76	0/6	0.00	100.00
CRE 1500	14.00± 1.47*	2/6	33.33	66.66
DZP 1.5	17.00 ± 1.00**	4/6	66.66	33.33
Flu 10	5.80 ± 0.60	0/6	00.00	100.00
Flu +CRE1500	11.50 ±0.97[a]	1/6	16.67	83.33
DZP1.5 + CRE1500	18.00 ± 0.00	5/6	83.33	16.67
Flu 10 + DZP 1.5	6.00 ± 0.86[#]	2/6	33.33	50.00

NS= Normal Saline, DZP= Diazepam, Flu= Flumazenil, CRE= Methanol leaf extract of *Cassia mimosoides*. CRE was administered 60minutes orally before injection of PTZ (90mg/kg), Flumazenil 10mg/kg was administered 15minutes before CRE or DZP. Data presented as Mean ± SEM and percentage, n=6, $P<0.05$One-way ANOVA) followed by post hoc test (Turkey) for multiple comparison. *=$P<0.01$ **=$P< 0.001$ compared to control group N/S, [a]= $P<0.001$ compared to CRE 1500, [#] = $P< 0.01$ compared to DZP 1.5mg/kg group.

4.9.2 Effect of Naloxone on the Anticonvulsant Activity of Methanol Extract of *Cassia mimosoides* and Diazepam against Pentylentetrazole Induced Seizure Model in Mice

Administration of naloxone (0.3 mg/kg) 15 minutes prior to the administration of the methanol leaf extract of *Cassia mimosoides* (1500 mg/kg) increase percentage mortality 66.66% of the extract to 83.33%. There was significant decrease ($p<0.05$) in mean onset of seizure of extract group pretreated naloxone (0.3 mg/kg) as compared with extract alone group. Diazepam (1.5 mg/kg) offered 66.66% protection against seizure but when pretreated with naloxone the protection against seizure was reduced to 16.67%. There was significant difference ($p<0.05$) increase in mean onset of seizure of extract group pretreated with naloxone as compared with extract alone group. There was an enhanced protection against seizure when diazepam and extract were administered (83.33%) as compared with diazepam (1.5 mg/kg) group (66.66) alone but there was no significant difference ($p<0.05$) in mean onset of seizure between the two group. However, naloxone alone did not offer any protection against seizure or significant difference ($p<0.05$) in mean onset of seizure as compare to normal saline (10 ml/kg) group (Table 8).

Table 8: Effect of Naloxone on the Anticonvulsant Activity of Methanol Extract of *Cassia mimosoides* and Diazepam against Pentylentetrazole Induced Seizure Model in Mice

Treatment (mg/kg)	Mean Onset of Seizures (min.)	Quantal protection	% Protection against seizure	% Mortality
NS 10 mls/kg	5.50± 0.76	0/6	0.00	100.00
CRE 1500	14.00± 1.47*	2/6	33.33	66.66
DZP 1.5	17.00 ± 1.00**	4/6	66.66	33.33
Nal. 0.3	6.00 ± 0.58**	0/6	00.00	100.00
Nal.0.3 +CRE1500	9.00 ±0.86[a]	1/6	16.67	83.33
DZP1.5 + CRE1500	18.00 ± 0.00	5/6	83.33	16.67
Nal. 0.3 + DZP 1.5	7.17 ± 0.95[#]	3/6	50.00	50.00

Control = Normal Saline, DZP= Diazepam, Nal.=Naloxone, CRE= Methanol extract of *Cassia mimosoides*. CRE was administered 60minutes orally before injection of PTZ (90mg/kg), Naloxone 0.3mg/kg was administered 15minutes before CRE or DZP. Data presented as Mean ± SEM and percentage, n=6, $P<0.05$One-way ANOVA) followed by post hoc test (Turkey) for multiple comparison. *=$P< 0.01$, **=$P<0.001$ compared to control group N/S, [a]= $P<0.01$ compared to CRE 1500,[#] = $P<0.01$ compared to DZP 1.5mg/kg group.

4.9.3 Effect of Sildenafil on the Anticonvulsant Activity of Methanol Extract of *Cassia mimosoides* and Diazepam against Pentylentetrazole Induced Seizure Model in Mice

Administration of sildenafil (5 mg/kg) 15 minutes prior to the administration of the methanol leaf extract of *Cassia mimosoides* (1500 mg/kg) decrease percentage protection against seizure 33.33% of the extract to 16.67%. There was no significant difference ($p<0.05$) in mean onset of seizure of extract group pretreated sildenafil (5 mg/kg) with as compared with extract alone group. The diazepam (1.5 mg/kg) offered 66.66% protection against seizure but when pretreated with sildenafil (5 mg/kg) the protection against seizure was reduced to 50.00%. There was no significant difference ($p<0.05$) in mean onset of seizure of extract group pretreated with sildenafil (5 mg/kg) as compared with extract (1500 mg/kg) alone group. There was an enhanced protection against seizure when diazepam and extract were administered (83.33%) as compared with diazepam (1.5 mg/kg) group (66.66) but there was no significant difference ($p<0.05$) in mean onset of seizure between the two group. However, sildenafil (5 mg/kg) alone does not offer any protection against seizure or significant difference ($p<0.05$) in mean onset of seizure as compare to normal saline (10 ml/kg) group (Table 8).

Table 9: Effect of Sildenafil on the Anticonvulsant Activity of Methanol Extract of *Cassia mimosoides* and Diazepam againstPentylentetrazole Induced Seizure Model in Mice

Treatment (mg/kg)	Mean Onset of Seizures (min.)	Quantal protection	% Protection against seizure	% Mortality
NS 10 mls/kg	5.50± 0.76	0/6	0.00	100.00
CRE 1500	14.00± 1.47*	2/6	33.33	66.66
DZP 1.5	17.00 ± 1.00**	4/6	66.66	33.33
SIL 5	6.00 ± 0.37	0/6	00.00	100.00
SIL. +CRE1500	13.20 ± 1.39	2/6	16.67	16.67
DZP 1.5 + CRE1500	18.00 ± 0.00	5/6	83.33	16.67
SIL 5 + DZP 1.5	9.67 ± 1.45[a]	3/6	50.00	50.00

Control = Normal Saline, DZP= Diazepam, SIL=Sildenafil, CRE= Methanol extract of *Cassia mimosoides*. CRE was administered 60minutes orally before injection of PTZ (90mg/kg), Sildenafil 5mg/kg was administered 15minutes before CRE or DZP. Data presented as Mean ± SEM and percentage, n=6, $P<0.05$One-way ANOVA) followed by post hoc test (Turkey) for multiple comparison. **=$P<0.001$ compared to control group N/S, [a] = $P<0.001$ compared to DZP 1.5mg/kg group.

CHAPTER FIVE

5.0 DISCUSSION

Phytochemical screening provides basic information about the different classes of secondary metabolites present in a plant and the medicinal importance of such plant (Shabbir *et al.,* 2013).

The thin layer chromatographic (TLC) analysis of methanol leaf extract of *Cassia mimosoides*revealed the presence of saponins, triterpenes and steroids, glycosides, cardiac glycosides, tannins, flavonoids and alkaloids while anthraquinones were absent. These chemical constituents might be responsible for the observed pharmacological activities of the extract. However, it is not possible to attribute with certainty the observed anticonvulsant effect of *Cassia mimosoides*to one or several active principles amongst those detected in the phytochemical screening. (Shabbir *et al.,* 2013).

Median lethal dose (LD_{50}) determination is of importance because it is a valuable tool employed to compare toxicities of compounds relative to their therapeutic doses (Berkowitz, 2004). It provides information regarding the margin of safety of a particular plant. The LD_{50} determination of the methanol extract of *Cassia mimosoides*was carried out in rats, mice and chicks via oral route. The oral LD_{50} values for the three species was found to be greater than 5000 mg/kg using OECD25 method. Doses of 30%, 15% and 7.5% of 5000 mg/kg were used for the research. Route of administration plays a key role in determining toxicity as the oral route has shown to be relatively safer than the intra peritoneal route in the species (Matsumura, 1985). Doses of less than or equal to 30% of the LD_{50} which have been demonstrated to be relatively safe for

ethnopharmacological research were used throughout the research procedure (Vongtau *et al.*, 2004).

Maximal electroshock test (MES) is a model for generalized tonic-clonic seizures and partial seizures (Raza *et al.,* 2001). The MES test model for anticonvulsant screening has a clearly defined (consistent) end point (inhibition of the tonic hind limb extension phase) and it is highly reproducible (Ambawade *et al.,* 2002). Protection against tonic hind limb extension in the MES predicts anticonvulsant activity of antiepileptic drugs (e.g phenytoin, carbamazepine, oxcarbazepine and lamotrigine) that prevent the spread of seizure discharge from an epileptic focus during seizure activity (Browning, 1992), this indicates the ability of the antiepileptic agent to serve in the treatment of generalized tonic clonic and partial seizures (Raza *et al.,* 2001). The methanol extract of *Cassia mimossoides* demonstrated activity against MES-induced seizure in a dose independent manner. Evidently, the 1500 mg/kg appeared to be most effective. The ability of the extract to inhibit seizures induced by electroshock stimulus and also shorten the recovery time of the convulsed chicks infers that it is likely to exhibit activity against generalized tonic-clonic seizures (Ambawade *et al.,* 2002). Though the model is not specific to one mechanism, it could be confirmed by the efficacy of the standard drug (phenytoin) used which is known to act via sodium channel. Inhibition of sodium channels would invariably stabilize neuronal membranes thereby leading to inhibition of neuronal excitability.

Pentylenetetrazole (PTZ), a tetrazole derivative is the prototype agent in the class of systemic convulsants (DeDyn *et al.*, 1992). The scPTZ seizure test is a model that predicts compound ability to raise seizure threshold and the behavioral seizure is not typical of absence epilepsy but clonic in nature. PTZ is believed to be an antagonist at GABA pathway in the CNS resulting in an imbalance between the ionic concentrations of the membrane (Nagakannan *et al.,* 2011).

Pentylenetetrazole has been shown to diminish GABAergic tone (Macdonald and Barker, 1977). Gamma amino butyric acid is the major inhibitory neurotransmitter in the brain while glutamic acid is the major excitatory neurotransmitter in the brain. The enhancement of GABA neurotransmission is reported to antagonize seizures while its inhibition promotes seizure (Rang *et al.,* 2005).

Compounds which are able to suppress PTZ-induced seizures are presumed to be effective in the treatment of absence seizures (McNamara, 2006). It has also been shown that seizures induced by PTZ, can be blocked by drugs such as ethosuximide that reduces T-type calcium currents (Rho and Saukar, 1999), and standard drugs such as diazepam and phenorbarbitone are thought to produce their effects by enhancing GABA-mediated inhibition in the brain (Rogawski and Porter, 1990). Antiepileptic drugs effective in the therapy of generalized seizures of petitmal type such as phenobarbitone and benzodiazepines are capable of raising the seizure threshold induced by pentylenetetrazole (Loscher *et al.,* 1991). The methanol extract of *Cassia mimosoides*showed activity against pentylenetetrazole induced seizures. The anticonvulsant activity of the extract suggest that it might be acting through activation of GABA neurotransmissions or blockade of glutamatergic neurotransmission mediated by NMDA receptor in the CNS.

Strychnine (STN) is a competitive glycine receptor antagonist (Rajendra *et al.,* 1997). The ability of the extract to protect the mice against strychnine-induced seizure suggests that the plant may contain compound (s) that interact with the glycine receptors probably as agonist or enhancing the binding of glycine to its receptors (Ya'u *et al.,* 2008)

Picrotoxin is used in determining mechanism of action of anticonvulsants (Vogel and Vogel, 1997). Picrotoxin-induce convulsions by blocking the inhibitory synaptic action of GABA on $GABA_A$ receptor chloride channels, although, not competitively (Rang *et al.,* 2007). As an

antagonist of GABA inhibitory action in different areas in the central nervous system, picrotoxin produces general clonic-tonic convulsions which can lead to death in most cases (Abdul-Ghani *et al.,* 1980). Drugs effective against picrotoxin-induced seizures have been shown to enhance GABA mediated neurotransmission. Antiepileptic agents such as sodium valproate, phenobarbitone, benzodiazepine suppreses seizures induced by picrotoxin (Porter *et al.,* 1984). Similarly, among the new antiepileptic drugs, gabapentin and tiagabine suppress seizures induced by picrotoxin (Taylor, 1995). The ability of the methanol extract of *Cassia mimosoides*to protect the animal against picrotoxin-induced seizures suggest that the anticonvulsant action of the extract may involve interaction with the picrotoxin site on the GABA-$_A$ receptor complex.

Kindling is a well-established model of abnormal plasticity leading to prolonged seizures and to epilepsy (Rivara *et al.,* 2012). It is a model of epilepsy and epileptogenesis where repeated administration of subconvulsive dose of PTZ produced a progressive increase in convulsant activity, culminating in generalized seizures in animals (Dhir *et al.,* 2007). Several studies have established that progression of seizures in kindling is associated with decreased numbers of $GABA_A$ receptor binding sites in hippocampus (Bazyan *et al.,* 2001), amplification in glutamate release, and elevated nitric oxide level (Riazi *et al.,* 2006). On the other hand, decreased serotonin level in brain also result in the inhibition of serotonin mediated release in kindled animals (Kailash *et al.,* 2013). Calcium channel of the NMDA glutamate receptor has been implicated in epileptogenesis after kindling and is a main target for new antiepileptic drugs like felbamateand topiramate (Armijo *et al.,* 2000). The chloride channel of the $GABA_A$ receptor is responsible for the rapid hyperpolarization of paroxysmal depolarizing state involved in kindling leading to increase in seizure severity (Armijo *et al.,* 2000). The extract at all doses was able to

reduce the severity of the seizure by not allowing the seizure to reach the classical seizure stage and this suggest that the extract could have antiepileptogenic activity.

Serotonergic and histerminergic pathways play a very important role in neurology in the sense that decrease neurotransmission of serotonin and histamine in the brain reduces seizure threshold. Cyproheptadine block both serotonine ($5HT_1$ and $5HT_2$) and Histamine (H_1). It interferes with serotonergic and histerminergic pathways via antagonizing subtypes of $5HT_1$, $5HT_2$ and H_1 receptors (Singh and Goel, 2010). From the mechanistic study, the extract seems to act via histaminergic and serotonergic pathways since when interacted with cyroheptadine, which was a blocker of these pathways, the quantal protection against seizure by the extract decreased. Therefore, the extract can be said to act via histaminergic and serotonergic pathways.

In order to establish the role of BDZ receptors participation in the *Cassia mimosoides*extract-induced anticonvulsant activity, flumazenil, a specific antagonist of the benzodiazepine site in the $GABA_A$-BDZ receptor complex (File *et al.,* 1982; File and Pellow, 1986; Brogden and Goa, 1988) was used. Antagonism of anticonvulsant activity of the extracts by flumazenil suggests that the extracts may mediate their anticonvulsant activity against PTZ-induced seizures in part through the facilitation of the inhibitory activity of the GABAergic system probably through a competitive agonistic action in the BDZ site of the GABA receptors.

Naloxone, a non-specific opioid receptors antagonist (Mannino and Wolf, 1974; Cowan *et al.,* 1979; Lauretti *et al.,* 1994) has been shown to antagonize the anticonvulsant activity of the *Cassia mimosoides.* Selective κ-opioid receptor activation produces a dose-dependent suppression of the bicuculine-induced seizures (Yajima *et al.,* 2000). Bicuculine produces convulsions through its antagonism of $GABA_A$ receptor-mediated inhibition (Macdonald and Baker, 1978) and was completely blocked by pretreatment with nor-binaltorphimine. It is well

documented that κ-opioid receptor agonists affect mostly Ca^{2+} channels, resulting in the blockade of Ca^{2+} entry (Werz and Macdonald, 1985). Although, the mechanism of the anticonvulsant effect with κ-opioid receptor agonists is presently unclear, one possibility is that postsynaptically localized κ- opioid receptor may contribute to the inhibition of excitability induced by postsynaptic blockade of $GABA_A$ receptor by PTZ through the reduction of Ca^{2+} entry. Since it is also known that at cellular level, one of the basic mechanisms of actions of AEDs such as ethosuximide and Valproic acid is the suppression of T-type calcium currents in thalamic neurons (Rho and Sankar, 1999; Meldrum, 1996; Macdonald and Kelly, 1994). The suppression of the T-type calcium channel may be involved in the extract's activity. On the basis of this, it would be suggested that κ-opioid receptor may participate in the *Cassia mimosoides*extracts mediated PTZ anticonvulsant effect since naloxone reduced the effect of the extracts on seizures (Macdonald and Kelly, 1994).

Nitric oxide pathway involvement in the anticonvulsant action of *Cassia mimosoides* was studied in mice using sildenafil as an antagonist of Phosphodiestrase-5 enzyme which affect cGMP, dose of the sildernafil was chosen based on pilot experiments and previous reports (Riazi et al. 2006; Akula et al., 2008; Bahremand et al., 2010). Based on the research findings there was no involvement of nitric oxide pathway in the anticonvulsant activity of *Cassia mimosoides.*

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

The thin layer chromatographic (TLC) analysis of methanol extract of *Cassia mimosoides*revealed the presence of saponins, triterpenes and steroids, cardiac glycosides, tannins, flavonoids and alkaloids while anthraquinones were absent. These chemical constituents might be responsible for the observed pharmacological activities of the extract. Median lethal dose (LD_{50}) determination is of importance because it is a valuable tool employed to compare toxicities of compounds relative to their therapeutic doses (Berkowitz, 2004). It provides information regarding the margin of safety of a particular plant. The LD_{50} determination of the methanol extract of *Cassia mimosoides*was carried out in rats, mice and chicks via oral route. The oral LD_{50} values for the rat, mice and chick species was found to be greater than 5000 mg/kg using OECD425 method.The methanol extract of *Cassia mimossoides* demonstrated activity against MES-induced seizure in a dose independent manner.The methanol extract of *Cassia mimosoides*showed activity against pentylenetetrazole induced seizures. The anticonvulsant activity of the extract also protects the animals against Strychnine (STN) induced seizure as well as against picrotoxin-induced seizures. Based on the mechanistic studies the extract may act via gabarergic, serotonergic and histaminergic pathways.

6.2 CONCLUSION

Based on the research results presented herein, it may be concluded that the methanol leaf extract of *Cassia mimosoides*contain bioactive component(s) that possess anticonvulsant and antiepileptogenic activities which may be responsible for the use of the plants in the management of epilepsy. Furthermore, methanol leaf extract of *Cassia mimosoides* may be acting through GABAergic mediated neural inhibition and partly via κ-opioid receptors system. The extract also seems to operate via histaminergic and serotonergic pathways.

6.3 RECOMMENDATIONS

The following recommendations were made after the study:

1. a) The effect of the extract on oxidative stress markers should be investigated in kindling model.

   b) Hippocampal slices to compare changes in the brain amongst groups

2. Bioassay of guided fractionation of the crude extract should be carried out and possibly isolate and characterise the bioactive compounds responsible for the anticonvulsant activity.

3. Chronic toxicity studies should be done to assess toxicity.

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APPENDIX

ETHICAL CLEARANCE

DIRECTORATE OF ACADEMIC PLANNING & MONITORING

AHMADU BELLO UNIVERSITY, ZARIA

Vice Chancellor *Prof. Ibrahim Garba, B.Sc. (Hons) Zoology, M.Sc. (Nnamdi Azikiwe) A.B.U., Ph.D Biology (London), D.I.C., FIBIOL*
Director: Prof. M.F. Ishiyaku, B.Sc. (Hons) Botany (ABU), M.Sc. Plant Breeding (Wales), Ph.D Agriculture (University of Reading, U.K.), MABN, MBSN

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Appl No.: ABUCAUC/2018/Pharmacology and Therapeutics/074 20th June, 2019
Approval No: ABUCAUC/2018/074

Dr. Jamilu Ya'u,
Department of Pharmacology and Therapeutics,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria.

Dear Sir,

EVALUATION OF ANTICONVULSANT EFFECTS OF METHANOL EXTRACT OF CASSIA MIMOSSOIDES IN LABORATORY ANIMALS

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Pharmacology and Therapeutics. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

S.L Usman
For: Chairman, ABUCAUC.

Cc. Director, DAPM
" Director, IC&ICT
" Head, Department of Pharmacology and Therapeutics
" Prof. C.A. Kudi, Chairman, ABUCAUC