EVALUATION OF THE ANTI-CONVULSANT EFFECT OF *Pennisetum glaucum* SUPPLEMENT IN SOME LABORATORY ANIMALS

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DECLARATION

I Hafsat Danmallam MUHAMMAD declare that the work in this dissertation entitled **EVALUATION OF THE ANTI-CONVULSANT EFFECT OF *Pennisetum glaucum* SUPPLEMENT IN SOME LABORATORY ANIMALS** has been carried out by me in the Department of Human Physiology, Faculty of Medicine and Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. 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.

Hafsat Danmallam MUHAMMAD _____ _____

Signature **Date**

CERTIFICATION

This dissertation entitled **EVALUATION OF THE ANTI-CONVULSANT EFFECT OF *Pennisetum glaucum* SUPPLEMENT IN SOME LABORATORY ANIMALS by I, Hafsat Danmallam MUHAMMAD** meets the regulations governing the award of Master degree of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Pennisetum glaucum has been pharmacologically studied for various activities like antidiabetic, anti-microbial and anti-tumor activities. The present study was aimed at investigating the anti-convulsant effect of *Pennisetum glaucum* supplement in some laboratory animals. Quantitative phytochemical and proximate tests were carried on *Pennisetum glaucum* using standard laboratory techniques. In PTZ-induced seizures in mice, 20 swill albino mice were group into 4 negative control which received normal saline, 25% *Pennisetum glaucum* supplemental group, 50% *Pennisetum glaucum* supplemental group and diazepam (2 mg/kg) treated group. All the 4 groups received their respective interventions for 14 day. Thirty minutes after the 14th administration freshly prepared PTZ (85 mg/kg) was subcutaneously injected to all the mice and observed for seizure onset, seizure duration, percentage seizure protection and percentage mortality protection for 30 minute post PTZ injection. Induction of PTZ kindling seizures in wistar rats involves the grouping of 20 rats into 4 (normal saline, 25% *Pennisetum glaucum* supplemental group, 50% *Pennisetum glaucum* supplemental group and 200 mg/kg valporic treated group) with each group receiving PTZ (35 mg/kg) on every alternate day for 30 days. Thirty minutes after each PTZ injection rats were observed for the presence/absence of seizures and seizure severity which were evaluated using Racine scale. In Maximal electroshock study, 40 chicks were equally grouped into 4: negative control, 25% *Pennisetum glaucum* supplemental group, 50% *Pennisetum glaucum* supplemental group and phenytoin (20 mg/kg) treated group. Maximal electroshock seizures in chicks was induced by applying an electrical stimulus 30 minutes after grouping and administration which was maintained at a frequency of 100pulse/sec, current 80 mA, pulse width 0.6ms for a duration of 0.8 sec using ugo basile convulsiometer with corneal electrodes placed on the upper eyelids of the chicks. Seizure duration, recovery from seizure, seizure protection and mortality protection were the parameters recorded. The results of the study showed a high presence of flavonoids, saponins, tannins, phytate with the least of alkaloids. Also, a high carbohydrates, protein, moisture, fibre, lipids with the least of ash, Magnesium and Phosphorus were revealed. *Pennisetum glaucum* at 50% supplement offered 40% protection from death and significantly ($p \leq 0.05$) decrease the duration of seizure (41.20 ± 3.34 vs 137.00 ± 13.13 seconds) whereas diazepam a standard anticonvulsant proved more effective in delaying seizure onset (182.00±10.00 vs 72.80 ± 1.32 seconds), offering 60% protection from death and decreasing seizure duration (72.20±19.98 vs 137.00 ± 13.13 seconds) in mice. The supplement was able to suppress the progression of seizure to most severe stages 4.4 reached by the control by retarding seizure severity to only stage 3.8 and 2.5 at 25% and 50% *Pennisetum glaucum* supplemental groups respectively in wistar rats. The supplement also, offered 40% vs 20% protection from seizure when compared to the control. However, phenytoin a standard anticonvulsant offered 100% seizure protection in chicks. The present study demonstrates that oral administration of *Pennisetum glaucum* supplement for 14 days, 30 days and 1 day caused anticonvulsant effect against subcutaneous PTZ-induced seizures in swiss albino mice, intraperitoneal PTZ-induce kindling in wistar rats and MES-induced seizures in chicks respectively. However, the efficacy of the anticonvulsant activity offered by *Pennisetum glaucum* supplement at both doses were less when compared to standard antiepileptic drugs used.

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LIST OF ABBREVIATIONS

AED-antiepileptic drug

AMPA-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ATP-adenosine triphosphate

$_{C}$AMP-cyclic adenosine monophosphate

CNS-central nervous system

CSWS-syndrome of conntinous spikes and waves during sleep

CT- computerized tomography

CYP-cytochrome P450

DHA-docosahexaenoic acid

ECF-extracellular fluid

ECS-electroconvulsive shock

EEG-electroencephalography

EPA-eicosapentanoic acid

GABA-gama amino butyric acid

GABA-T-GABA transaminase

GAD-glutamate decarboxylase

GSH-glutathione

ILAE-International League Against Epilepsy

KGD-ketoglutarate dehydrogenase

MEST-maximal electroshock test

$_{M}$GluR-metabotropic glutamate receptor

MRI-magnetic resonance imaging

NMDA-N-methyl-D-aspartate

NREM-non rapid eye movement

OS-oxygen spacies

PHE-phenytoin

PNS- peripheral nervous system

PTZ-pentylenetetrazole

RMP-resting membrane potential

ROS-reactive oxygen species

SE-status epilepticus

SOD-superoxide dismutase

SPECT-single positron emission computerized tomography

SSD-succinate semialdehyde dehydrogenase

THLE-Tonic hind limb extension

TLE-temporal lobe epilepsy

VGSCs-voltage-gated sodium channels

VPA-valproic acid

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to The Study

Epilepsy is a brain disease involving recurrent unprovoked seizures characterized by an enduring predisposition to generate epileptic seizure and by the neurobiological, cognitive, psychological and social consequences of this condition (Fisher *et al*., 2005).

The overall world prevalence is 10/1,000 with an incidence of 24 to 53 per 100,000. In the African region the prevalence is ranging from 2.2 to 58 per 1000 with an average prevalence of 15.83 (Shakhirullah *et al*., 2014). The incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing countries is 100 per 100,000 (Diniz *et al*., 2015).

In Nigeria, the prevalence of epilepsy varies from 5.3 to 37/1000 (Akinsulore and Adewuya, 2010) and major causes are attributed to congenital malformations, persistent high fever, meningitis, tumours and trauma sustained from automobile traffic accidents (Olubunmi, 2006) which is persistently increasing day by day. Also, parasitic infestations such as *Onchocerca volvulus, Taenia solium and Toxoplasma gondii* are believed to increase the risk of epilepsy and these infections are common in most parts of Sub-Saharan Africa (Ae-Ngibise *et al.*, 2015).

Pennisetum glaucum popularly known as pearl millet in English, *gero* in *Hausa*, *oka* in *Yoruba* is a member of the grass family (poaceae) (Amadou *et al*., 2013) that is widely grown around the world especially in tropical semi-arid regions for fodder and food (Odusola *et al*., 2013)

and well adapted to production systems characterized by low rainfall, low soil fertility and high temperature (Nambiar *et al*., 2012).

Current anticonvulsant drugs are largely aimed at decreasing neuronal excitability to prevent or control the occurrence of epileptic seizures. However, the role of oxidative stress in seizures led to the proposition that antioxidant compounds may be considered as promising candidates for preventing the development of epilepsy (Zaeri and Emamghoreishi, 2015). *Pennisetum glaucum,* being gluten free with high antioxidant activity and polyunsaturated fatty acids (Daniel *et al*., 2012; Mahanty *et al.,* 2012) may play a vital role in salvaging this devastating neurological condition.

1.2 STATEMENT OF RESEARCH PROBLEM

Approximately 1-2% of the world population suffer from epilepsy (Kamboj *et al.,* 2009) making it the second most common neurological disease after stroke. 80% of epileptic patients are found in resource poor (developing) countries with 25% in Africa among which 90% go untreated due poverty, lack of awareness and poor accessibility to required treatment (Bierbeck, 2012). Antiepileptic Drugs (AEDs) remain the most commonly utilized treatment and continuous medication for long period and sometimes throughout lifetime is necessary even after the seizure have long been suppressed (Kwan *et al*., 201). Also, concomitant administration of AEDs with other drugs predispose the patient to the risk of drug interaction, all of which limit the patients school achievements, employment prospects and participation in all of life experience (EFA, 2012; Nóbrega *et al*., 2014). The available Antiepileptic Drugs (AEDs) are confronted with problems including pharmaco-resistance in 30% of epileptics even with adequate dose, severe adverse effect including cognitive and motor disturbances, and chronic toxicities including teratogenicity and hepatotoxicity (Nobrega *et al.,* 2014). Similarly herbs that are used sometimes causes unpleasant side effects such as headaches, rashes and gastrointestinal problems (Herbal therapy, 2013). Thus, the search for new medicines from

plant origin that are readily available, efficacious and safer for the control of seizures becomes crucial and *Pennisetum glaucum* supplement may play a role in salvaging the situation.

1.3 JUSTIFICATION

Research have shown that Free radical generation in the brain inactivate glutamate decarboxylase (preventing the conversion of glutamate to GABA), inhibit glutamate synthetase (prevent glutamate inactivation to glutamine) increasing glutamate activity and subsequent seizure generation (Diniz *et al*., 2015). Gluten free diet are reported to decrease glutamate receptor activation, decreasing cortical hyperexcitability thereby modulating GABAergic neurotransmission (Bella *et al*., 2015). Fatty acids of poly unsaturated class have been shown to activate peroxisome proliferator activated protein which inhibit proinflammatory transcription factor and subsequently inhibit inflammation a contributing factor in epileptogenesis (Sancheti *et al*., 2013). Hence, *Pennisetum glaucum* being gluten free with high antioxidant, polyunsaturated fatty acids (Thilagavathi *et al*., 2015) and common all over the world may be beneficial in the treatment of epilepsy though documented reports are lacking and thus, the reason for this study.

1.4 AIM AND OBJECTIVES

1.4.1 AIM

This study was aimed at evaluating the anti-convulsant effect of *Pennisetum glaucum* supplement in some laboratory animals.

1.4.2 OBJECTIVES

i To assess pentylenetetrazole induced seizures in mice feed with different doses of *Pennisetum glaucum*.

ii To assess pentylenetetrazole induced kindling seizures in wistar rats feed with different doses of *Pennisetum glaucum* .

iii To assess maximal electroshock induced seizures in chicks feed with different doses of *Pennisetum glaucum* .

iv To compare the efficacy of *Pennisetum glaucum* supplement with the standard anticonvulsant used in this study.

1.5 RESEARCH HYPOTHESIS

Pennisetum glaucum supplement does not have significant anticonvulsant effect in laboratory animals.

CHAPTER TWO

2.0 Literature Review

2.0 Epilepsy is one of the most prevalent non communicable brain disorders characterized by recurrent unprovoked seizures that result from excessive disorderly discharge of cerebral neurons either due to over excitation of glutamatergic or under inhibition of GABAergic neurons resulting to disability (neurobiological, cognitive, psychological and social consequences) and mortality (Fisher *et al.*, 2014; Ali-Reda *et al.*, 2015). A seizure manifest intermittently in various forms ranging from dramatic convulsive activity to experiential phenomenon not readily discernible by an observer (strange smell/taste, lightning spot, blurred vision, crawling/tingling sensation on the skin, lip smacking, increase eye blink) that normally last for less than 5minutes (**Diniz *et al.*, 2015)**.

2.1 Epidemiological variations

Age

The incidence rates are highest in childhood, plateaus from the age of 15 to 65 years, and rise again among the elderly (Bierbeck, 2012).

Gender

Many studies report a higher incidence in males than females in both developed and developing countries which may be due the preponderance of the male sex hormone that increases their occupational and social exposure to epileptogenic insults such as cranial trauma and alcohol (Olubunmi, 2006). It has also been proved scientifically that two female sex hormones (estrogen and progesterone) raise the threshold of seizure to some extent (Shakirullah *et al.*, 2014).

2.1.2 Historical perspective of Epilepsy

The word Epilepsy was derived from the Greek word "epilambanein" which literary means "to seize upon" or "to attack" (Hozayen *et al.,* 2012). It is one of the oldest recorded neurological disorders in man with at least 3000 years of written history (Diniz *et al* 2015) with one of the earliest descriptions been secondarily generalized tonic clonic seizure that was attributed to the god of the moon as recorded in mesopotamus (Cavazos, 2012).

There are many superstitious beliefs concerning the cause of seizure disorder which cut across all social strata. Epilepsy was believed to be a sacred disease that is the result of the invasion of the body by a god as was thought that only a god could deprive a healthy man of his senses, throw him to the ground, convulse him and then rapidly restore him to his former self again (Shakirullah *et al.,* 2014). It was also believed to results from visitation of the devil, effect of witch-craft, the revenge of an aggrieved ancestral spirit and punishment for sins by traditional healers (Akpan *et al*., 2013).

These beliefs have resulted in patients with epilepsy being ostracized, stigmatized and misunderstood. The social implications are serious including patients with epilepsy are refused burial in the family grave, dropping out of school, losing job and impossible to marry among others. This historical legacy has continued to influence public attitude to epilepsy making it a dreaded disease (Olubunmi, 2006).

About 2500 years ago, a remarkable issue was raised by Hippocrates in 'famous treatise on the Sacred Disease' where he believed that epilepsy was not sacred and attributed it to excessive phlegm that causes abnormal brain consistency (Cavazos, 2012; Shakirullah *et al.*, 2014).

2.1.3 Causes of epilepsy

Epilepsy has been associated with different aetiologies such as **persistent High fever (due to infections), heatstroke, Insufficient oxygen to the brain (from** strokes **or carbon monoxide poisoning), Destruction of brain tissue (from head injury,** birth defects, genetic disorder, cerebrovascular diseases like haemorrhage, infestation like meningitis, **or brain tumour),** metabolic disorders **(diabetes mellitus, hypoglycaemia) (**Orellana-Paucar *et al*., 2013**). Use of recreational agents (such as excess alcohol and cocaine),** electrolyte imbalance (**low levels of magnesium,** high level of **sodium in the blood,** aminoaciduria, water intoxication and deficiency of pyridoxine) (Toni, 2015). **In rare cases, excessively high doses of certain nutrients and** herbs **may cause a seizure or make it more likely to occur such as ginkgo biloba herb** (Orellana-Paucar *et al*., 2013).

2.1.4 Symptoms and Phases of Epilepsy

The Symptoms of epilepsy include altered levels of consciousness, disturbances of movements, abnormal sensory phenomena, autonomic changes, transient disturbances of behaviour (like mood) and physical problems (such as fractures and bruises from injuries related to seizures) (WHO, 2015).

The phases of seizures are often grouped into ***Prodromal Phase*** which is characterized mostly by emotional symptoms that occurs days/ hours before seizure ensues. Some of the symptoms observed during this phase includes depression, insomnia, irritability, light headedness and increased agitation (Degirmenci and Kececi, 2016).

Aura (subjective feeling that act as warning sign that leads to behavioural and mood changes) that proceeds seizure by hours/ days which may be emotional (e.g. fear, anxiety, depression), physical (e.g. dizziness, nausea), or sensory (e.g. tingling or crawling sensation on the skin, spots of colors before the eyes, strange tastes or smells) (Maranhao *et al*., 2011).

Ictal Phase which is the seizure itself that start from the first sign of aura and last to the end seizure activity. Ictus may be triggered by loud noise, screams or cries, television flashing, sleep deprivation, poor diet, stress, menstruation, ingestion or withdrawal of alcohol and drug use. Some of the common symptoms during seizure include disturbances in movement, behaviour, alteration of consciousness and memory lapses (Degirmenci and Kececi, 2016).

Post-ictal Phase: is the period immediately after seizure. Some of the observed responses are numbness, muscle weakness, paralysis, psychiatric symptoms, aphasia, anxiety, fear, shame, sleepiness, bruises, lethargy and confusion (Maranhao *et al*., 2011).

2.1.5 Pathophysiology / Aetiology of Epilepsy

The Pathophysiology of seizure has been associated with enhanced excitatory amino acid transmission, impaired inhibitory transmission or abnormal electrical properties of the affected cells such that the excitatory forces supercedes those of the inhibitory. The electrical signal then spreads to the surrounding normal neuronal cells which then begin to fire in concert with the abnormal cells leading to neuronal cell death, scar tissue formation, neurogenesis, gliosis, axonal and dendritic plasticity, olecular reorganization of cellular membranes, extracellular matrix and sprouting of new axons and thus increasing the risk of future seizures (Hozayen *et al*., 2012; Maheswari *et al*., 2015; Toni, 2015).

Synaptic neurotransmitters implicated in epileptic seizures are mostly glutamate, glycine and gamma-aminobutyric acid (GABA).

Gamma-aminobutyric acid (GABA)

Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter of the human brain that is formed by the decarboxylation of glutamate in the presence of pyridoxine (vitamin B6) and act by opening negatively charged chloride channels leading to it increase permeability

into the excited cell and thus, prevents it from second discharge (John, 2005) by binding to either

1) Ionotropic receptors ($GABA_A$ and $GABA_C$) that act by opening ligand gated chloride channel increasing chloride permeability that decrease calcium influx into the presynaptic neurone resulting into hyperpolarization and no discharge

2) Metabotropic receptor ($GABA_B$) which causes the opening ligand gated potassium channel increasing it efflux thereby decreasing calcium influx and thus, hyperpolarizing and inhibiting neuronal conduction (Jentsch *et al*., 2002).

$GABA_A$ receptor is agonised by muscimol and antagonised by bicuculline and picrotoxin while $GABA_B$ receptors are agonised by baclofen and antagonised by 2-OH Saclofen. $GABA_A$ receptor also possesses binding sites for compounds that allosterically modify chloride channel gating GABA such as benzodiazepines and barbiturates (Diniz *et al.*, 2015).

Glycine

This is another inhibitory neurotransmitter that is predominantly used in spinal cord and brainstem. Binding of glycine to its receptors opens intrinsic anion channels where in adult brain it leads to chloride ion influx resulting to hyper polarization and inhibition of neuronal activity. Early in development both glycine and GABA induce strong depolarizing response that can cause calcium influx via voltage gated calcium channels and thus, triggers transmitter release (Reichling *et al*., 1994).

Glycine receptors are activated by analine and taurine in addition to glycine itself but selectively antagonized by strychnine. Low concentrations of zinc, ethanol and isoflurane anaesthetics are found to potentiate glycine's affirnity.

Glutamate

Glutamate is the principal excitatory neurotransmitter in all rapidly conducting pathways of the brain. It is synthesized from glutamine by glutaminase (deactivated to glutamine by glutamine

synthetase) which when release due to arrival of action potential binds to its receptors to facilitate sodium and calcium influx and efflux of potassium ions from neuronal cell resulting into fast /prolonged neuronal excitation (Bienvenu *et al*., 2002). Frequent/sustain activation of glutamate receptors causes hyperexcitability and thus, increase the risk of seizure occurance (John *et al*., 2005).

1) Ionotropic Glutamate receptors include N-Methyl-D-Aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and Kainate subtypes. Both AMPA and Kainate (which are agonised by domoic acid) subtypes mediates fast excitatory signalling as they exhibit rapid activation and desensitization. NMDA receptors play a more modulatory role as their activation requires concurrent glutamate binding and membrane depolarization resulting to slower and lasting excitations. NMDA receptors channels are highly permeable to calcium ions in addition to sodium and potassium and are antagonised by dizocilpine and 2-amino-5- phosphonovalerate (Aamodi and Constantine, 1999)

2) Metabotropic Glutamate receptors are 8 in number and include $_{M}GluR_{1-8}$ which when activated causes the binding of alpha subunit with ion channel increasing ion influx and producing postsynaptic potential or the alpha subunit activate enzymes (like adenylyl cyclase) that produces second messenger which then opens calcium ion channel by cAMP or potassium channel by phosphoinositide hydrolysis (inositol triphosphate and diacylglycerol) resulting into ion influx and development of postsynaptic potential (Riveri *et al*., 2012). Post synaptic activation of phosphoinositide hydrolysis receptors causes an increase in intrinsic excitability of principal neurones particularly those of hippocampus mainly by down modulation of voltage gated potassium channels and therefore activation is likely to promote seizure activity. Conversely, presynaptic activation of group 2 tends to depress excitatory synaptic transmission by inhibiting glutamate release and therefore there activation is likely to inhibit seizure activity (Aamodi and Constantine, 1999).

Epilepsy and Oxidative Stress

Recent evidence implicated an important role for oxidative stress in the pathophysiology of neurological disorders including Epilepsy. Thus, a number of insults that make brain prone to seizure such as trauma can produce oxidative stress and mitochondrial dysfunction (Zaeri and Emamghoreishi, 2015).

Oxidative damage to susceptible targets protein, lipids, and DNA, caused by persistent seizures (status epilepticus) contribute to significant mitochondrial dysfunction, harming neuronal excitability through electron transport chain (ETC) dysfunction, changes redox potential and decreases adenosine triphosphate (ATP) level, which can lead to a collapse in brain energy production and supply (Kurosinski and Götz, 2002; Uttara *et al.*, 2009).

Several studies have suggested that reactive oxygen species (ROS) may increase cellular excitability and result in abnormal neuronal discharge by inhibiting Na^+/ K^+-ATPase pumps. Accordingly, it seems that inhibition of free radicals by antioxidants either directly or indirectly by increasing levels/activities of glutathione (GSH), GSH peroxidase, superoxide dismutase (SOD) or catalase could prevent abnormal neuronal discharge and in turn generation of seizure (Zaeri and Emamghoreishi, 2015)

Seizure activity during epilepsy decreases the antioxidant defence mechanism in the brain and increases the amount of free radicals, which further induces the oxidative stress that is involved in causation of lipid peroxidation, brain oedema, epilepsy, coma and death (Diniz *et al*., 2015)

2.2.0 Classification of Epilepsy

2.2.1 Base on origin

1) Partial otherwise called focal or local seizures are the result of an abnormal electrical discharge in a specific part of the brain that is originate from a single location within one cerebral hemisphere which may or may not spread to other brain areas. It account for about 80% of adult epilepsies. These can be

- Simple which may be associated with motor, sensory, psychic or autonomic symptoms but typically consciousness is preserved
- Complex also known as psychomotor seizures characterised by initial subjective feeling (aura), automatism with decrease or loss of consciousness. It originate preferentially in temporal lobe structures.
- Partial seizures evolving secondary to Generalised: is a type of partial seizure that later spread to the whole cortex and thus, become generalized (EITF, 2015).

2) Generalized seizures: this type of seizure arises from sub cortical structures (brainstem or thalamus) and project to both cerebral hemispheres hence, occurs due to widespread seizure activity in the brain. It further divided base on behavioural effect into

- Absence formerly known as petit mal which occurs more often in children and associated with interruption of consciousness (the patient experiences vacant/unresponsive) for usually 30 seconds with slight muscle twitch. It could also be typical which is characterized by sudden brief momentary loss of consciousness without loss of postural control or atypical where the period of unconsciousness is

longer with slight loss of postural control. It can recur numerous times in a day. The possibility of absence seizures should be considered in kids who have difficulty paying attention (EITF, 2015).

- Tonic clonic formerly known as grand mal is the main type of generalized seizure that occurs in approximately 10% of epileptics. It involves an initial contraction (tonic phase) like tongue biting, apnea, followed by a rhythmic contraction (clonic phase), muscle flaccidity, excessive salivation and noisy breathing due partial obstruction of airways are among its post-ictal characteristics (Berg *et al.,* 2010).
- Tonic seizures involves tonic contraction of the face, neck, axial or appendicular muscles and may involve extension/ flexion of the muscles. It often occur out of sleep and may lead to injuries (Leslie and Jerry, 2011).
- Clonic seizure is characterized by repetitive rhythmic clonic jerks with impairment of consciousness (Leslie and Jerry, 2011).
- Myoclonic seizures: they are characterized as sudden, brief, shock-like seizures. They can affect facial or torso muscles, extremities, muscle group or isolated muscles, and can be generalized, occurring isolated or repeatedly. Myoclonic seizures commonly occur after sleep deprivation, waking or sleeping (Moranhao *et al*., 2011).
- Atonic also called drop attack is characterized by sudden loss of postural muscle tone leading to falling. Consciousness is briefly impaired with injuries particularly to the face (Leslie and Jerry, 2011).

2.2.2 Base on aetiology

The aetiology of epilepsy is not well understood but genetic, physical and metabolic causes have been attributed.

- Idiopathic- epilepsy having a predominantly or presumably genetic cause with no significant neuroanatomical or neuropathological anomalies. Mutation in some genes

encoding voltage-gated Na^+ and K^+ channel respectively plays an important role in molecular pathogenesis of some kinds of epilepsy (Shorvon, 2011; Soltani *et al*., 2016).

- Symptomatic- Physical causes such as trauma, stroke, infection and tumours are also involved in the aetiology of symptomatic epilepsy (Soltani *et al*., 2016).
- Important metabolic causes result in reduction of oxygen supply in blood and mitochondrial disorders which can leads to the lack of ATP needs for cellular metabolism (Soltani *et al*., 2016).
- A known mechanism involved in pathogenesis of seizure relate2wd to nutritional status, is the imbalance between free radicals and antioxidant agents (Soltani *et al*., 2016)
- Other aetiological category like autoimmune is currently being proposed (EITF, 2015).

Continuous generalized seizures (Status Epilepticus)

Status Epilepticus is an emergency condition that makes up about 1% visit to the emergency department occurring in up to 40 per 100,000 people per year. It denotes a very prolonged seizure for more than 5 minutes or series of seizures occurring so frequently within 5 minutes such that full recovery of brain function does not occur inter-ictally hence, consciousness does 9not return between seizures for more than 30 minutes. It could be convulsive or nonconvulsive base on the presence of rhythmic jerking of the extremities (Al Mufti and Claasen, 2014).

It may be life threatening with development of pyrexia, deepening coma, circulatory collapse and death in 5-10% cases with 10 to 30% dying within 30 days. This condition may occur in those with a history of epilepsy as well as those with an underlying problem of the brain like frontal lobe lesion as in strokes, head injuries, substance withdrawal or intoxication, metabolic disturbances (low blood sugar). Movement disorders, meningitis and delirium are among other conditions that may appear to be Status Epilepticus (Moghaddasi *et al*., 2015).

Diagnosis often involves checking the blood sugar, imaging of the head, a number of blood tests and an electroencephalogram. Immediate suppression of convulsions: diazepam 2-4 mg/min IV to a total dose of 10-15 mg with monitoring of Vital signs. Initiation or reloading with anticonvulsants: slow infusion of phenytoin 15-20 mg/kg (25-50 mg/min, diluted in normal saline). If convulsions continued, the patient is considered to have refractory SE; then Midazolam 0.2 mg/kg loading dose followed by infusion at 0.1-0.4 mg/kg/h or Propofol 2 mg/kg/h were administrated; and if convulsions persist after several hours, Valproate or Phenobarbital 10 mg/min to total dose of 20 mg/kg should be administered intravenously. Alternatively, Carbamazepine or Levetiracetam be administered via nasogastric tube if bowel activity present, and if convulsions persist, neuromuscular paralysis must be done by any means possible. Intubation may also be required to help maintain the patient's airway (*Al-Mufti and Claasen, 2014;* Moghaddasi *et al*., 2015).

Refractory Status Epilepticus is a condition in which ongoing seizures fails to respond to first and second line anti convulsant drug therapy and account for about 30-43% cases of SE. Super Refractory Status Epilepticus fails to even third line anti convulsant drug therapy (Al Mufti and Claasen, 2014).

2.3.0 Diagnosis and Investigation

2.3.1 Diagnosis

According to International League Against Epilepsy (ILAE) 2005 the criteria for diagnosis include

1) At least 2 unprovoked (reflex) seizures occurring greater than 24 hours apart
2) An unprovoked and a probability of further seizure similar to the general recurrence risk (at 60%) after 2 unprovoked seizures occurring over the next 10years.

3) Diagnosis of an epilepsy syndrome (Fischer *et al.*, 2014)

In partial seizures, differential diagnosis should be performed with transient ischemic attack, aggressiveness and panic attacks. In generalized seizures, differential diagnosis should be done with syncope, cardiac arrhythmias, brainstem ischemia and pseudoepilepsia (due to psychiatric illness) (Maranhao *et al*., 2011).

2.3.2 Investigation of Epilepsy

Despite the fact that the diagnosis of epilepsy is largely based on clinical data, the evaluation of patients with suspected seizures requires the use of investigative modalities such as electroencephalography and neuroimaging facilities.

2.3.2.1 Electroencephalography (EEG)

This is a technique use in studying the electrical activity of the brain and a functional clinical tool for assessing disorders of cerebral function thus, plays a vital role in formulating prognosis. Epileptiform EEG changes are essential in the diagnosis and therapeutic management in the epileptic patient. EEG is a simple and inexpensive diagnostic method that determines the physiological manifestations of abnormal cortical excitability associated with epilepsy (Maranhao *et al*., 2011).

The EEG shows three types of information: confirm abnormal electrical activity, seizure focus and the type of seizures. It also indirectly help in the selection of appropriate antiepileptic drugs. However, EEG analyzes only the superficial layers of the cerebral cortex and short duration of routine EEG (Maronhao *et al*., 2011).

Preferably, EEG should be performed during sleep, light stimulation and hyperventilation because certain types of seizures occur in these conditions (Maronhao *et al*., 2011). Depth, nasopharyngeal and sphenoidal electrodes are also used to facilitate the demonstration of EPs due to temporal lobe abnormalities. In patients with unclassifiable seizures, video recording of

attacks with simultaneous EEG recordings are employed to facilitate direct observation of seizures (Olubunmi, 2006).

2.3.2.2 Computerized Tomography

It is very useful in the detection of the commonest structural abnormalities demonstrated in epileptics (cerebral atrophy, Hydrocephalus, infarct, cerebral tumours and vascular lesions) and determination of the exact location of such lesions despite high incidence of abnormal findings reported among those with partial seizures (Maronhao *et al*., 2011).

2.3.2.3 Magnetic Resonance Imaging (MRI) and MR spectroscopy

These techniques are more sensitive and accurate than CT in the detection of structural lesions, demyelinating diseases, many white matter disorders and pre-surgical localization. Cerebral angiography retains an important place in the investigation of intracranial tumours and vascular lesions. Biomagnetic imaging, however, using biomagnetometer provides information about the body's electrical activity, and is entirely non-invasive and can map the sources of electrical activity in three dimensions with resolution. It is of potential in the diagnosis of epilepsy (Maronhao *et al*., 2011).

2.4.0 Treatment of Epilepsy

The treatment of epilepsy can be divided into 3 main categories: the use of antiepileptic medications, surgical measures and behavioural treatment

2.4.1 Treatment gap

Antiepileptic drugs (AEDs) commonly called Anticonvulsants are a diverse group of pharmacological agents used in the treatment of epileptic seizures. 70% of children and adults with epilepsy can be successfully treated (seizures completely controlled) with antiepileptic drugs (AEDs). Nevertheless, around 30% of patients with epilepsy remain resistant to AEDs (both mono- and combinatorial therapy). Despite In low- and middle-income countries where

80% cases of epilepsy are reported 75% of it patients do not have access to these AEDs due to their low availability, lack of awareness and poverty (WHO, 2015).

Several antiepileptic medications are employed to manage epilepsy. However, they exhibit serious side effects (depression, ischemia, impaired cognition and motor disability), dose- and duration-related toxicity (teratogenicity, neurotoxicity and hepato-toxicity) and predisposes the patient to the risk of drug interactions with concomitant administration of other drugs (Dhande *et al*., 2009).

Approximately 10% of the population have a single convulsive episode during their lifetime (Engel, 2001). In this situation, the use of anticonvulsants is not indicated. After the occurrence of a second seizure, the diagnosis of epilepsy is confirmed, initiating then the routine treatment with antiepileptic drugs. The choice of the anticonvulsant should be made according to the type of crisis, effectiveness, side effects and used as monotherapy whenever possible since the goal of pharmacological treatment is to control seizures without side effects. It is important to emphasize that most of the adverse effects of antiepileptic drugs is dose-dependent hence, decreasing the dose of the drug reduces the severity of side effects, with no need to discontinue the drug. The appropriate prescription of anticonvulsants requires knowledge of the pharmacokinetics of the drug. Once the pharmacological treatment is established anticonvulsant can only achieve metabolic balance after a period of at least four half-lives which is the time required to obtain tissue saturation (Maronhao *et al*., 2011).

2.4.2 Initiation of Antiepileptic Drugs

Monotherapy is the most desirable as it decreases the likelihood of adverse effects, avoids the risk of drug interactions and less expensive (David *et al*., 2014) hence, the pharmacological treatment of epilepsy starts with the administration of a single antiepileptic drug (AED) suitable for the specific epilepsy syndrome. Selection of the appropriate AED is based on its efficacy, tolerability and safety according to the type of seizure. Treatment with AED in patients with

epilepsy aims to provide the best quality of life with no seizures and fewest adverse effects from treatment. Decision to initiate AED in patients with newly diagnosed epilepsy (including patients not currently on AED) should be based on the discussion between the physician and the patient (or legal guardian) which should include:

• Risk of seizure recurrence • Cost of treatment • Type of seizures • Potential duration of treatment • Type of epilepsy and it natural course • Negative effects of seizures • AED options • Goals of treatment (EITF, 2015).

Choice of Antiepileptic Drugs depends on multiple factors including

• Patient specific variables: age, gender, co-medications, co-morbidities, affordability, and compliance (ability to swallow pills/tablets). • AED specific variables: seizure or epilepsy syndrome, adverse effects, ease and speed of drug initiation, teratogenicity, interactions, pharmacokinetics and availability (EITF, 2015).

2.4.3 Antiepileptic Drugs to be avoided or used with caution

1. Absence seizures: Carbamazepine, Oxcarbazepine, Phenytoin, and Gabapentin (avoided) may precipitate or aggravate the seizure.

2. Myoclonic seizures/Juvenile Myoclonic Epilepsy: Carbamazepine, Oxcarbazepine and Phenytoin (avoided) as they may worsen them.

3. Children less than 1 year of age: Valproic acid (avoided) due to teratogenicity, oedema, encephalopathy and hepatotoxicity.

4. Children 1-2 years: Valproic acid (use with caution due to hepatotoxicity). This risk is high in combination with phenobarbital.

5. Women of child bearing age group: Valproic acid (avoided due to teratogenicity) (EITF, 2015)

2.4.4 General principles of Antiepileptic Drugs Treatment

1. It is recommended that epileptic patients should be treated with a single AED (monotherapy) whenever possible.

2. If the initial treatment is unsuccessful, then polytherapy using another drug or add-on treatment with a second drug can be tried. Caution is needed during the changeover period. If an AED has failed because of adverse effects or continued seizures, a second drug should be started (which may be an alternative first-line or second-line drug) and built up to an adequate or maximum tolerated dose and then the first drug may be tapered off slowly.

3. If the second drug is unhelpful, either the first or second drug may be tapered, depending on relative efficacy, side effects and how well the drugs are tolerated before starting another drug. Some patients are required to be on more than 2 AEDs.

4. It is recommended that combination therapy should only be considered when attempts at monotherapy with the tolerated dose of AED have not resulted in seizure freedom.

5 If trials of combination therapy do not bring about worthwhile benefits, treatment should revert to the regimen (monotherapy or combination therapy) that has proved most acceptable to the patient in terms of providing the best balance between effectiveness in reducing seizure frequency and tolerability of side effects. AED interactions and comorbidities should be taken into consideration when choosing combination therapy.

6. If there is no improvement after two adequate trials of AEDs, the patient should be referred for epilepsy surgery evaluation (EITF, 2015).

2.4.5 Mechanism of action of Antiepileptic Drugs

The strategies for the development of antiepileptic drugs have heavily relied on the basic premise that epilepsies are due to an imbalance between excitatory and inhibitory transmission in the brain (Tanko *et al*., 2012). Generally, currently available AEDs exert their effect by antagonizing sodium (Na+) or calcium (Ca2+) channels, facilitating GABAergic inhibition or reducing glutamatergic neurotransmission.

AEDs are classified base on the mechanism they Act into

1) Sodium channel blockers like carbamazepine, phenytoin, lamotrigine.
2) Activators of slow Sodium channel blockers like rufinamide, ladosamide
3) Glutamate blockers- Although inhibition of AMPA, NMDA, Kannate receptors are not use for developing AEDs because such compounds have been associated with unacceptable side effects for clinical use. Example, NMDA Blockers influence memory, cognition and learning impairment However, felbamate, remacemide (NMDA blockers), low dose phenobarbital, topiramate, perampanel (AMPA blockers) are used (Surges *et al*., 2008).
4) Calcium channel blockers- calcium channels regulate neuronal excitability and neurotransmitter release. The voltage gated calcium channel expressed in the brain exist in three known forms in the human brain L, N and T types. In resting state influx of calcium current produces partial rapid depolarization of membrane that facilitates development of action potential. L-type is a high voltage channels activated by significant depolarization are mainly expressed post synaptically and permit sustain calcium influx as they are slowly deactivated following depolarization. N-type is also a high voltage channels calcium influx that triggers exocytosis of presynaptic vesicles. that is expressed at synaptic boutons where it mediate calcium influx necessary for transmitter release, these channels rapidly closes resulting in brief Low voltage activated calcium current T-type which opens by relatively small depolarization, rapidly deactivate are responsible for rhythmic thalamo-cortical

spikes wave patterns of generalized absence seizure example valporoate, ethosuximide bind and stabilize the inactivated T-channels to inhibit the slow depolarizations while carbamazepine antagonize L-type channels (Surges *et al.*, 2008; David *et al.*, 2015).

5) GABA Activators/enhancers- AEDs that enhance GABA system act via binding directly to GABA receptors to mediate chloride influx (pre synaptic inhibition), Increase GABA synthesis, Inhibiting GABA transaminase (it metabolic enzyme) or Block GABA pre synaptic reuptake leading to hyperpolarization of neuronal cells resulting into inhibition of neuronal transmission example phenytoin, benzodiazepine.

6) Carbonic anhydrase inhibitors- inhibition of Carbonic anhydrase increase intra cellular concentration of hydrogen ion, decrease pH and consequently shift potassium ions to ECF to buffer the acid-base status resulting into hyperpolarization that increases seizure threshold of the cells. Acetazolamide has been used as an adjunct therapy in refractory seizure with catamenial pattern (seizures clustering around menstrual period) other examples include zonisamide, topiramate (David *et al*., 2015).

2.4.6 Pharmacology of major anticonvulsants, mechanism of action and adverse effects

Carbamazepine (CBZ), Phenytoin (PHE), Valpoaric acid (VPA) and Phenobarbitone were the first-line antiepileptic drugs commonly used because of their efficacy and low cost (Bialer and Wheeler, 2010). These drugs induce hepatic microsomal enzymes, cytochrome P450 (CYPs) thereby complicating the use of multiple antiseizure drugs as well as impacting metabolism of oral contraceptives, warfarin and many other drugs. They also enhance metabolism of endogenous compounds including gonadal steroids and vitamin D (Abubakar *et al*., 2013)

Carbamazepine: stabilizes voltage-gated sodium channels (VGSCs), minimizes VGSCs in the rest status subsequently to be excited, and reduce polysynaptic responses to block post-tetanic

potentiation. These actions make CBZ a widely used AED for partial and secondary generalized seizures. Additionally, CBZ's structure is similar to that of the tricyclic anti-depressants and a function of CBZ is a GABA receptor agonist. These may partially explain the effects of CBZ on bipolar disorder and the treatment of pain in trigeminal neuralgia. CBZ may cause several adverse effects, including sedation, ataxia, dizziness, nausea, vomiting, constipation, diarrhea, interference with the metabolism of lipids and sex hormones, hyponatremia, weight-gain, anemia (Fan *et al.*, 2016).

In addition to accelerating its own metabolism, carbamazepine exacerbates hepatic oxidation and conjugation of other liposoluble drugs especially oral contraceptives, anticoagulants and antipsychotic drugs. Drugs that inhibit the metabolism of carbamazepine may increase its toxicity and include cimetidine, verapamil, erythromycin and isoniazid (Suleiman *et al.*, 2015).

Oxcarbazepine- Trileptal®, Apydan®: is a keto-analogue of carbamazepine, with a similar spectrum of anticonvulsive activity and efficacy.it blocks voltage-gated sodium channels, potentiates potassium conduction and inhibits calcium channels and N-methyl- D-aspartate (NMDA) receptors. It is generally well tolerated except by patients with myoclonic and absence epilepsy, which can be worsened under oxcarbazepine treatment. It most common adverse effects are sedation, amnesia, ataxia, depression, insomnia, anxiety, diarrhea, and hyponatremia (Due to its effect on antidiuretic hormone in the distal convoluted tubule of the kidney) (Gschwind and Seeck, 2016).

Phenytoin (5, 5-Diphenyl-Imidazolidine-2, 4-Dione**)** inhibits GABA and glutamate transport, reduces calcium influx into neurons to decrease the release of neurotransmitters, and reduces synaptic post-tetanic potentiation, and excitatory synaptic transmission to stop the cortical abnormal current propagation. Moreover, PHE can bind to and stabilize the inactive VGSCs.

VGSCs are highly conserved and responsible for the upstroke of the action potentials in neurons involving the propagation of the electrical impulse in the CNS, PNS, cardiovascular and skeletal muscle tissue. After binding, PHE prevents further generation of action potentials, which initiate seizures. These mechanisms may significantly prevent generalized tonic-clonic seizures, complex partial seizures, and status epilepticus, but not absence seizures. It side effects include nystagmus, diplopia, gingival hyperplasia, depression, hyperglycemia, hepatotoxicity, hirsutism and teratogenicity. Phenytoin can induce the oxidative metabolism of several liposoluble drugs like carbamazepine, valproic acid, ethosuximide, corticosteroids and anticoagulants (Fan *et al*., 2016).

Phosphofenitoin: a prodrug of phenytoin with the same pharmacological properties. Its main indication is the replacement of intravenous phenytoin in the treatment of status epilepticus.

Phenobarbital: a barbiturate considered as a second-line treatment and effective against all epilepsies except generalized non-convulsive type. Phenobarbital exerts its antiepileptic effect by modulating the postsynaptic action of gamma aminobutyric acid (GABA) as well as blocking postsynaptic excitatory effect induced by glutamate especially those mediated by the activation of the AMPA (α-amino-3-hydroxy- 5-methyl-4-isoxazolepropionic acid) receptor. Despite the low cost, it presents both cognitive and behavioural effects which limit it use. Some of it adverse effects are sedation, depression, hyperactivity (in children), confusion (in elderly), Congenital defects (with chronic use in pregnancy) dependence, tolerance and low therapeutic index. Interaction of phenobarbital with other drugs can exacerbate hepatic metabolism of many liposoluble drugs including anticonvulsants, oral contraceptives, beta-blockers, corticosteroids, digoxin, anti-inflammatory drugs and anticoagulants. It may also increase the

biotransformation and toxicity of inhaled anaesthetics which may persist for 30 days after it discontinuation (Maranhao *et al*., 2011).

Valproic acid: a carboxylic acid (2-propylpentanoic acid) affects the GABAergic system, inhibits _-ketoglutarate dehydrogenase (KGD), GABA transaminase (GABA-T), and succinate semialdehyde dehydrogenase (SSD), and enhances glutamate decarboxylase (GAD) to elevate GABA levels in plasma and in several brain regions. Consequently, VPA may affect cerebral metabolism, activate GABA receptors to block sodium channels, and modulate calcium and potassium conductance and dopaminergic and serotoninergic transmission. These mechanisms make VPA a multi-functional medication for absence, partial, and tonic-clonic seizures, bipolar disorder, depression, migraine, personality disorders or mental retardation, dementia and cognitive problems, and a potential chemotherapeutic agent. It adverse effects include Hepatotoxicity, pancreatitis, teratogenicity, and endocrine disturbance, such as menstrual abnormalities, increased total testosterone levels, teratogenicity, obesity, and polycystic ovary syndrome (Fan *et al*., 2016).

2.4.7 Surgical treatment

In cases of pharmacoresistant epilepsy (which should be considered early in the disease, when there is a lack of response to the first antiepileptic drug, since only 14% of those will respond to a second drug, and only 2% to a third drug if the second fails too) where patients are at high risk of additional morbidity and mortality surgical procedures such as vagal nerve stimulation and deep brain stimulation in the anterior thalamic nuclei are employed (Gschwind and Seeck, 2016).

2.4.8 Behavioural Treatment

Antioxidant

Antioxidants play a major role in protection against molecular oxidative damage and may be relevant in epilepsy treatment since free radical generation can induce seizure activity by direct inactivation of glutamine synthase, thereby permitting it abnormal build-up. Flavonoids are structurally similar to benzodiazepines and exert antiepileptic activity by modulating GABAA-Cl-channel complex. The onset of oxygen-induced convulsions in animals is correlated with a decrease in the brain cortex content of neurotransmitter GABA because of the inhibition of enzyme glutamate decarboxylase by oxygen free radicals (Diniz *et al*., 2015).

Induced seizures may be partially prevented with treatment using antioxidant substances, such as SOD mimetics, melatonin e vitamins A, C. and E. Investigation on the role of RNA oxidation in epileptogenesis using pilocarpine to induce SE, reported a significant increase in RNA oxidation in vulnerable neurons in rat brains immediately after SE followed by neuronal death. However, a daily supplement of antioxidants (coenzyme Q10) significantly reduced RNA oxidation and protected rats from SE and neuronal loss. Hence, there is evidence that suggests that antioxidant therapy may reduce lesions induced by oxidants in some animal seizure models (Kurosinski and Götz, 2002).

Flavonoids exerts a multiplicity of neuroprotective actions within the brain, including a potential to protect neuron against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning and cognitive function. These effects appear to be underpinned by two common processes. Firstly, they interact with critical protein and lipid kinase signalling cascade in the brain leading to an inhibition of apoptosis triggered by neurotoxic species and to a promotion of neuronal survival and synaptic plasticity. Secondly, they induce beneficial effects on the vascular system leading to changes in cerebrovascular blood flow capable of causing angiogenesis, neurogenesis and changes in neuronal morphology. Through these mechanisms, the consumption of flavonoid-rich foods throughout life holds the potential to limit neuro-degeneration and to prevent or reverse age-

dependent loses in cognitive performance. The intense interest in the development of drugs capable of enhancing brain functions means that flavonoids may represent important precursor molecules in the quest to develop of a new generation of brain enhancing drugs (Vauzour *et al.*, 2008).

Fatty Acids

Fatty acids mainly of the polyunsaturated class (PUFAs) such as docosahexanoic acid, arachidonic acid, eicosapentanoic acid were found to inhibit fast voltage gated sodium channels, L-type Calcium channels and can activate potassium channels thereby dampening neuronal excitability. PUFAs have to property to induce the expression of mitochondrial uncoupling protein (UCPs) thereby uncouples the electron transport from ATP production as well as inhibits the production of reactive oxygen species (ROS) (Sancheti *et al*., 2013).

Gluten free Diet

Gluten free diet have been shown to reduce glutamate activation particularly by blocking AMPA receptors which are present on motor cortex thereby decreasing cortical hyperexcitability. Thus, gluten free diet are thought to produce anticonvulsant effect by modulating GABAergic neurotransmission. Also, studies have shown that the efficacy of gluten free is inversely proportional to the duration of epilepsy and the age of patient at the start of the diet (Bella *et al.*, 2015).

Ketogenic Diet

Ketogenic diet is one of the oldest forms of medical treatment for epilepsy. It is a high fat, adequate protein, low carbohydrate diet with different versions available, but of the same basic principles. KD leads to rise in plasma ketone bodies which play a helpful role in lowering the excitability of neurons and modifying seizure threshold. Moreover, ketone bodies can alter the amount of fluid, electrolytes and lipids intake on the way to help control of seizure Attacks (Soltani *et al*., 2016). Main proposed mechanisms include (1) increased GABA synthesis; (2)

increased adenosine-mediated neuronal inhibition; (3) increased activity of ATP-sensitive potassium channels leading to membrane hyperpolarization; and (4) reduced uptake of glutamate by synaptic vesicles resulting in reduced synaptic glutamate release (Klein *et al*., 2014)

Vitamins

Supplementation with vitamins especially Thiamine (B1), Pyridoxine (B6), Folic acid (B9), Vitamin D, Vitamin E and Vitamin K have been reported to help prevent, reduce seizure frequencies and reverse seizures induced by their severe deficiencies and improve other aspects of health in patients with epilepsy (Asif, 2013).

2.5 Prevention of Epilepsy

Idiopathic epilepsies are not preventable. However, preventive measures can be applied to the known causes of epilepsy. Preventing head injury is the most effective way to prevent post-traumatic epilepsy. Adequate perinatal care can reduce new cases of epilepsy caused by birth injury. Lowering body temperature of a feverish child can reduce the chance of febrile seizures. Elimination of parasites in tropical areas can reduce Central nervous system infections thereby preventing the occurrence of epilepsy (Megiddo *et al*., 2016).

2.5.1 Human Rights

Imposing Legislation based on internationally accepted human rights standards can prevent discrimination, stigmatization and rights violations as well as improving access to health-care services raising the quality of life for people with epilepsy (WHO, 2015). Proper diagnosis, patient and family education and timely referrals are among other Quality epilepsy care (Fountain *et al.*, 2015).

2.5.2 First Aid Treatment of Epilepsy

This provides a step wise quick guide to help epileptic patient under attack

1. Stay calm.
2. Ensure that the surrounding is danger free by keeping harmful objects away from them.
3. Don't hold them down or put anything in their mouth
4. Cushion their head with soft cloth if collapsed to the ground.
5. Note the duration of the seizure.
6. Stay with them until they are fully recovered. If they are injured, have breathing difficulties, doesn't stop after 5 minutes or have another seizure without recovering fully from the first seizure, call for an ambulance (Chesham *et al.,* 2015).
7. Put the patient by his side for draining oral discharge.

2.6.0 Animal models used to assess anticonvulsant activity

Because different models could simulate dissimilar kinds of seizures or epilepsy, selecting the appropriate model is a key factor in AED screening. Prediction of a drug's or any agent's efficacy on epilepsy cannot rely on one chronic model, but rather a battery of models should be used. The screening of antiepileptic drugs is based mainly on two types of models; acute and chronic seizure models. Maximal electroshock test (MEST) and Pentylenetetrazole (PTZ) injection model among others are commonly used acute seizure models whereas the kindling model is considered as chronic model primarily used for evaluating the test drugs for antiepileptogenic activity (Ishola *et al.,* 2015).

2.6.1 Maximal electroshock seizure test is a predictive animal tool to study anticonvulsant efficacy and has been documented as a major end point used to assess the antiepileptic drugs (AEDs) effective potentials against generalized tonic-clonic seizures (Danjuma *et al*., 2009).

MES-induced convulsion model causes facilitation of Ca^{2+} and Na^{+} into the cells, and the blockade of them can prevent MES-induced tonic extension. Potentiation of GABA receptor may offer protection against MES-induced seizures. Seizures are induced in animals (e.g. rats and chicks) by delivering electroshock at specific current, voltage and frequency by means of a convulsiometer through a pair of ear clip or corneal electrodes. All established antiepileptic drugs have anticonvulsant activity in at least maximal electroshock seizure (MES) model.

MES-induced seizure can be prevented either by drugs that inhibit voltage-dependent Na^{+} channels such as phenytoin, valproate, felbamate, and lamotrigine or by drugs that block glutamatergic receptor such as felbamate. Many neurotransmitter systems other than GABA, such as norepinephrine, dopamine, and serotonin (5-HT), are related to the occurrence of electroshock seizure. The ability of an intervention to inhibit the HLTE in MES as compared to currently available antiepileptic drugs that are clinically effective in the management of generalized tonic–clonic and partial seizures, such as carbamazepine, phenytoin and clonazepam in this model suggests anticonvulsant activity (Ishola *et al.*, 2015).

2.6.2 Pentylenetetrazole (PTZ) the most popular chemo-convulsant used for evaluating antiepileptic drugs is a GABAA receptor antagonist and a well-established model to assess efficacy of drugs against generalized myoclonic and absence seizures in humans. PTZ causes seizure through the activation of glutamate receptors (NMDA) and stimulation of the calcium ions influx into the nerve cells. Accordingly, drugs that block glutamatergic excitation mediated by NMDA receptor such as felbamate, reduce T-type calcium currents, enhance GABAA receptor mediated neurotransmission such as benzodiazepines (e.g. diazepam) and phenobarbitone (Kenechukwu *et al*., 2012) have anticonvulsant activity against PTZ-induced seizures.

The anticonvulsant activity of an intervention against PTZ-induced seizures raises seizure threshold in the brain by modifying excitatory and inhibitory neurotransmission through effects on voltage gated ion channels, $GABA_A$ receptors and glutamate mediated excitatory neurotransmission (Tanko *et al*., 2012; Zaeri and Emamghoreishi, 2015)

2.6.3 Kindling is a repeated chemical (e.g. subconvulsant doses of PTZ, cocaine or carbomylcholine) or electrical (supramaximal or minimal threshold and psychomotor electroshock) stimulation of the brain that persistently reduce seizure threshold and eventually leads to the occurrence of seizures (Morimoto *et al.*, 2004) and a widely accepted chronic animal model of epilepsy (Lekha *et al*., 2014).

Kindling model has been widely studied both as a tool for understanding chronic epileptogenesis and as a model for testing AEDs with a potential of treating complex partial seizures (Diniz *et al*., 2015).

2.7 Description of *Pennisetum glaucum*

Pennisetum glaucum is an erect annual grass that is about 0.5- 4m high with profuse root system. It culms are slender and about 1.3cm wide with alternate, simple, blade, linear, pubescent and minutely serrated leaves reaching 1.5cm long and 8cm wide. It fruits are grains whole shape differ according to cultivars (usually ovoid) nearly white, pale yellow, brown or purple in colour (Odusola *et al*., 2013; Hauze *et al.,* 2015).

2.7.1 Classification of *Pennisetum glaucum*

Kingdom Plantae

Subkingdom Tracheobionta (vascular plant)

Super division Spermatophyte (seed plant)

Division Magnoliophyta (flowering plant)

Class Liliopsida (monocotyledon)

Sub class	Commelinidae	
Order	Cyperales	
Family	Poaceae (grass family)	
Genus	Pennisetum (fountain grass)	
Species	*glaucum*	(Hauze *et al*., 2015)



Plate 1: Picture of *Pennisetum glaucum* (snapped by Hafsat Muhammad Danmallam)

2.7.2 Constituents of *Pennisetum glaucum*

Pennisetum glaucum is a rich source of energy (361 Kcal/100g) consisting of proteins, fat, crude fibre, starch, ash (Thilagavathi *et al*., 2015). It also contains various essential micro nutrients needed by the body including Calcium, Phosphorous, Iron, Zinc, Sodium, Magnesium, Vitamin A, Thiamine, Riboflavin, Niacin and Folic acid (Nambiar *et al*., 2012). Essential Amino Acids present in *Pennisetum* glaucum include Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Valine, Histidine and Tryptophan while Alanine, Arginine, Aspartic acid, Cystine, Glutamic Acid, Glycine, Serine, Tyrosine, and Proline are among the Nonessential Amino Acid present in *Pennisetum glaucum* (Amadou *et al*., 2013)

Phospholipids which are useful in brain function, behavioural disorders and stress are among its constituents with up to 75% of it fatty acids been of polyunsaturated class including docosahexaenoic, eicosapentaenoic acid, Omega 6 and 9 (Nambiar *et al.*, 2011) with linoleic acid, oleic acid, palmitic acid, erucic acid, stearic acid, arachidic acid, linolenic acid and myristic acid among others (Chughtai *et al.,* 2015).

Volatile compounds such as Aldehydes, Benzene derivitves, Alcohols, Ketonic, hydrocarbon, Acidic, ester and Sulphur containing compounds have also been reported present in *Pennisetum glaucum* (Nambiar *et al*., 2012; Chughtai *et al.,* 2015).

It's a gluten free starch with high amylose, D ribose, maltose but low fructose and glucose (Pushparaj and Urooj, 2014; Finkelstein *et al.,* 2015). It has also been reported to contain oxalic acid which forms an insoluble complex with calcium thereby impairing it biological availability (Leder, 2004).

It qualitative phytoscreening showed the presence of tannins, saponins, flavonoid, terpenoid, glycosides, alkaloid, phenols, steroid, phytic acid, goitrogens and oxalic acid. It changes colour reversibly from grey to yellow-green at alkaline pH, and partially reversibly from grey to creamy white under acidic conditions due to the presence of phenolic compounds (Nambiar *et al*., 2012; Ndiku and Ngule, 2015).

2.7.3 Uses of *Pennisetum glaucum*

Pennisetum glaucum has been reported to possess several biological activities including anti-inflammatory, anti-oxidant, anti-mutagenic, anti-thrombotic, anti-allergic, anti-diabetic, anti-tumour and anti-leukemic (Daniel *et al*., 2012; Mahanty *et al.,* 2012).

Due to its high fibre content, it has being recommended for the treatment of severe constipation, stomach ulcers and weight loss (Nambiar *et al*., 2012).

Being gluten-free and having cholesterol lowering potential, *Pennisetum glaucum* offers an excellent option for people suffering from celiac diseases, atherosclerosis and diabetic heart diseases. Flavonoids modify the body's reaction to pathogens, allergens and carcinogens. They are powerful antioxidants with greater effects than vitamin C, vitamin E, selenium, and zinc

that offer protection against oxidative and free radical damage, inhibit the oxidation of LDL cholesterol (prevent atherosclerosis), reduce platelet aggregation (reduce ischemic damage) promote relaxation of cardiovascular smooth muscle (antihypertensive, antiarrhythmic effects) (Middleton *et al.*, 2000).

Industrially, millets are used as raw materials for the production of biscuits and confectioneries, beverages, weaning foods and beer while Traditional consumption forms are *Kunu-zaki, Koko* (pap), *Fura, Dambu* and *Burukutu* among others (Amadou *et al*., 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Animals

Twenty swiss albino mice (weighing 18-29g) and twenty wistar rats (weighing 180-200g) of both sexes used for the study were purchased from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria however, animals of the same sex were grouped together while forty day old chicks (weighing 33-40g) were purchased from National Animal Production and Research Institute (NAPRI) Shika, Zaria. All animals were fed on standard *pellets* of growers mash (vital feeds, Plateau state) and given access to water *ad libitum.* This research was carried out in Ahmadu Bello University Zaria, Nigeria.

3.2 Instruments

Some of the equipment used include Ugo basile electroconvulsive machine (model 7801), atomic absorption spectrophotometer, stop watch and weighing balance.

3.3 Chemical and Drugs

Pentylenetetrazole (Sigma-Aldrich, St. Louis, USA), Sodium valproate (Epilim) (Sanofi Fawdon Manufacturing Centre, United Kingdom), Diazepam (Laborate Pharmaceutical, India), Phenytoin (Dilantin) (Parke – Davis, Mumbai, India) were the chemical and drugs used.

All drugs and chemical were freshly prepared in normal saline to the desired concentration. Eighty-five mg of Pentylenetetrazole was dissolved in 1 ml of normal saline, 140 mg of Pentylenetetrazole in 4 ml of normal saline, one tablet of Sodium valproate (200mg) in 1 ml of normal saline, one tablet of Diazepam (10mg) in 5 ml of normal saline, 1 tablet of Phenytoin (20mg) in 1 ml of normal saline.

3.4 METHODOLOGY

3.4.1 Plant material

Pennisetum glaucum was purchased from Samaru market, Sabon Gari Local Government, Kaduna State, Nigeria, in June 2016 and was identified as such by Namadi Sunusi a taxonomist at Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where it was given the voucher number 1824.

3.4.2 Preparation of supplement

The identified *Pennisetum glaucum* was soaked in water for 12 hours in a container after the impurities (such as stones) were sorted, grounded into paste using grinding machine, sieved into another container using muslin cloth and the residue was discarded and the filtrate was allowed to settle down. The water floating on top of the sediment was then decanted in order to concentrate the sample which was put into muslin cloth and allowed to drain completely and thereafter dried under the sun into powder (Odusola *et al.*, 2013).

A portion of the powder was collected for the assessment of quantitative phytochemical and proximate analyses while the remaining was weighed and added to pellets grower mash (feed for mice and rat) and pelletised broiler finisher feed (for chicks) at 25% and 50% respectively.

3.4.3 Phytochemical screening of *Pennisetum glaucum*

3.4.3.1 *Test for Flavonnoids*

Ten grams of *Pennisetum glaucum* powder was extracted with 100 ml of 80% aqueous methanol at room temperature and filtered. The residue was transferred into a crucible and dried to a constant weight in an oven. The percentage flavonoids was calculated as

$$\frac{\text{Weight of dried residue}}{\text{Weigh of original sample}} \times 100$$ (Allen *et al*., 1973).

3.5.3.2 *Test for Alkaloids*

Two and a half grams of *Pennisetum glaucum* powder was weighed into a 250 ml beaker and 100 ml of 10% acetic acid in ethanol was added, covered, allowed to stand for 4 hours and then filtered. The filtrate was concentrated on a water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid which was dried and weighed (Harborne, 1973).

3.4.3.3 *Test for Tannins*

Two grams of *Pennisetum glaucum* powder was poured into a beaker containing 50 ml distilled water and heated at 60^0C. It was thereafter filtered and the residue was discarded. 10 ml of 4% copper acetate solution was added to the hot filtrate and boiled again for 10 minutes. The precipitate was filtered and the filtrate discarded. The residue was dried using filter paper and scraped into pre-weighed crucible and weighed. The residue containing crucible was

incinerated in a muffle furnance at 550 ^{0}C, cooled in a dessicator and weighed. The difference between the weight of sample before ashing and ashed residue after incineration represent the tannin content (Joslyn, 1970).

3.4.3.4 *Test for Saponins*

Two grams of *Pennisetum glaucum* power were folded in a filter paper and put in a thimble and extracted by refluxing in a soxhlet extractor. Extraction was done with acetone in a 250 cm^3 capacity round bottomed flask for 3 hours after which the apparatus was dismantled and another 150 cm^3 capacity flask containing methanol was fitted to the extractor and extraction sustained for another 3 hours. The weight of flask before and after the second extraction were recorded. Methanol was recovered by distillation after the second extraction and the flask oven dried, allowed to cool at room temperature and weighed. Saponin content was calculated as:

$$\frac{\text{Mass of flask and extract- mass of empty flask}}{\text{Mass of sample}} \times 100$$

(AOAC, 1990)

3.4.3.5 *Test for Phytate*

The method described by Wheeler and Ferrel 1971 was adopted. This method relies on the solubilisation of phytate by diluted acid and it subsequent precipitation with ferric ion. 4 g of *Pennisetum glaucum* powder was soaked in 100 ml of 2% HCl for 3 hours and then filtered. 25 ml of the filtrate were dispensed into a conical flask and 5 ml of 0.3% ammonium thiocyanate solution was added as indicator. Thereafter, 53.5 ml distilled water was added for proper acidity and then titrated with standard $FeCl_3$ solution until a brownish-yellow colour that persist for 5 minutes was obtained.

3.4.4 Proximate analysis of *Pennisetum glaucum* supplement

3. 4.4.1 *Test for Ash content*

Two grams of *Pennisetum glaucum* powder was added into a pre-weighed crucible and incinerated in muffle furnace at 600 °C.

$$\text{Ash (\%)} = \frac{W2-W3}{W2-W1} \times 100$$

Where W1 was the weight of cleaned, dried, ignited, and cooled crucible, W2- the weight of the crucible and *Pennisetum glaucum* powder after incinerating at 600 °C, and W3- the weight of the crucible and *Pennisetum glaucum* powder after cooling in an air tight homogenized vessel (Association of Analytical Chemists, 2005).

3. 4.4.2 *Test for Crude protein*

Ten mililitters of H_2SO_4 was added to 3 g of *Pennisetum glaucum* powder and digested for 90 minutes. 40 mL water was added and then distilled. Liberated ammonia was collected in 20 mL boric acid with bromocresol green and methyl red indicators and titrated against 0.04 $NH2SO_4$. A blank (without sample) was likewise prepared (Pearson, 1976).

Percent protein was calculated as:

$$\text{Crude protein (\%)} = \frac{\text{Sample titre - blank titre x 14 x 6:25}}{\text{Sample weight}} \times 100$$

Where 14 is the molecular weight of nitrogen and 6.25 is the nitrogen factor.

3. 4.4.3 *Test for Crude fibre*

A weighed crucible containing 1 g of defatted *Pennisetum glaucum* powder was attached to the extraction unit and into this 150 mL of hot 1.25% H2SO4 was added and digested for 30 min. The acid was drained and *Pennisetum glaucum* powder washed with hot distilled water for 90 minutes. The crucible was removed and oven dried at 105 °C, cooled, weighed, and incinerated at 550 °C in a muffle furnace overnight and reweighed after cooling. Percentage extracted fibre was calculated as:

$$\text{Crude fibre (\%)} = \frac{\text{Weight of digested sample-Weight of ashed sample}}{\text{Weight of samples}} \times 100$$

3. 4.4.4 *Test for Lipids*

One and a half grams of *Pennisetum glaucum* powder mixed with 2.3 g anhydrous sulfate was weighed into a thimble and covered with absorbent cotton, while 40 mL of petroleum ether at 40–60 °C boiling point was added to a pre-weighed cup. Both thimble and cup were attached to the extraction unit. The *Pennisetum glaucum* powder was extracted using ethanol for 30 minutes and rinsed for 90 minutes. Thereafter, the solvent was evaporated from the cup to the condensing column. Extracted fat in the cup was then placed in an oven at 105 °C for 1 hour, cooled and weighed. Percent fat was calculated as:

$$\text{Lipid (\%)} = \frac{\text{Initial cup weight - Final cup weight}}{\text{Weight of sample}} \times 100$$

3. 4.4.6 *Test for Carbohydrate*

The carbohydrate content was determined by subtracting the sum of all the percentages of moisture, lipid, crude protein, ash, and crude fibre from 100%. Two grams of *Pennisetum glaucum* powder was ashed at 500 ^{0}C and dissolved in 10% 25 ml of hydrochloric acid and filtered. The filtrate was made up to 100 ml with deonized water in a volumetric flask. The filtered solution was loaded to an Atomic Absorption Spectrophotometer. The standard curve for phosphorus and magnesium were prepared from known standards and the mineral value of *Pennisetum glaucum* powder was estimated against that of the standard curve (AOAC, 1995). These were done by Dr. M. D. Katung of the Product Development Research Programme Unit, Institute for Agricultural Research, Ahmadu Bello University, Zaria.

3.5 Experimental Design

3.5.1 Pentylenetetrazole induced seizures in mice

This was done according to the method described by Swinyard *et al.*, 1989. Twenty mice were divided into 4 groups of 5 mice each

Group 1- Standard feed + Normal Saline (orally)

Group 2- Diet containing 25% *Pennisetum glaucum* (Tornekar *et al*., 2009)

Group 3- Diet containing 50% *Pennisetum glaucum*

Group 4- Standard feed + Diazepam 2mg/kg (orally) (Umokoro *et al*., 2007).

Mice were pre-treated for 14 days, 30 minutes after the 14^{th} administrations all mice received freshly prepared pentylenetetrazole (PTZ) 85mg/kg subcutaneously and were observed for a 30minutes period for onset, duration, quantal protection and death protection (Swinyard, 1989). An episode of clonic spasm of at least 5 seconds was considered as seizure while Lack of threshold convulsion during 30 minutes of observation was regarded as protection

3.5.2 Pentylenetetrazole induced kindling seizures in wistar rats

Twenty wistar rats were divided into 4 groups of 5 each and given various interventions as shown below. On every alternate day, 30 minutes after receiving their interventions they were injected sub-convulsant dose of PTZ (35 mg/kg) and observed for 30 minutes for the presence or absence of seizure and seizure severity for 30 days.

Group 1- Standard feed + Normal Saline (orally) + PTZ 35mg/kg (Ip)

Group 2- Diet containing 25% *Pennisetum glaucum* (Tornekar *et al*., 2009) + PTZ 35mg/kg (Ip)

Group 3- Diet containing 50% *Pennisetum glaucum* + PTZ 35mg/kg (Ip)

Group 4- Sodium valpoarate 200mg/kg (orally) + PTZ 35mg/kg (Ip) (Xu *et al.*, 2014).

Seizure behaviour was evaluated using the Racine scale of 0-5 with stage 5 showing the most severe seizure activity.

Score 0-rats show no response

Score 1-hyperactivity, vibrissae twitching

Score 2-head nodding, head clonus and myoclonic jerk

Score 3-unilateral shaking of fore limbs

Score 4-rearing with bilateral shaking of fore limbs

Score 5-generalized tonic-clonic seizures with loss of righting reflex (Racine, 1972).

Rats were considered fully kindled when they showed stages 4 and 5 on two consecutive trials which happened on 15 administration of PTZ (30^{th} day of the experiment).

3.5.3 Maximal electroshock induced seizure in chicks

The method described by Swinyard and Kupferberg 1985 as modified by Sayyah *et al.* (2002) was used in the study. Forty day old chicks were divided into 4 groups of 10 each as indicated below:

Group 1- Standard feed + Normal Saline (Ip)

Group 2- Diet containing 25% *Pennisetum glaucum* (Tornekar *et al*., 2009)

Group 3- Diet containing 50% *Pennisetum glaucum*

Group 4- Standard feed + Phenytoin 20mg/kg (Ip) (Yau *et al*., 2014)

Administrations were done for a day. Thirty minutes after the administrations an electrical stimulus was applied to induce seizure to the chicks using Ugo Basile electroconvulsive machine connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids. The shock duration, frequency, current and pulse width were set and maintained at 0.8 seconds, 100 pulse/sec, 80mA and 0.6 ms respectively. Seizure was manifested as tonic hind limb extension. Parameters observed were duration of seizure, seizure protection, recovery time from seizure and percentage mortality.

The ability of *Pennisetum glaucum* supplement to prevent seizures or prolong the latency and/or onset was considered as an indication of anticonvulsant activity.

3.6 Statistical Analysis

Data obtained from Pentylenetetrazole-induced seizures in mice and MES in chicks were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests while data from Pentylenetetrazole induced Kindling in wistar rats were analysed with Kruskall Wallis test (to assess the presence or absence of seizure and the severity of seizure) followed by Mann-Whitney U-test (to compare between 25%, 50% *Pennisetum glaucum* supplemental groups and valporic treated group) using SPSS version 20.0. Values of $P \leq 0.05$ was considered significant.

CHAPTER FOUR

4.0 RESULT

4.1 Quantitative phytochemical screening and proximate analysis of *Pennisetum glaucum*.

Quantitative phytochemical screening of *Pennisetum glaucum* has revealed high flavonnoids (63.77%), saponins (7%), tannins (0.95%), phytate (0.28%) and least alkaloids (0.08%) (As shown in appendix 1).

Quantitative proximate analysis of *Pennisetum glaucum* showed a high presence of carbohydrates (65.92%), crude protein (12.03%), moisture (10.20%), crude fibre (7.40%), lipids (2.00%), magnesium (0.025%) and phosphorus (0.028%) (As shown in Appendix 2).

4.2 Effect of *Pennisetum glaucum* supplement against Pentylenetrazole-induced seizure in swiss albino mice.

The results showed that 25% and 50% *Pennisetum glaucum* supplemental groups showed significant ($P \leq 0.05$) decrease in seizure onset (66.40 ± 3.17 and 81.80 ± 2.11 seconds)

respectively when compared to diazepam treated group (182.00 ± 10.00 seconds). However, no significant difference was observed between *Pennisetum glaucum* supplemental groups when compared to the negative control (72.80 ± 1.32 seconds).

All treated groups including 25% and 50% *Pennisetum glaucum* supplemental groups (83.00 ± 2.49 and 41.20 ± 3.34 seconds) and diazepam treated group (72.20 ± 19.98 seconds) showed significant ($P \leq 0.05$) decrease in seizure duration when compared to the negative control (137.00 ± 13.13 seconds). However, no significant difference was observed between the 25% and 50% *Pennisetum glaucum* supplemental groups and diazepam treated group.

Only diazepam treated group offered 40% protection against seizure when compared to 0% offered by 25%, 50% *Pennisetum glaucum* supplemental groups and the negative control.

Diazepam treated group offered 60% protection against death as to 40% death protection offered by 50% supplemental group when compared to zero protection offered by 25% *Pennisetum glaucum* supplemental group and negative control (Table 4.1).

Mice that did not convulse within or survived for more the 30 minutes observation period were qualified protected.

4.3 Effect of *Pennisetum glaucum* supplement against Pentylenetetrazole-induced Kindling seizures in wistar rats

Seizure activity was less severe at 25% *Pennisetum glaucum* supplemental group (stopped at stage 3.8), much less severe at 50% *Pennisetum glaucum* supplemental group (reached only to stages 2.5) and stages 2.2 observed by valporic treated group when compared to the most severe seizure activity attained by the negative control group (reached stage 4.4)

However, no significant difference was observed between the supplemental groups and the valporic treated group in terms of seizure severity (figure 1).

4.4 Effect of *Pennisetum glaucum* supplement against maximal electroshock in chicks.

No significant ($P \leq 0.05$) difference was observed in seizure duration between and across all the groups (negative control 3.50 ± 0.78, 25% supplemental group 3.60 ± 0.54, 50% supplemental group 2.30 ± 0.65 and phenytoin treated group 0.00 ± 0.00 seconds).

However, a significant ($P \leq 0.05$) decrease in recovery from seizure was seen in the 50% *Pennisetum glaucum* supplemental group (2.96 ± 1.06min) when compared to 25% *Pennisetum glaucum* supplemental group (8.93 ± 2.11min).

Only 10% of chicks in 25% *Pennisetum glaucum* supplemental group and 40% of chicks in 50% *Pennisetum glaucum* supplemental group did not underwent seizure when compared to 20% in negative control. In contrast, 100% of chicks in phenytoin treated group did not underwent seizure at all.

None of the chicks from negative control, 25% *Pennisetum glaucum* supplemental group and phenytoin treated group died (100% mortality protection were recorded in these groups) nevertheless 10% dead was recorded in 50% *Pennisetum glaucum* supplemental group (Table 4.2).

Chicks that did not convulse within or survived for more the 30 minutes observation period were qualified protected.

Table 4.1: Effect of *Pennisetum glaucum* supplement against pentylenetetrazole induced seizures in mice.

Parameters/Group	**N.S + PTZ**	**25% PG + PTZ)**	**50% PG + PTZ**	**Diazepam + PTZ**

Seizure onset/sec	72.80 ± 1.32[d]	66.40 ± 3.17[d]	81.80 ± 2.11[d]	182.00 ± 10.00[a,b,c]
Seizure Duration/sec	137.0 ± 13.13[bcd]	83.00 ± 2.49[a]	41.20 ± 3.34[a]	72.20 ± 19.98[a]
Seizure protection %	0	0	0	40
Mortality protection %	0	0	40	60

Values with different superscripts a, b, c and d indicate significant difference ($P \leq 0.05$) compared to N.S + PTZ, 25% PG + PTZ, 50% PG + PTZ and Diazepam + PTZ respectively.

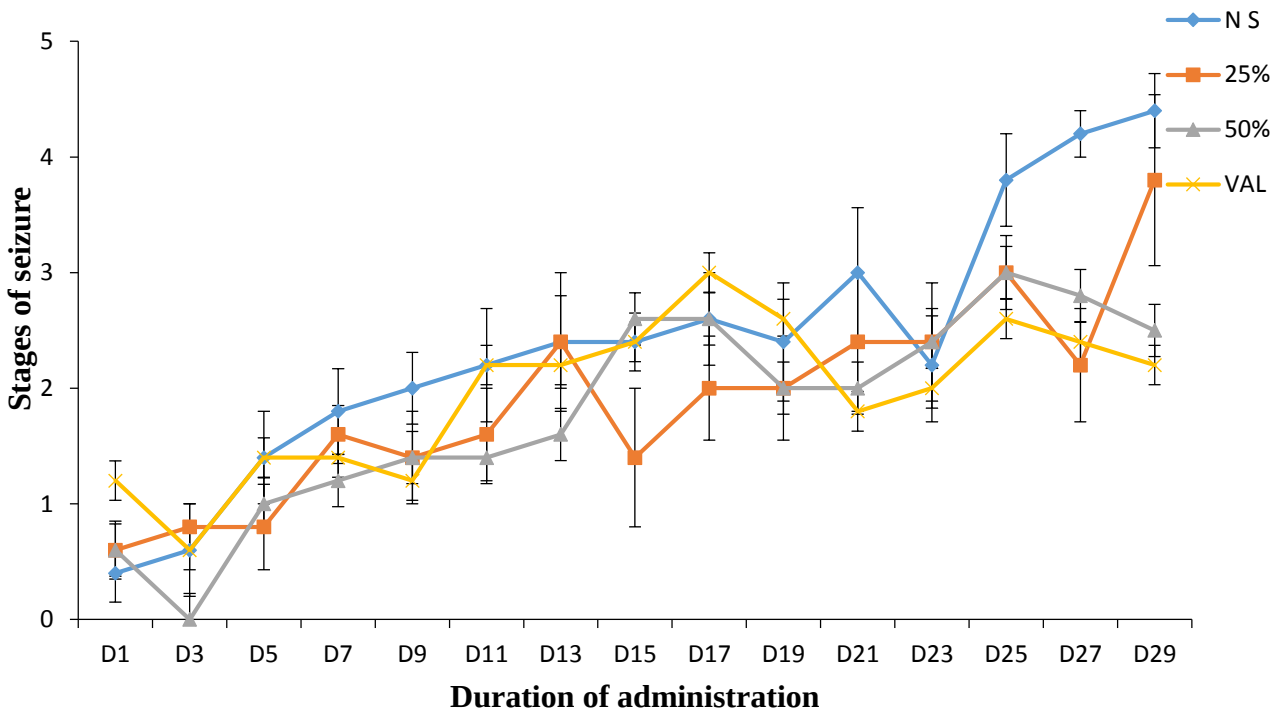


Figure 4.1: Effect of *Pennisetum glaucum* supplement against Pentylenetetrazole induced Kindling in wistar rats.

Table 4.2: Effect of *Pennisetum glaucum* supplement against maximal electroshock-induced seizures in chicks.

Treatment/Group	N. S + MES	25% PG + MES	50% PG + MES	PHE + MES
Duration/sec	3.50 ± 0.78	3.60 ± 0.54	2.30 ± 0.65	0.00± 0.00
Recovery/min	3.39 ± 0.78	8.93 ± 2.11[a ,c]	2.96 ± 1.06[b]	0.00 ± 0.00
Seizure protection%	20	10	40	100
Mortality protection%	100	100	90	100

Values with different superscripts a, b, c and d indicate significant difference ($P \leq 0.05$) compared to N.S + MES, 25% PG + MES, 50% PG + MES and Phenytoin + MES respectively.

CHAPTER FIVE

5.0 Discussion

Epilepsy is a chronic and often progressive disorder characterized by the periodic and unpredictable occurrence of epileptic seizures caused by disorganized and sudden electrical activity in the brain that is associated with cognitive, psychological and social consequences (Ali-Reda *et al.*, 2015).

Pennisetum glaucum considered a poor man‘s cereal (Nambiar *et al*., 2012), may be a repository of dietary antioxidants, especially flavonoids which provide bioactive mechanisms to reduce free radical-induced oxidative stress and play a role in the prevention of ageing and various diseases associated with oxidative stress including epilepsy (Diniz *et al*., 2015).

Therefore, the present study was aimed at investigating the anti-convulsant effect of *Pennisetum glaucum* supplement in some laboratory animals.

Pentylenetetrazole is a known convulsant that induces seizure similar to the symptoms observed with the human condition (absence seizure) (Malawska, 2005) and also drugs that are useful in absence seizures are found to suppress PTZ-induced seizures (McNamara, 2006). Pentylenetetrazole produces convulsions by blocking GABA-A receptors thereby impairing GABA-mediated inhibitory neurotransmission (De Deyn *et al*., 1992). Hence, AEDs such as diazepam and phenobarbitone are thought to produce their effect by enhancing GABA-mediated inhibition in the brain (Rogawski and Porter, 1990). In addition, activation of NMDA receptor system appears to be involved in the initiation and propagation of PTZ-induced seizure

(Velisek *et al*., 1999). Anticonvulsant activity of subcutaneous PTZ test identifies compound that raises seizure threshold in the brain (White *et al*., 1998).

Hence, the ability of *Pennisetum glaucum* at 50% to decrease seizure duration and offer protection from death in mice suggests its possible interaction with GABAergic neurotransmission and blockade of glutamatergic neurotransmission mediated by NMDA receptor in system in the brain.to produce anticonvulsant activity against absence or myoclonic seizures (Vida, 1995). Since anticonvulsant activity of a novel compound is not measured only by its ability to prevent convulsions but also to delay the onset of seizures or to reduce death rate and/or decrease the frequency of the episodes (Kendall *et al*., 1981).

Phytoconstituents such as flavonoids, tannins and saponins have been shown to modulate central nervous system (CNS) activities. It is found that many flavonoids could act as benzodiazepine- like molecules in the CNS and modulate GABA-generated chloride currents in animal models of anxiety, sedation and convulsion (Danjuma *et al*., 2009). Also, flavonoids have overall health promoting functions with particular importance in the prevention of diseases associated with oxidative damage of membrane, proteins and DNA (Diniz *et al.,* 2015). Flavonoids and tannins have been shown to interact with various voltage gated ion channel which are targets of synthetic AEDs (Nicholas *et al*., 2015). Hence, Modulation of the activity of ligand gated and voltage gated ion channels by these secondary metabolite of *Pennisetum glaucum* is likely to be an explanatory basis for the seen anticonvulsant effect. Also, Saponins in plants are believed to have sedative potential as well as the ability to inhibit spontaneous motor activity in mice (Aiyelero *et al*., 2012) Thus, there presence in high concentrations in *Pennisetum glaucum* supplement may be responsible for the observed effect.

Kindling is an established model of abnormal plasticity that leads to prolonged seizure and epilepsy (Rivera *et al*., 2012). Pentylenetetrazole induced kindling seizure involves repeated

administration of a subconvulsive dose of PTZ (35mg/kg) in wistar rats which produces a progressive increase in convulsant activity concluding to generalized seizure (Takemiya *et al*., 2003). The generalized tonic clonic seizure in this model resembles that of epilepsy in humans (Becker *et al*., 1992). The results obtained from this study demonstrated a trend towards improvement in seizure management in 25 and 50% *Pennisetum glaucum* supplement treated groups as seizure progression to classical convulsive stages (stages 4.4) were suppressed reaching only to stages 3.8 and 2.5 respectively. This was also described by Malami *et al.* (2015) who demonstrated that 3-synthesized dichloro-substituted phenylpropanalmides retarded seizure progression from reaching most severe stages of 4 and 5.

Since, PTZ induces seizure by blocking the major inhibitory pathways mediated by GABA at all levels of the CNS (De Sarro *et al*., 1990), this study further suggests the potentials of *Pennisetum glaucum* supplement in enhancing GABA transmission or inhibit seizure generation following kindling.

Also, seizure activity leads to mitochondrial dysfunction causing decrease ATP level needed for cellular metabolism, collapse in brain energy level resulting to abnormal neuronal excitability. Hence, the presence of Phosphorus which is an essential component of adenosine triphosphate (ATP) in addition to high carbohydrate content in *Pennisetum glaucum* supplement may help in boosting brain energy level thereby counteracting abnormal neuronal excitability. The Magnesium in *Pennisetum glaucum* supplement may also have contributed to it anticonvulsant activity since it is known to slow the spread of electrical discharge from one area of the brain to another and it deficiency has been shown to decrease seizure thresholds which were reversed by it supplementation (Jahangir *et al*., 2014).

Maximal electroshock (MES) causes several changes at the cellular level which can interrupt neuronal transduction causing cellular damage. One of the mechanisms by which MES causes

this damage is via facilitation of large amount of calcium ions Ca^{2+} entry into the cells thereby prolonging seizure duration (Inan and Buyukafsar, 2008). Facilitation of other positive ions including Na^{+} influx has also been reported in MES and it blockade prevent MES-induced tonic hind limb extension in animal models (Bum *et al*., 2010). Potentiation of GABA receptors are also reported to protect against MES-induced seizure (Sandrani, 1992) and currently available AEDs like phenytoin and sodium valproate act by modulating these ion channels (Rang *et al*., 2003).

The behavioural and electrographic seizure generated in this model are consistent with the human disorder (generalized tonic clonic seizure) Thus, MES assay is used primarily as indication for compounds that are effective in the treatment of generalized tonic clonic seizure (Rang *et al*., 1995). Protection against THLE from MEST suggest anticonvulsant effect that prevent the spread of seizure activity from a focus (Swinyard, 1972).

It has been suggested that seizure induction by electroshock machine is through inhibitory current breakdown and voltage-dependent Na-channel in these electrically induced stimuli (McNamara, 1996) hence, MES seizure can be prevented by sodium channel blockers like phenytoin, felbamate, valpoarate and lamotrigine which limit repetitive firing of action potential by slowing the rate of recovery of voltage activated sodium channels from inactivation (Rho and Sankar, 1999) or agent that block glutamatergic transmission mediated by NMDA receptors (Subramaniam *et al*., 1995) thereby suppressing tonic hind limb extension in chicks.

The protein content in *Pennisetum glaucum* is high and consists of all varieties of essential amino acids including Tryptophan (Odusola, *et al*., 2013) which can raise serotonin level and helps in stress reduction which is implicated in the pathophysiology of epilepsy.

Also, the lipid content of *Pennisetum glaucum* which was reported to contain phospholipids including omega 3 and decosahexanoic acid (DHA) (Nambiar *et al*., 2012) which plays a vital

role in management of stress, behavioural disorders, strengthen the brain and ensure smooth brain function (Bandero *et al*., 2013) thereby reducing seizures might have also contributed to its anticonvulsant activity.

Despite showing lesser efficacy than the commonly used anticonvulsants used in this study *Pennisetum glaucum* supplement at 25% and 50% offers several advantages over anticonvulsants due to its lack of rapid initiation and relatively low cost and serves dual purposes (food and drug).

CHAPTER SIX

6.0 Summary, Conclusion, Recommendations and References

6.1 Summary

The present study demonstrates that oral administration of *Pennisetum glaucum* supplement for a period of 14 days to decrease seizure duration at both 25% and 50%, offer 40% protection from death at 50% against subcutaneous PTZ-induced seizures in mice.

This study also demonstrates the ability of *Pennisetum glaucum* supplement to delay the progression of seizure to most severe stage at 50% against intraperitoneal PTZ induce kindling in rats.

Pennisetum glaucum supplement at 50% offered 40% seizure protection against MES induce seizures in chicks.

6.2 Conclusion

The present study demonstrates that oral administration of *Pennisetum glaucum* supplement for 14 days, 30 days and 1 day caused anticonvulsant effect against subcutaneous PTZ-induced seizures in swiss albino mice, intraperitoneal PTZ-induce kindling in wistar rats and MES-induced seizures in chicks respectively. Though the efficacy of the anticonvulsant activity

offered by *Pennisetum glaucum* supplement at both doses were less when compared to standard antiepileptic drugs used.

6.3 Recommendations

- Mechanistic study using picrotoxin, strychnine, isoniazid and 4-aminopyridine should be carried out to further assess how *Penisetum glaucum* exert it anticonvulsant effect.
- Assessment of neurotransmitters like GABA, glycine and glutamate will provide more insight as to possible mechanism via which *Penisetum glaucum* exert it anticonvulsant effect.
- Consumption of *Penisetum glaucum* should be encouraged especially in epileptics in addition to taking their anticonvulsant drugs.

6.4 Contributions to Knowledge

i. This study expose new frontiers by improving the current applications of this plant to assess the effect of the *Pennisetum glaucum* supplement using laboratory seizure models

ii This study demonstrated the ability of *Pennisetum glaucum* supplement at

- 50% to decrease seizure duration (41.20 ± 3.34 vs 137.00 ± 13.13 seconds), offer 40% vs 20% protection from death and delay seizure progression to severe stages (stopping at stages 2.5 vs 4.4).
- 25% to decrease seizure duration (83.00 ± 2.49 vs 137.00 ± 13.13 seconds).

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APPENDIX

Table 1: Quantitative preliminary phytochemical screening of *Pennisetum glaucum*

Phytoconstituents	**Percentage Composition**
Flavonoids	63.77%
Alkaloid	0.08%
Tannins	0.95%
Saponins	7.00%

Phytate	0.28%

APPENDIX

Table 2: Quantitative proximate analysis of *Pennisetum glaucum*

Parameters	**Percentage Composition**
Ash content	2.85%
Crude protein	12.03%
Crude fibre	7.40%
Lipid	2.00%

Moisture	10.20%
Carbohydrate	65.92%
Phosphorus	0.028%
Magnesium	0.025%