ANTICONVULSANT STUDIES ON METHANOL LEAF EXTRACT OF *LAGGERAAURITA* LINN.F.IN LABORATORY ANIMALS

BY

Hadiza KYARI

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

AHMADU BELLO UNIVERSITY, ZARIA,NIGERIA.

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BY

Hadiza KYARI, B Sc. Biochemistry (A.B.U) 2011
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NIGERIA.

DECEMBER, 2015

DECLARATION

I declare that the work in this Dissertation entitled "**ANTICONVULSANT STUDIES ON METHANOL LEAF EXTRACT OF *LAGGERAAURITA* LINN.F. IN LABORATORY ANIMALS"** has been carried out by me in the Department of Pharmacology and Therapeutics,Faculty of Pharmaceutical Sciences**.** 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 another degree or diploma at this or any other Institution.

Hadiza Kyari	_____	_____
Name of Student	**Signature**	**Date**

CERTIFICATION

This dissertation entitled "**ANTICONVULSANT STUDIES ON METHANOL LEAF EXTRACT OF *LAGGERAAURITA* LINN.F. IN LABORATORY ANIMALS" by** Hadiza KYARI, meets the regulations governing the award of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Dr. N.M. Danjuma _____ _____

Chairman, Supervisory Committee Signature Date

Professor I.M. Hussaini _____ _____

Member, Supervisory Committee Signature Date

Dr. S. Malami _____ _____

Member, Supervisory Committee Signature Date

Dr. N. M. Danjuma _____ _____

Head of Department Signature Date

Professor K. Bala _____ _____

Dean, School of Postgraduate Studies Signature Date

DEDICATION

This dissertation is dedicated to Almighty Allah (S.W.T), then to my Husband, Alhaji Ishaq Tijjani Giazhe and my children, Abdur-Rahman and Maryam (Afrah).

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Finally, to my blessed children, Abdur-Rahman and Maryam (Afrah). May the peace, blessing and guidance of Allah (S.W.T) always be with you.Amiin.

ABSTRACT

*Laggeraaurita*Linn. belongs to the family of Asteraceae. It is an annual herb which is found growing as weeds in Nigeria and spread throughout the sub-Saharan Africa. In Nigeria, *Laggeraaurita* is used asa remedy for pediatric malaria and in the management of epilepsy in Niger state, Nigeria Safiya, M, (personal communication, October 10, 2014). The study was conducted to evaluate the anticonvulsant potential of the methanol leaf extract of *Laggeraaurita*(LAME) using pentylenetetrazole induced seizure in mice, maximal electroshock induced seizure in chicks, picrotoxin induced seizure in mice and strychnine induced seizure in mice. Model of epilepsy involvingpentylenetetrazole induced kindling in ratsas well as interaction studieswas also conducted using fluphenamic acid and cyproheptadine. Extraction of 500g of the powdered leaves afforded a 20.4% yield. The preliminary phytochemical screening of the methanol leaf extract of *Laggeraaurita* revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids/terpenoids, glycosides, carbohydrates, and cardiac glycosides. The oral median lethal dose (LD_{50}) values for both species (rats and mice) was found to be greater than 5000 mg/kg while the *i.p* median lethal dose values for both species also was found to be 2154.06 mg/kg. In the maximal electroshock induced seizure model, the extract did not protect the animals against tonic hind limb extension (THLE), however it decreases the mean recovery time of the convulsed chicks. In the pentylenetetrazole-induced seizure model, the extract protected the mice by delaying the mean onset of seizure at all doses tested with significant $p<0.05$ increase at 600 mg/kg. The extract produced protection against both strychnine and picrotoxin induced seizure in mice by delaying the mean onset of seizure in the convulsed mice at all doses but there was minimal protection against seizure seen with picrotoxin induced seizure model.In the interaction studies, co-administration of fluphenamic acid (FFA) (5 mg/kg) and the extract (600 mg/kg) showed an enhanced effect with percentage protection of 70% indicating the possible modulatory effect of the extract via sodium channel. Single intraperitoneal(*i.p)* dose administration of the extract (600 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 the extract (600 mg/kg) as well as the

co-administration of cyproheptadine (4 mg/kg) and phenytoin (20 mg/kg) offered a reduced protection of 20% and 50% protection against seizurerespectively, these also indicate theinvolvement of serotonergic and histerminergic pathways in the anticonvulsant effect of the extract. The extract at all doses tested reduced the severity of seizure episodes induced by kindling. The results suggest that the methanol leaf extract of*Laggeraaurita* possessessignificant anticonvulsant effects and has antiepileptogenic property.

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LIST OF ABBREVIATIONS

The Following Abbreviations were used in The Work

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
CYPT	Cyproheptadine
DAG	Diacyl Glycerol
DC	Decarboxylase
DZP	Diazepam
EEG	Electroencephalography
FFA	Fluphenamic Acid
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
IP_3	Inositol Triphosphate
K^+	Potassium Ion
LAME	*Laggeraaurita* Methanol Extract
LD_{50}	Median Lethal Dose
MAO_A	Monoamine Oxidase A
MEST	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
PBT	Phenobarbitone
PHT	Phenytoin
PLC	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
$VGCC_S$	Voltage Gated Calcium Channels
W.H.O	World Health Organization

CHAPTER ONE

1.0INTRODUCTION

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) and 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 chronic neurological condition seen by neurologist worldwide (Sridharan, 2002). Ithas 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 1% of the global burden of diseases (Reynolds, 2002). Among the 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).Treatment of epilepsy with herbal drugs seems to be more beneficial and is gaining more popularity due to their fewer side effects and herbal drugs are acting at the target site having same mechanism of action as the synthetic drugs. 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).

1.1Statement of Research Problem

Epilepsy is one of the most common serious neurological disorders (Hirts *et al.,* 2002) .affecting about 65 million people globally (Thurman *et al.,* 2011). Over 80 percent of the cases are from the developing world (Scott*et al*., 2001), about 10 million from Africa (Coleman *et al*., 2002). The prevalence of active epilepsy in Nigeria is 3-14 per 1,000 populations (WHO, 2004). The world health organization (WHO) estimates that of the 10 million people living with epilepsy in Africa, 8 million (80%) are not receiving adequate treatment (WHO, 2004). Around thirty thousand develop epilepsy every year 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 in

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. Only a little is known on the exact mechanism of epilepsy (Noebels and Avoli,2012). The scientific understanding of seizure pathogenesis and propagation is far from complete and the mechanism of action of most available antiepileptic drugs (AEDs) is either unknown or involves multiple interactions (Gerlach and Krajewski, 2010). However, it is unknown under which circumstances the brain shifts into the activity of a seizure with its excessive synchronization (Lopez *et al.*,2003).

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, 2012).People with epilepsy are at an increased risk of deathand 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, *statusepilepticus*, suicide, trauma, and sudden unexpected death in epilepsy (SUDEP)(Hitiris *et al.*,, 2007).Death from *statusepilepticus* 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).

1.2Justification

Despite the development of various new antiepileptic drugs (AEDs) in recent decades, they only supress seizures, but they do not affect the underlying epileptogenic process itself (Shinner and Berg, 1996). The medical treatments for epilepsy are not uniformly effective, they are expensive and possess chronic adverse effects and do not meet the criteria for an ideal antiepileptic drug. Due to the chronic nature of the disorder, compliance with therapy is a major problem for most patients because of the need for long term therapy coupled with unwanted effects ranging in severity from minimal effects like gingival hyperplasia to death from aplastic anaemia or hepatic failure (McNamara, 2006).There is a shift to the use of herbal remedies in the management of epileptic seizures, probably because these measures fit into the cultures of people and are not usually as expensive as the more refined orthodox drugs (Balamurugan *et al*., 2009). There are a number of plants being used in traditional medicine for treatment of epilepsy. Presently many of these plants are being explored scientifically to ascertain theiranticonvulsant activity (Malvi*et al.,* 2011). *Laggera aurita* is a herbplant with many ethnomedicinal uses in asthma, pain, stomatitis, paediatric malaria among others (Dibala *et al.*, 2014). Anti-inflammatory and antinorcicative property of the plant has been repoted in literature (Olurishe and Mati, 2014). The use of the leaf of

*Laggeraaurita*alsoacclaimed to have anticonvulsant activity used mostly in Niger state, Nigeria. Safiya, M,(personal communication, October 10, 2014). Literature survey has shown that no work has been carried out to scientifically scrutinize the claim of the therapeutic benefit of *Laggera aurita* in the management of epilepsy.

1.3Theoretical Framework

The method used to determine the median lethal dose (LD_{50}) was as previously described by Lorke (1983). 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 every route of administration applicable to drugs, industrial and agricultural chemicals.In experimental epilepsy studies, animal models have been developed according to the classification of epileptic seizures (Ilgaz and Nilufer, 2011).

Different chemical models of epilepsy mimic different clinical seizure types, developed to assess the pathophysiology of epileptic seizures and to search for new effective anti-epileptic drugs. It is important to note that one experimental model can represent more than one seizure types. A number of animal models have demonstrated utility in the search for more efficacious and more tolerable antiepileptic drugs (Smith*et al.*, 2007).

Commonly used *invivo* animal models of epilepsy employed by most AED discovery programs include the maximal electroshock (MES) test, the Pentylenetetrazole (PTZ) seizure test, and the kindling model. Among these, the MES and the subcutaneous PTZ seizure models represent the two models most widely used in the search for new AEDs (White *et al.*,2002). The maximal electroshock test (MEST),subcutaneous Pentylenetetrazole (scPTZ) induced seizure model, strychnine induced seizure model,

kindling induced seizure modelswere used in the screening of the methanol leaf extract of *Laggeraaurita* to determine its anticonvulsant activity.

1.4 Aims and Objectives

1.4.1 Aim of the study

The aim of this study is to provide some pharmacological rationale for the ethnomedicinal use of *Laggera aurita* in the management of epilepsy and epileptogenesis.

1.4.2 Objectives of the study

The specific objectives of this study are;

i. To establish the acute toxicity profile of *Laggera aurita* in mice and rats.
ii. To establish the preliminary phytochemical constituents present in the leaf of *Laggera aurita.*
iii. To conduct anticonvulsant study using acute and chronic models of epilepsy
iv. To establish the involvement of sodium channel, Serotonergic and Histaminergic system in the anticonvulsant activity of the methanol leaf extract of *Laggera aurita.*

1.5 Statement of Research Hypothesis

The methanol leaf extract of *Laggeraaurita* possesses 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, sometimesconvulsions, 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 changesin 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 phenomenonwhich 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 thosewith 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 commontype 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 precipitatingfactors 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 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

Optimum treatment of seizure disorders requires accurate classification of seizure type as well as appropriate choice and use of medication. The seizure classification used is based on the international classification of epileptic seizures (Dreifuss, 1989).

2.4.1 Partial seizures

Partial seizures arise from electrical activity in discrete regions of the brain. Hippocampal sclerosis is the most common focal lesion in adults with partial seizures.

Partial seizures (focal seizures) occur in a single region of the brain but may spread to other regions. These seizures are sometimes accompanied by an aura (awarning sign, such as a smell, sound, or sensation) (Chang and Lowenstein, 2003).

There are two types of partial (focal) seizure which are simple partial seizure and complex partial seizures.

I. Simple partial seizures is a type of partial seizure where one's consciousness is intact throughout the attack. It can cause motor (e.g., progressive jerking, mild paralysis), sensory (e.g., hallucinations), autonomic (e.g., flushing, sweating), and psychic (e.g., fear) symptoms.

II. Complex partial seizures are similar to simple partial seizures, but patients consciousness is impaired during the attack. In addition, complex partial seizures are associated with automatisms (e.g., lip smacking, chewing,

"picking" movements of the hand). Electroencephalography (EEG) shows a burst of repetitive spikes in the right temporal region (Vincent *et al.*,2007).

2.4.2 Generalized seizures (Convulsive or Non- Convulsive).

Generalized seizures affect the entire brain regions and may result in a loss of or some alteration of consciousness. Generalized seizures arise from both cerebral hemispheres, with no detectable focal deficits as found in partial seizures. Within the hemispheres, alterations can be found within neuronal networks or intrinsic neuronal function (Chang and Lowenstein, 2003).

There are six main types of generalized seizure.

2.4.2.1 Absence Seizure

Absence seizures mainly affect children. They cause the child to lose awareness of their surroundings for up to 20 seconds. The child will seem to stare vacantly into space, although some children will flutter their eyes or smack their lips. The child will have no memory of the seizure.Absences can occur several times a day. Although they are not dangerous, they may affect the child's performance at school.

2.4.2.2 Myoclonic seizures

These seizures consist of sudden, brief, shock-like muscle contractions,either occurring in one limb, or more widespread and bilateral. They maybe single jerks, or jerks repeated over longer periods. They are often seenin combination with other seizure types occurring in special epilepticsyndromes (WHO, 2002)

2.4.2.3 Clonic seizures

These seizures are generalized seizures, where the tonic component is notpresent, only repetitive clonic jerks (clonic jerks are repetitive rhythmicflexing and stretching of limbs) (WHO, 2002).

2.4.2.4 Tonic seizures

Tonic seizures are sudden sustained muscle contractions, fixing the limbsin some strained position. There is immediate loss of consciousness. Oftenthere is a deviation of the eyes and head towards one side, sometimesrotation of the whole body. They are seen mainly in paediatric practice (WHO, 2002).

2.4.2.5 Tonic -clonic seizure/Grand mal seizure

A tonic-clonic seizure has two stages. The patient has muscle stiffening throughout the body (in the tonic phase), often causing a fall, followed by rapid and rhythmic muscle jerking in the arms and usually the legs (in the clonic phase). Tongue biting and incontinence often are noted (Vincent *et al.*,2007).

2.4.3Unclassified Epileptic Seizures

These include seizures that cannot be classified as they do not fall under any of the categories previously mentioned. These include;

I. Febrile convulsions (e.g. accompanied by high grade fever in children aged 5years and below, characterized by rhythmic eye movements and swimming movements)
II. *Status epilepticus*: This refers to continuous or intermittent (but frequent) seizures lasting more than 5 minutes in which one fit follows another so closely without full recovery of consciousness.

2.5Ion 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 Potentialand 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).

2.7Mechanism of Epileptogenesis

Epileptogenesis is the process by which the previously normal brain is functionally altered and biased towards the generation of abnormal increasein electrical activity that subserves chronic seizures (Goldberg and Douglas, 2013). A widely accepted hypothesis holds that during the interval between brain injury and the appearance of clinically obvious seizures (latent period) which characterizes many (if not all) cases of epilepsy, there is a cascade of poorly understood changes that transform the non-epileptic brain into one that generates spontaneous recurrent seizures (Pitkanen and Lukasiuk, 2009). This insult-induced process, which is of variable length in different patients and ultimately leads to chronic epilepsy is called epileptogenesis.

2.7.1Epileptogenesis 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).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).

2.8Neurotransmitters and Epilepsy

2.8.1Serotonin 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 *etal*., 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 TPH_1 and TPH_2. The TPH_2 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 ($5HT_{1A}$), ($5HT_{1B}$), and ($5HT_{1D}$), 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 $5HT_1$ receptor subtypes is via coupling of G protein alpha subunit. This interaction

decreases cyclic AMPformation by inhibiting adenylate cyclases (ADC) (Bockaert *et al.*, 2006). After interaction with 5-HT, the main signaling linkage for the $5HT_2$ receptor sub-population is to activate phospholipase C (PLC) through coupling of Gq/11 protein alpha (GNAQ) (Bockaert *et al.,* 2006). PLC catalyzes the formation of myoinositol-1, 4, 5-trisphosphate (IP_3) and diacylglycerol (DAG) (Raymond *et al.*, 2001). The ionotropic $5HT_3$ 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 $5HT_2$ receptors. $5HT_4$, $5HT_6$, and $5HT_7$ primarily couple G protein alpha, which results in an activation of adenylate cyclase, and consequently in an increase of cyclic AMP levels Raymond (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 $5HT_3$ 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).

2.8.2Histamine 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) 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 physiologicalfunctions 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 H_3 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.

2.9Basic 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 Ca^{2+}, 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 Ca^{2+} concentration (Marom *et al.,* 2001).Changes in

ionic concentrations observed during hyperexcitation such as increasedextracellular K^+or decreased extracellularCa^{2+}may be caused by decreases in extracellularsize or volume. Failure of Na^+-K^+ pump due to hypoxia or ischemia is known to promoteepileptogenesis in animal models, and interference with Cl^--K^+ transport, which controlsintracellular$Cl^-$and regulates GABA-activated inhibitory Cl^-currents, may lead to enhancedexcitation. Excitability of synaptic terminals depends on the extent ofdepolarization and the amount of neurotransmitter released. Synchronization followingabnormal bursts of spikes in the axonal branching of thalamocortical relay cells plays a keyrole 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).

2.9.2.1Gamma 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. In the adult brain, $GABA_A$ receptors are permeable toCl^-

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 $GABA_A$ 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 decreasedinhibition (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 receptors has been reported in human brain tissue, and low glutamic acid

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 (AMPA) 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 cause 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).

2.10Diagnosis 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) which 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 antiepilepticdrug(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 fourth method of diagnosing epilepsy is neuroimaging which looks at the structure of the brain. 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 inthe brain which may suggest a reason for seizure (www.Epilepsy.com). 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).

2.11Treatment 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).

2.12Classification 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 cellwhich 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 Ca^{2+} channels is the critical link between cell depolarization and Ca^{2+} entry which can

result in its high concentration. The subsequent binding of Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} (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 ofGABA receptors, $GABA_A$, $GABA_B$ and $GABA_c$ 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 $GABA_A$ receptors whereas slow inhibition is mediated by $GABA_B$ receptors (Kayal-Brooks *et al.,* 2009). When GABA binds to a $GABA_A$ receptor, the passage of chloride, a negatively charged ion into the cell is facilitated via chloride channels. This influx of chloride increases the negativity of the cell (i.e., a more negative resting membrane potential) 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.

2.13The Plant: *Laggera aurita* Linneus(Asteraceae)

Description

Laggera aurita Linneus(Asteraceae) also known as *Blumea aurita* is a viscid, crisped-pubescent annual plant. The upper leaves are amplexicaul, while lower ones are spathulate, all with sharply dentate margins. Flowers are yellow, tubular, sometimes mixed with a few broad ligulate. *Laggera aurita* Linneus(Asteraceae)belongs to the family of Asteraceae and it is an annual herbacerous plant which is found growing as weeds in Nigeria and spread throughout the sub-Saharan Africa (Burkill, 1985).

2.13.1 Taxonomic classification

Kingdom Plantae

Phylum Magnoliophyta

Class Magnoliopsida

Order Asterales

Family Asteraceae

Genus *Laggera*

Specie*Aurita*

Name *Laggera aurita*

2.13.2 Local Names

HausaTaba-taba

Yoruba Eru-taba

Fulani Tabanya

2.13.3 Ethnomedicinal Uses

In Burkina Faso, ethnobotanical investigations in the central region have shown that.*Laggera aurita* is widely used in traditional medicine to treat various kinds of diseases such as malaria, fever, pain, stomatitis, asthma, bronchitis and nasal congestion and has also antibacterial activity (Nacoulma, 1996). The plant has been reported for use in cereal grains preservation in Cameroon and in Nigeria as remedy for pediatric malaria (Njan *et al.*, 2007; Okhale *et al.*, 2010). The plant has also acclaimed to have anticonvulsant activity Safiya, M, (personal communication, October 10, 2014).

The Asian researchers have reported various bioactive properties of the plant(Okhale *et al.*, 2010).

Previous studies have shown that *Laggera aurita* possesses antiviral, antibacterial and hepatoprotective properties (Wu *et al.*,2006; Shi *et al.*,2007).Essential oils from the leaves are used for the treatment of different diseases including cancer and cardiovascular diseases, in atherosclerosis and thrombosis (Edris,2007). Anti-inflammatory and antinorcitative properties of the plant has also been reported (Olurishe and Mati, 2014).

2.13.4Reported Phytochemical Constituents of the Plant

Phytochemical analysis showed that sesquiterpenoids, monoterpenoids and some flavonoids with some bioactivity have been isolated from the whole plant of *Laggera aurita*(Yang *et al.*, 2006; Wu *et al.*, 2006; Xiao *et al.*, 2003). Recent study byOlurishe and Mati (2014) revealed thepresence of tannins, saponins and carbohydrates.

2.13.5 Medicinal Plants with Anticonvulsant Properties

Several medicinal plants have been reported to possess anticonvulsant properties and some of these plantshave been reviewed in literature:*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 activities for species of *Bauhinia outimouta, Rauvolfia ligustrina* and *Ximenia americana* (Quintans-Júnior *et al.,* 2008),



Plate 1: *Laggeraaurita* Linn. in its Natural Habitat

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Material, Collection and Authentication

The leaves of *Laggera aurita* were collected from Dajin Kudingi, Zaria, KadunaState Nigeria, in the month of January 2015. The plantwas identified by Mallam Namadi Sunusi of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria, where a voucher specimen (No. 2002) was deposited for future reference.

3.2Materials and Equipment

Electroctroconvulsive machine (Ugo Basile, model no. 7801), Mortar and Pestle, Animal cages, Spatula, Stop watch, Syringes, Micropippette, Water bath (Gallenkamp), Weighing balance (Ohio, New York, USA)

3.3Chemicals and Drugs

Phenytoin (Sigma-aldrich, St.louis U.S.A.), Diazepam (Swipa Product Ltd. Welnyn Garden city), Pentylenetetrazole (Sigma Aldrich, St.Louis U.S.A.), Strychnine (Sigma-Aldrich, St.Louis U.S.A.), Phenorbarbitone (Sigma-Aldrich, St.Louis U.S.A.), Fluphenamic acid (Sigma-Aldrich, St.Louis U.S.A.), Cyproheptadine (PT Kalbe Pharma, Bakesi, Indonesia), Sodium Valproate (Sanofi St.Surrey, Canada), Methanol (Sigma-Aldrich, St.Louis U.S.A.), Tween 80 (Cole-Parmer Illinois U.S.A). Picrotoxin (Sigma-Aldrich, St.Louis U.S.A.)

3.4 Experimental Animals

The pharmacological experiments were conducted using adult Swiss Albino mice of both sexes (20-26 g),Wister rats of both sexes(160-240g)obtained from Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria-Nigeria. Day old Ranger cockerels (20 – 35 g), were obtained from the National Animal Production Research Institute (NAPRI). The animals were maintained 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.

3.4 Plant Extraction

The leaves of *Laggera aurita* plant were air dried under shade.The leaves were then crushed into a coarse powder with the aid of a mortar and pestle. A portion (500g) of the powdered leaves wasextracted with 1 Litre of absolute methanol for 8 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 of *Laggera aurita* was stored in an airtight container. The solutions of the extract were always freshly prepared for each study by dissolution of the appropriate amount required in deionized water under standard laboratory conditions.

3.6Preliminary Phytochemical Studies

Preliminary phytochemical screening of the methanol leaf extract of *Laggera aurita* was carried out according to the methods described by Trease and Evans, 2002.

3.6.1Test for Saponins

3.6.1.1 Frothingtest

Aqueous solution of the extract (2 ml) was diluted with distilled water twice its volume and shaken in a test tube for 2 minutes. The occurrence of foam column of at least 1 cm in height persisting for a minimum of 15 minutes indicated the presence of saponins.

3.6.2Test for Flavonoids

3.6.2.1. Shinoda Test

An alcoholic solution of the extract (3 ml) was evaporated to dryness. The residue was dissolved in 2 ml of 70% methanol with heat. Four (4) drops of conc. Hydrochloric acid were added followed by some chips of magnesium metal., Immediate appearance of orange colour denote flavones, a red-crimson colour denotes flavonols while pink-magenta colour denotes flavonones(Trease and Evans, 2002).

3.6.3Test for Tannins

3.6.3.1 Ferric ChlorideTest

The extract was dissolved with distilled water and filtered. Few drops of ferric chloride solution were added to the filtrate. A blue-black colouration indicated the presence of tannins.

3.6.3.2 Lead Sub-acetate Test

Three (3) drops of lead sub-acetate solution was added to a solution of the methanol leaf extract of *Laggera aurita*. Appearance of a light brown precipitate indicated the presence of condensed tannins.

3.6.3.3 Brominewatertest

Three (3) drops of bromine were added to aqueous solution of the extract. Appearance of a coloured precipitate indicates the presence of condensed tannins only. (Trease and Evans, 2002)

3.6.4Test for Steroids and Terpenoids

3.6.4.1 Liebermann-Burchard'stest

The portion (0.5g) of the extract was dissolved in 5ml of methanol and then filtered. The filtrate was evaporated to dryness at 45 °C on water bath. The residue was shaken with chloroform and filtered into a clean and dry test tube. 2 ml of acetic acid anhydride was added to the filtrate and shaken after which 1 ml of conc. Sulphuric acid was added carefully down the side of the tube to form a lower layer. The appearance of a brownish-red or violet ring at the zone of contact of the two liquids and the upper layer turning green indicated the presence of sterols and terpenes (Trease and Evans, 2002).

3.6.5Test for Cardiac glycosides

*3.6.5.1 KellerKiliani*test: Aqueous solution of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution and then 1 ml of conc. sulphuric acid was added down the side of the tube. A pure ring colour at the interface indicates the presence of cardiac glycosides (Trease and Evans, 2002).

3.7Acute toxicity study(LD_{50} determination)

Median lethal dose (LD_{50}) was investigated in rats and mice using the method of Lorke (1983). The study was divided into 2 phases. In the first phase, nine animals were divided into 3 groups of 3 animals per group. Group 1 received the extract at a dose of 10 mg/kg body weight while groups 2 and 3 received extract at doses of 100 and 1000 mg/kgbody weight respectively. The animals were observed for signs and symptoms of toxicity including death within 24 hours after treatment.In the second phase, 4 groups each comprising of one mouse/rat was treated with the methanol extract of *Laggera aurita* usingfour specific doses of the extract which was dependent on the outcome of the first phase.The LD_{50} value was calculatedas the square root of the product of the lowest lethal dose and the highest non-lethal dose i.e., the geometric mean of consecutive doses for which 0 and 100% survival was recorded.

3.8Anticonvulsant Studies

3.8.1Maximal Electroshock-induced seizure(MEST)test in chicks

The methods previously described by Swinyard andKupferberg (1985) and Browning (1992) were 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,* whilegroups 2 – 4 received the extracts (600, 300 and 150 mg/kg, *i.p* respectively). The fifth group received phenytoin 20 mg/kg (*i.p.).* Thirty minutes later, maximal electroshock was delivered toinduce seizures in the chicks using Ugo basile electroconvulsivemachine (model 7801) with corneal electrodes placed on the uppereyelid of the chick after dipping them in normal saline.

Thecurrent, shock duration, frequency and pulse width were set andmaintained at 90mA, 1.0 sec, 200 Hz and 1.0 ms^{-1} respectively.

Anepisode of tonic extension of the hind limbs of the chicks wasconsidered as full convulsion while lack of it was regarded as protection. The recovery time was also recorded forthe unprotected animals.

3.8.2Pentylenetetrazole-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 controlwas treated with normal saline (*i.p.*), while groups 2 – 4 received graded doses of the aqueous ethanol extract reconstituted in water (600, 300 and 150 mg/kg, *i.p.* respectively). Group 5 whichserved as positive control was treated with 200 mg/kg *i.p.*sodium valproate. Thirty minutes later, 90 mg/kg of freshly preparedsolution of pentylenetetrazole was administered subcutaneously toall the mice. The mice were observed for 30 minutes for onsetand incidence of seizures. An episode of clonic spasm of at least 5seconds duration was considered as seizure. Lack ofclonic spasmduring 30 minutes of observation was regarded as protection. Thenumber of mice protected was noted andthe anticonvulsantactivity of the extract expressed as percentage protection.

3.8.3 Strychnine – induced seizure test in mice

The method described by Krall*etal* (1978) was adopted. Thirtymice 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.*), whilegroups 2 – 4 received the extract at a dose of 600, 300 and 150 mg/kg *(i.p.)* respectively. Group 5 which served as the positive control recieved phenobarbitone (30 mg/kg *i.p*). Thirty minutes later, 1.0 mg/kg of

freshly prepared solution of strychnine was administered subcutaneously to all the mice.

The mice were observed for presence or absence of tonic convulsion and latency to death within a 30 minutes period.

3.8.4Picrotoxin – induced seizure test in mice

The method described by Swinyard *etal*(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.*), whilegroups 2 – 4 received the extract at a dose of 600, 300 and 150 mg/kg (*i.p.)* respectively. Group 5 which served as the positive control received Diazepam (10 mg/kg, *i.p).* Thirty minutes later, 4 mg/kg of freshly prepared solution of picrotoxin was administered subcutaneously to all the mice. The mice were observed for presence or absence of tonic hind limbextension within a 30 minutes period. Prolongation of the latency of tonic hind limb extension was also considered as indication of anticonvulsant activity.

3.8.5Pentylenentetrazole – induced kindling model in rats

The method described by Gupta *etal*(2001); Dhir *etal*(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 20 days. Fourty 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 – 4) received the extract at a dose of (600, 300 and 150 mg/kg *i.p.)* respectively. Group 5 received Sodium Valproate (100 mg/kg *i.p.).* Thirty minutes post treatment, all the groups were administered pentylenetetrazole (PTZ) and the rats were observed forseizure intensities within a 20 minutes period and classified as follows: Stage 0: no

response, stage 1: ear and facialtwitching, stage 2: convulsivewaves throughout the body, stage3: myoclonic jerks, rearing, stage 4: turning over onto one side, stage 5: turning over onto the back, generalized tonic-clonic seizures (Wu *et al.,* 2006).

3.9 Interaction Study Using Maximal Electroshock Test

3.9.1 Effect of co-administration of methanol leaf extract of *Laggera aurita* and fluphenamic acid (FFA) on maximal electroshock-induced seizures in chicks

The chicks were divided into ten groups of ten chicks each. Group 1 served as control and was given normal saline (10 ml/kg *i.p.*), while groups 2 and 3 received the methanol leaf extract of *Laggera aurita* at doses of600 and 300 mg/kg *i.p.* respectively, Groups 4 and 5 received FFA at doses of 10 and 5 mg/kg *i.p.* respectively, Groups 6 and 7 received Phenytoin 10 and 5 mg/kg respectively. Thirty minutes post treatment, these groups including the control were electroshocked via their corneal electrodes. The dose where least quantal protection was obtained for the FFA and Phenytoin were used for the interaction study, and the dose where highest quantal protection was obtained for the extract was also used. Thus, groups 8 and 9 received FFA at the dose of 5 mg/kg and group 10 received phenytoin at the dose of 5 mg/kg. Five minutes later, groups 8 and 10 received the extract at a dose of 600 mg/kg and group 9 received Phenytoin (5 mg/kg *i.p.*) and allowed for 30 minutes. Seizure was then induced to these groups using the MES machine as previously described by Swinyard and Kupferberg (1985).

3.9.2Effect of co-administration of methanol leaf extract of *Laggera aurita* and cyproheptadine against maximal electroshock-induced seizures in chicks

The chicks were divided into seven groups of ten chicks each. Group 1 served as control and was given normal saline (10 ml/kg *i.p*), groups 2 and 3 received the extract at doses of600 and 300 mg/kg *i.p*. respectively. Group 4 received phenytoin 20 mg/kg and group 5 received Cyproheptadine at a dose of 4 mg/kg *i.p*. Thirty minutes post treatment, these groups including the control were electroshocked via their corneal eyes.

Groups 6 and 7 received cyproheptadine at the dose of 4mg/kg each. Five minutes later, group 6 received the extract (600 mg/kg) and 7received Phenytoin (20 mg/kg *i.p.)* and allowed for 30 minutes. Seizure was then induced to these groups as previously described by Swinyard and Kupferberg (1985).

3.10 Statistical Analysis

Statistical analysis were carried out using SPSS (Version 20) and data obtained were expressed as Mean ± SEM. All analysis were done using Analysis of Variance (ANOVA), and followed by Scheffe post hoc test for multiple comparison. Differences were considered significant at P values less than or equal to 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1` Percentage Yieldof the Plant Extract of *Laggera aurita*

The extraction of the powdered leaf of *Laggera aurita*with 90% methanol afforded a yield of 20.4% w/w.

4.2 Phytochemical Constituents of the Methanol Leaf Extract of *Laggera aurita*

Preliminary phytochemical screening of the methanol leaf extract of *Laggera aurita* revealed the presence of alkaloids, flavonoids, saponins, steroids/terpenoids, and tannins (Table 4.1).

Table 4.1: Phytochemical Constituents of the Methanol Leaf Extract of *Laggera aurita.*

Constituents	Inference
Alkaloids	+
Anthraquinones	_
Flavonoids	+
Saponins	+
Steroids/Terpenoids	+
Tannins	+
Cardiac glycosides	+

Key: + = Present, - = absent

4.3 Median Lethal Dose (LD_{50}) Values of Methanol Leaf Extract of *Laggera aurita* in Mice and Rats

The intraperitoneal median lethal dose (LD_{50}) values of the methanol leaf extract of *Laggera aurita* in both mice and rats was found to be 2154.05 mg/kg while the oral (LD_{50}) value was found to be greater than 5000 (Table 4.2)

Table 4.2: Median lethal dose Values of Methanol Leaf Extract of *Laggera aurita* in Mice and Rats

Specie	Route of Administration	LD_{50} Values (mg/kg)
Mice	Intraperitoneal	2154.06
Mice	Oral	>5000
Rats	Intraperitoneal	2154.06
Rats	Oral	>5000

4.4 Effect of Methanol Leaf Extract of *Laggera aurita* on Maximal Electroshock-Induced Seizure in Chicks

The extract showed dose dependent protection against Tonic Hind Limb Extension (THLE). There was a significant ($p<0.05$) reduction in themean recovery time in unprotected animals at all the doses of the extract. The 600 mg/kg offered 40%protection against seizure,however, phenytoin (20 mg/kg), the standard drug protected all the animals against THLE with 100% protection against MEST. There was no mortality observed in all the experimental groups (Table 4.3)

Table 4.3: Effect of the Methanol Leaf Extract of*Laggera aurita* on Maximal Electroshock Induced Seizure in Chicks

Treatment (mg/kg)	% Protection against seizure	Mean Recovery time (min)
N/S(10ml/kg)	0.0	15.00±2.07
LAME(600)	40.00	7.83±0.87*
LAME(300)	20.00	8.75 ±1.29*
LAME(150)	10.00	9.11±0.82*
PHT(20)	100.00	-

Data presented as Mean ± SEM and Percentage, n = 10; *p< 0.05 (One-way ANOVA) followed by Posthoc test (Scheffe) for multiple comparison;N/S = normal saline, PHT = Phenytoin, LAME = *Laggera aurita* Methanol Extract.

4.5 Effect of Methanol Leaf Extract of *Laggera aurita* on Pentylenetetrazole-induced Seizure in Mice

The methanol leaf extract of *Laggera aurita* offered protection against seizures induced by pentylenetetrazole (90 mg/kg). At 600 mg/kg and 300 mg/kg, the percentage protection against seizures were 50% and 16.7% respectively. There was a significant increase ($p< 0.05$) in the mean onset at the dose of 600 mg/kg compared to the normal saline treated group. The standard drug, (sodium valproate) afforded 66.7% protection at the dose of 200 mg/kg. There was a significant difference ($p<0.05$) in the mean onset of seizure in the sodium valproate group. There was a reduction in the mortality rate in all the doses with 66.67% at 600 mg/kg and 300 mg/kg each and 50% at 150 mg/kg of the extract compared to the control group (Table 4.4)

Table 4.4: Effect of Methanol Leaf Extract of*Laggera aurita* on Subcutaneous Pentylenetetrazole-induced Seizure in Mice

Treatment (mg/kg)	Mean Onset of Seizure (min)	%Protection against Seizure	% Protection against Mortality
N/S(10ml/kg)	6.83±1.11	0.00	16.67
LAME(600)	15.67±1.67*	50.00	66.67
LAME(300)	10.60±0.93	16.67	66.67
LAME(150)	11.17±0.87	0.00	50.00
SV(200)	13.50 ±1.50*	66.67	100

Data presented as Mean ± SEM and Percentage, n = 6; *p< 0.05 (One-way ANOVA) followed by Posthoc test (Scheffe) for multiple comparison;N/S = Normal saline, SV = Sodium valproate, LAME = *Laggera aurita* Methanol Extract.

4.6 Effect of Methanol Leaf Extract of *Laggera aurita* on Strychnine-Induced Seizure inMice

The methanol leaf extract of *Laggera aurita* at 300 mg/kg offered 50% protection against strychnine-induced seizure.Percentage protection against mortality at 600 mg/kgand the300 mg/kgwasfound to be 33.3% while there was no protection against mortality at 150 mg/kg. There was a statistically significantincrease ($p<0.05$) in the mean onset of seizure in all the treated groups compared with the control group.However, phenobarbitone (30 mg/kg) offered 100%protection against seizure and 83.33% protection against mortality (Table 4.5).

Table 4.5: Effect of Methanol Leaf Extract of *Laggera aurita* on Strychnine Induced Seizure in Mice

Treatment (mg/kg)	Mean Onset of Seizure (min)	% Protection against Seizure	% Protection against Mortality
N/S(10ml/kg)	5.17±0.17	0.00	16.67
LAME(600)	12.00 ± 1.00 *	16.67	33.33
LAME(300)	12.67±0.67*	50.00	33.33
LAME(150)	12.20 ±1.07	16.67	16.67
PBT. (30)	-	100	100

Data presented as Mean ± SEM and Percentage, n = 6; *p< 0.05 (One-way ANOVA) followed by Posthoc test (Scheffe) for multiple comparison;N/S = Normal saline, PBT = Phenobarbitone LAME = *Laggera aurita* Methanol Extract.

4.7 Effect of Methanol Leaf Extract of*Laggera aurita* on Picrotoxin-induced Seizure in Mice

The methanol leaf extract of *Laggera aurita* at the doses of (600 mg/kg) and (300 mg/kg) protected the animals against both picrotoxin-induced seizure andmortality with 33.33% and 16.67% respectively. However, there was no protection at the dose of 150 mg/kg.Similarly, there was statistically significant ($p<0.05$)increase in the mean onset of seizure at 600 mg/kg and 300 mg/kg. Diazepam (10 mg/kg), the standard drug used offered100% protection of the mice against picrotoxin-induced seizure (Table 4.6).

Table 4.6: Effect of Methanol Leaf Extract of *Laggera aurita* on Picrotoxin Induced Seizure in Mice

Treatment (mg/kg)	Mean Onset of Seizure (min)	%Protection against Seizure	% Protection against Mortality
N/S(10ml/kg)	9.33 ±0.99	0.00	16.67
LAME(600)	20.50 ± 1.44 *	33.33	33.33
LAME(300)	20.00 ±1.45*	16.67	16.67
LAME(150)	15.00 ±1.15	0.00	16.67
DZP(10)	0.0	100	100

Data presented as Mean ± SEM and Percentage, n = 6; *p< 0.05 (One-way ANOVA) followed by Posthoc test (Scheffe) for multiple comparison;N/S = Normal saline, DZP= Diazepam LAME = *Laggera aurita* Methanol Extract.

4.8 Effect of Co-administration Administration of Methanol Leaf Extract of *Laggera aurita* and Fluphenamic Acid against Maximal Electroshock-Induced Seizures in Chicks

Fluphenamic acid at the doses of 10 mg/kg and 5 mg/kg offered 60% and 20% protection respectively, against MEST-induced tonic hind limb extension (THLE), whereas the group treated with fluphenamic acid (5 mg/kg) and the extract (600 mg/kg) concurrently produced an enhanced protection of 70% when compared to groups treated with the extract (40%) and fluphenamic acid (20%) alone. Co-administration of fluphenamic (5 mg/kg) and phenytoin (5 mg/kg) offered 60% protection against seizure, while that of phenytoin (5 mg/kg) and the extract (600 mg/kg) produced 80% protection.There was a statistically significant ($p<0.05$) decrease in the mean recovery time from seizures in groups co-administered with FFA and LAME; FFA and PHT; PHT and LAME(Table 4.7).

Table 4.7: Effect of Co-administration Administration of Methanol Leaf Extract of *Laggera aurita* and Fluphenamic Acid against Maximal Electroshock-Induced Seizures in Chicks

Treatment (mg/kg)	% protection against seizure	Mean Recovery time (min)
N/S(10ml/kg)	0.00	12.01± 0.89
LAME(600)	40.00	7.83 ± 0.83
LAME(300)	20.00	7.50 ± 0.68
FFA (10)	60.00	8.38 ± 0.89
FFA (5)	20.00	7.25 ± 1.38
PHT (10)	70.00	8.00 ± 1.15
PHT (5)	40.00	9.17 ± 1.11
FFA(5) + LAME(600)	70.00	3.33± 0.33*
FFA (5) + PHT (5)	60.00	5.50± 0.65*
PHT (5) + LAME (600)	80.00	5.00± 1.15*

Data presented as Mean ± SEM and Percentage, n = 10; *p< 0.05 (One-way ANOVA) followed by Posthoc test (Scheffe) for multiple comparison;N/S = normal saline, PHT = Phenytoin, FFA= Fluphenamic acid, LAME = *Laggera aurita*Methanol Extract.

4.9Effect of Co-administration of Methanol Leaf Extract of *Laggera aurita* andCyproheptadine against Maximal Electroshock-Induced Seizures in Chicks

Cyproheptadine at the dose of 4 mg/kg offered0.00% protection against maximal electroshock induced seizure. *Laggera aurita* methanol extract at 600 mg/kg offered 40% protection which was reduced to 20%, when co-administered with Cyproheptadine.Co-administration of cyproheptadine (4 mg/kg) and phenytoin (20 mg/kg) offered 50% protection against seizure. There was a statistically significant($p<0.05$)decrease in the mean recovery time in the group treated with the extract (300 mg/kg) and the group where cyproheptadine (4 mg/kg) was co-administered with the extract (600 mg/kg). There was 100% protection against mortality for all the experimental groups (Table 4.8).

Table 4.8: Effect of Co-administration of Methanol Leaf extract of *Laggera aurita* and Cyproheptadine against Maximal Electroshock-Induced Seizures in Chicks

Treatment (mg/kg)	% protection against seizure	Mean Recovery time (min)
N/S (10ml/kg)	0.00	12.10 ± 0.89
LAME (600)	40.00	7.83± 0.83*
LAME (300)	20.00	5.00± 0.68*
CYPT (4)	0.00	5.60± 1.21*
PHT(20)	100.00	-
CYPT (4) + LAME (600)	20.00	7.50 ± 0.89*
CYPT(4) + PHT (20)	50.00	9.00± 0.89

Data presented as Mean ± SEM, n = 10; *p< 0.05 (One-way ANOVA) followed by Posthoc test (Scheffe) for multiple comparison;N/S=Normal Saline, PHT = Phenytoin, CYPT = Cyproheptadine, LAME = *Laggera aurita* Methanol Extract.

4.10Effect of the Methanol Leaf Extract of *Laggera aurita* on Pentylenetetrazole Induced Kindling in Rats

The extract at all doses tested reduced the kindling scores induced by sub convulsive dose (35 mg/kg) of pentylenetetrazole. The reduction as recorded by seizure scoring model was generally statistically significant ($p<0.05$) throughout the treatment days. There was graded increase in the severity from the first to thefifthinjection where it reached its peak, thereafter, the severity dropped at thesixth and maintained through theseventh to theninthand increased on thelast injection. (Figure 4.1)

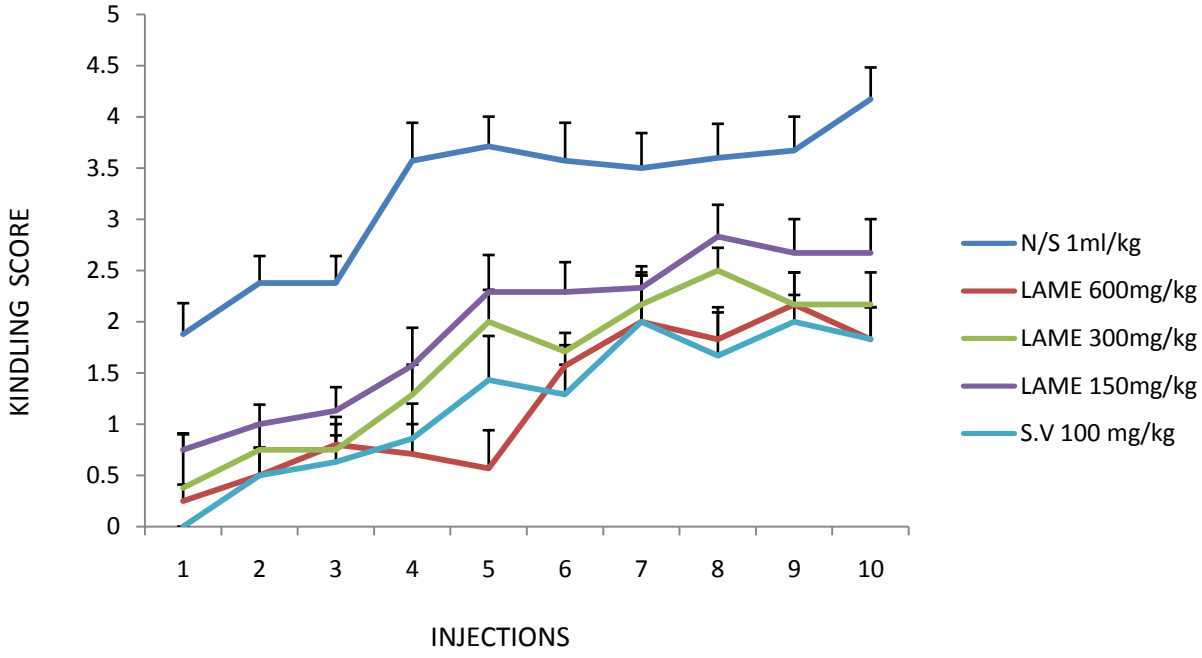


FIGURE4.1: Effect of the Methanol Leaf Extract of *Laggera aurita* on Pentylenetetrazole induced Kindling in Rats

LAME = *Laggeraaurita* Methanol Extract, N/S = Normal Saline, S.V = Sodium Valproate

CHAPTER FIVE

5.0DISCUSSION

Recent research by Abdullah *et al.,* (2013) established that *Laggera aurita*possesses triterpenes, flavonoids, saponin, cumarins, tannins and alkaloids as some of its active constituents.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 preliminary phytochemical screening of the methanol leaf extract of *Laggera aurita* revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids/terpenoids, glycosides, carbohydrates, and cardiac glycosides. Based on the results obtained from the phytochemical screening, it is not possible to attribute with certainty the observed anticonvulsant effect of *Laggera aurita* to one or several active principles amongst those detected in the phytochemical screening.

However, triterpenic steroids and saponins have been reported to possess anticonvulsant activity in experimental seizure models such as MEST and ScPTZ (Kasture *et al.,*, 2002; Chaunhan *et al.,*, 1988). Some alkaloids and flavonoids have also been shown to exhibit protective effects against PTZ convulsions. Studies have revealed that flavonoids have neuroprotective effect against electrical kindling in rats (Tourandokht *et al.*,2010). The presence of these phytochemical constituents might be responsible for the observed pharmacological activities of the crude methanol leaf extractof *Laggera aurita* in the tests conducted.

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 leaf extract of *Laggera aurita* was carried out in rats and mice via both oral and *i.p* routes. The oral LD_{50} values for both species was found to be greater than 5000 while the *i.p* median lethal dose values for both species also was found to be 2154.06 mg/kg .The result showed that the route of administration plays a key role in determining toxicity as the oral route has shown to be relatively safer than the *i.p.* route in both 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 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 MEST predicts anticonvulsantactivity 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), which indicate the ability of the antiepileptic agent to serve in the treatment of generalized tonic clonic and partial seizures (Raza *et al.,*2001). The methanol leaf extract of *Laggera aurita* demonstrated activity against MES-induced seizure in a dose dependent manner. Evidently, the 600 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 leaf extract of *Laggera aurita* 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 to protect the mice against strychnine induced seizure indicates 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.

Picrotoxin is used in determining mechanism of action of anticonvulsants (Vogel andVogel, 1997). Picrotoxin induced 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 leaf extract of *Laggera aurita*

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.

Fluphenamic acid modulates neuronal sodium channels there by reducing sodium current, thus, prevent seizure generation. Fluphenamic acid (FFA) has an interesting modulatory effect on neuronal sodium channels, reducing sodium current availability and slowing down inactivation and recovery from inactivation, leading to diminished repetitive and burst firing (Hau-Jie *et al.*,2010). Antiepileptic drugs that act via MEST model are able to limit the repetitive firing of action potentials by slowing the rate of recovery of voltage gated sodium ion channels from inactivation and suppress hind limb tonic extension in maximal electroshock seizures (Rho and Sankar, 1999). FFA showed dose dependent protection against MEST which confirmed its modulatory effect on neuronal sodium channel. The extract also showed dose dependent protection against MEST. Interaction between FFA and the extract produced an enhanced protection and quicker recovery.Additive effect was observed when FFA (5 mg/kg) was co-administered with phenytoin (5 mg/kg), and when phenytoin (5 mg/kg) was co-administered with the extract (600 mg/kg). These effects observed may predict that the extract, FFA and phenytoin might possibly acted via similar mechanism of action.

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 induces seizure by blocking both serotonine ($5HT_1$ and $5HT_2$) and histamine (H_1). It interferes with serotonergic and histerminergic pathways via antagonizingsubtypes 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.

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 haveestablishedthat progression ofseizures in kindling is associated withdecreasednumbersof$GABA_A$ receptor binding sites inhippocampus(Bazyan *et al.*, 2001), amplification inglutamaterelease, and elevated nitricoxidelevel(Riazi *et al.*,2006). On the other hand decreased serotonin level in brain also resultin 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.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Oral and intraperitoneal median lethal dose (LD_{50}) values in both mice and rats were found to be greater than 5000 mg/kg and 2154.06 mg/kg respectively. The preliminary phytochemical screening of the methanol leaf extract of *Laggera aurita* revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids/terpenoids, glycosides, carbohydrates, and cardiac glycosides.

The methanol leaf extract of *Laggera aurita* showed dose dependent protection against maximal electroshock and pentylenetetrazole-induced seizure models. It also significantly ($p<0.05$) reduced the mean recovery time of the convulsed chicks and prolonged PTZ models respectively. The extract also protected the animals against picrotoxin and strychnine induced seizure.

Interaction between FFA (5 mg/kg) and the extract (600 mg/kg) produced an enhanced protection with percentage protection against seizure of 70 % when compared to single administration of each. Additive effect was observed when FFA (5 mg/kg) was co-administered with phenytoin (5 mg/kg), and when phenytoin (5 mg/kg) was co-administered with the extract (600 mg/kg) with % protection against seizure of 60 % and 80 % respectively. These observed effects may predict that the extract, FFA and phenytoin might possibly be operating via similar mechanism of action.

The extract also seems to operate via histaminergic and serotonergic pathways since when co-administered with cyproheptadine, which was a blocker of these pathways, by antagonizing subtypes of $5HT_1$, $5HT_2$ and H_1 receptors, the percentage protection against seizure of the extract was reduced even though there was a significant ($p<0.05$) decrease in the mean recovery time. The extract at all doses tested 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.

6.2 Conclusion

The methanol leaf extract of *Laggera aurita* possesses significant anticonvulsant and has antiepileptogenic properties.

6.3 Recommendation

I. Further studies should be carried out to evaluate the action of *Laggera aurita* using bicuculine, NMDA among others.

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

III. 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.

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