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MECHANISM OF ACTION OF ETHANOLIC EXTRACT OF *Synedrella nodiflora* (ASTERACEAE) IN MODULATING SEIZURE INTENSITIES.

BY

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This thesis / dissertation is submitted to the University of Ghana, Legon in partial fulfillment of the requirement for the award of **MPHIL IN PHYSIOLOGY Degree**.

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DECLARATION

I, VINCENT ABOAGYE, hereby declare that with the exception of references made to other people's work which I have duly acknowledged, this dissertation which is my original work has neither in whole nor in part been presented to the University or elsewhere for another degree.

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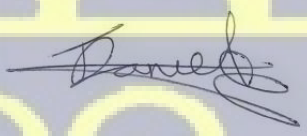
Signature: 

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DEDICATION

I dedicate this work to God Almighty and my Father, Mr. James Yeboah Asuamah.

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ABSTRACT

Introduction: Seizure is a neurological disorder which manifests as abnormal cortical nerve cell activity. This results from synchronous neuronal activity in the brain with some effects manifesting as uncontrolled jerky movements involving much of the body with loss of consciousness. Treatment of this neurological condition has been complicated by drug resistance and side effects. This has fueled the need to identify new compounds with properties which can be developed into drugs. Compounds from plant extracts such as *Synedrella nodiflora* (SNE) have previously been shown to exhibit anti-convulsive properties, making them worthy of further investigation. The aim of this study therefore is to explore the potential pathways with which the extract decreases seizure.

Methods: The anti-convulsant effect of ethanolic extract of *Synedrella nodiflora* was investigated using two animal seizure models; Acute Pentylenetetrazol (PTZ) and Chronic PTZ induced seizures. Liver and Renal function tests were assessed after Chronic PTZ induced seizures. Brain tissues were resected for histological studies. Flumazenil, a GABA receptor blocker, was used to determine whether or not the extract reduced seizures via the GABAergic pathway.

Results: SNE (1000 mg/kg/body weight) reduced PTZ induced kindling significantly ($P<0.05$). Also, SNE (100, 300, 1000 mg/kg/body weight) reduced the latency and frequency of acute PTZ induced seizures. Histology of the hippocampus of SNE- treated mice showed normal cell count when compared with control mice. Results of Renal and Hepatic Function were within normal limits. SNE had no significant effect after the mice were pretreated with Flumazenil (GABA receptor Blocker).

Conclusion: The result of this study provides evidence that the ethanolic extract of the whole plant of *Synedrella nodiflora* possesses anti-seizure activity probably mediated through GABAergic pathway in murine experimental models.

LIST OF ABBREVIATIONS

SNE	*Synedrella nodiflora*
GABA	Gama Amino Butyric Acid.
PTZ	Pentylenetetrazol
PHE	Phenobarbitone Sodium
ILAE	International League Against Epilepsy
AUC	Area under Curve
E/I	Excitation/Inhibition
ICH	Intracerebral Hemorrhage
SAH	Subarachnoid Hemorrhage
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
NMDA	N-Methyl-D-Aspartate Receptor
IPSP	Inhibitory Postsynaptic Potential
CSF	Cerebrospinal Fluid
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
GGT	Gamma-Glutamyl Transferase
LDH	Lactate Dehydrogenase
NADH	Nicotinamide Adenine Diphosphate
GFR	Glomerular Filtration Rate

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CHAPTER ONE

1.0 Introduction

1.1 Background of Study

A seizure is an overwhelming electrical disturbance in the brain that results in changes in behavior, movement, sensation, and awareness (Lawal *et al.*, 2018; Spray, 2015). This results from synchronous neuronal activity in the brain with some effects manifesting as uncontrolled jerky movements involving muscles of the body with loss of consciousness (tonic-clonic seizure), jerky movements involving only part of the body with variable levels of consciousness (focal seizures), and a subtle momentary loss of awareness (absence seizures)(Leibetseder *et al.*, 2020;Fisher *et al.*, 2017). These episodes usually last less than two minutes. During this process, loss of bladder control may occur (Brown, 2021).

Seizures may be either provoked or unprovoked. Provoked seizures are due to a temporary event such as low blood sugar, alcohol withdrawal, drug abuse, low blood sodium, fever, brain infection, or concussion (Delanty, 2001). Unprovoked seizures occur without a recognized cause, and they are likely to persist. Stress or sleep deprivation may also cause unprovoked seizures (Kotagal, 2001).

The underlying neurological cause of seizures is a disruption in the brain's normal pattern of excitation (E) and inhibition (I) (Bonansco & Fuenzalida, 2016; Stafstrom & Carmant, 2015; Ramamoorthi & Lin, 2011). The factors that alter E/I balance can be either genetic or acquired (Kang, 2021; Casillas-Espinosa *et al.*, 2020).

The developing brain is more susceptible to seizure activity because excitatory synaptic activity develops before inhibitory synaptic function, which thereby promotes excitation rather than inhibition (Nardou *et al.*, 2013; Owens & Kriegstein, 2002). Furthermore, the neurotransmitter GABA produces stimulation rather than inhibition in the cerebral cortex early in life. These

findings help to understand why the brain of a child is particularly vulnerable to seizures (Nardou *et al*., 2013; Bowery & Smart, 2006).

Treatment of this neurological disorder has mostly focused on the use of drugs (Landmark, 2008; Löscher, 2002). Drugs such as Phenytoin, carbamazepine, oxcarbazepine block repetitive activation of sodium channels (Rogawski & Löscher, 2004). Also, Phenobarbital, benzodiazepines, clobazam enhances activity of $GABA_A$ receptors and drugs such as Tiagabine inhibit GABA reuptake (Greenfield Jr, 2013; Porter & Meldrum, 2001). In summary, these medications prevent neuronal depolarization by blocking sodium or calcium channels, enhancing potassium channel function, decreasing glutamate-mediated excitation, or promoting GABA-mediated inhibition. However, this approach to the treatment of seizure disorders is complicated by drug resistance and side effects (Chen *et al.*, 2017; Lagae, 2006). Reactions to some of these traditional seizure medications have accelerated the search for new compounds, including herbal extracts that are effective against recurrent seizure episodes.

*Synedrella nodiflora (*SNE*)* has been demonstrated to have analgesic, antioxidant, and anticonvulsant properties (Amoateng *et al.*, 2017; Amoateng *et al.*, 2012). Its analgesic effect has been shown to be mediated in part by adenosinergic pathways (Woode *et al.*, 2009), however, the mechanism behind its anticonvulsant activity is unknown. The aim of this study therefore is to explore the potential pathways through which the extract decreases seizure activity.

1.2 Problem Statement

Seizures are more common in children under the age of one and the elderly, usually after the age of 65, as a result of various health issues such as stroke and heart disease (Van Win *et al.*, 2020; Liu *et al.*, 2016). According to a prospective multicenter study, the total incidence of seizures after intracerebral hemorrhage (ICH) and ischemic strokes are about 10.6% and 8.6%, respectively.

Seizures were present in about 10% –25% of individuals after an ICH, and 15.2% after a subarachnoid hemorrhage (SAH), (Constant Dit Beaufils *et al.*, 2021).

A meta-analysis point to a higher prevalence of seizures and epilepsy in children ranging from 3.2-5.5/1,000 in developed countries to 3.6-44/1,000 in underdeveloped countries (Camfield & Camfield, 2015). Prolonged seizures usually lead to active epilepsy. In a recent study, the prevalence rate of epilepsy was about 6.38 per 1,000 people, and a lifetime prevalence of 7.60 per 1,000 people (Beghi, 2020). This has been found more prevalent in in low- and middle-income nations (Kotsopoulos *et al.*, 2002). A major neurological defect has to do with the shrinking of the hippocampus of the brain. The hippocampus is reported to be liable to atrophy following repeated status epilepticus which may in learning defects (Hattiangady *et al.*, 2008; Marques *et al.*, 2007; Silvia *et al.*, 2003). A lot of drugs have been developed in attempting to resolve this neurological issue, However, many of the drugs have failed due to their side effects. This therefore has influenced the need to research into herbal extracts with their mechanism of action known in decreasing seizures.

1.3 Justification

Previous studies have looked into Property of *Synedrella nodiflora* to abort seizures, but there has been no study of the mechanism of action of the extract. Research is therefore necessary to establish the mechanism(s) of action of the extract. Also, given the high cost of anti-epileptic drugs and their numerous adverse effects, there is a pressing need to investigate this plant extract and the mechanisms involved in reducing seizure intensity. This study could lead to the development of a new anti-seizure drug.

1.4. Aim

The goal of the study is to investigate further the anticonvulsive effects, as well as the mechanism of action and safety profile of extracts of *Synedrella nodiflora.*

1.5 Specific Objectives

The specific objectives of the study include:

1. To determine how SNE affects the latency, frequency, and duration of both acute and chronic chemically induced seizures.
2. To determine the possible receptor pathway by which SNE mediates decreasing seizures.
3. To determine how SNE affects the viability of hippocampal neurons following a prolonged PTZ kindling.
4. To determine how prolonged SNE administration affects the body's organs (liver and kidney functions).

1.6 Hypothesis

Synedrella nodiflora extract will decrease seizures via the GABAergic pathway.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Neuronal and synaptic Physiology

In the mammalian brain, excitatory and inhibitory neurotransmission are mediated by the transmitter's glutamate and GABA, respectively (Wen *et al.*, 2022; Obata, 2013). Fast signaling between neurons occurs at specialized synaptic contacts formed between the axons of presynaptic cells and the soma or dendrites of postsynaptic target neurons (Jan & Jan, 2001; Isaacson, 2000). The narrow width of the synaptic cleft ensures that transmitters reach receptors quickly and at high concentrations. The lifetime of transmitter action at excitatory and inhibitory synapses is governed by a combination of diffusion of transmitter molecules away from receptors in the postsynaptic region, and uptake of transmitter molecules by specific transporters in neuronal and glial membranes (Farrant & Kaila, 2007; Cherubini & Conti, 2001).

Neurons form a complex biological neural network through which action potentials travel. Neurons do not touch each other except in the case of an electrical synapse through a gap junction; instead, neurons interact at close contact points called synapses (Hormuzdi *et al.*, 2004). A neuron transports its information by way of an action potential. When the nerve impulse arrives at the synapse, it may cause the release of neurotransmitters, which influence another postsynaptic neuron (Hormuzdi *et al.*, 2004). The postsynaptic neuron may receive inputs from many additional neurons, both excitatory and inhibitory. The excitatory and inhibitory influences are summed, and if the net effect is inhibitory, the neuron will be less likely to generate an action potential, and if the net effect is excitatory, the neuron will be more likely to generate action potential (Huang *et al.*, 2012; Yoshimura *et al.*, 2005). How likely a neuron is to fire depends on how far its membrane potential is from the threshold potential, the voltage at which an action potential is triggered because enough voltage-dependent sodium channels are activated so that the net inward sodium current exceeds all outward currents (Khaliq & Bean, 2010;

Denac *et al*., 2000). Excitatory inputs bring a neuron closer to threshold, while inhibitory inputs bring the neuron farther from threshold.

Neurotransmission is the process by which signaling molecules known as neurotransmitters are released by the axon terminal of a neuron (the presynaptic neuron), and bind to and react with the receptors on the dendrites of another neuron (the postsynaptic neuron) (Togashi *et al*., 2009; Süudhof, 2008; Stahl, 2000).

Glutamate is a divalent anion of glutamic acid and it acts as a neurotransmitter (Heath & Shaw, 2002). It is by a wide margin the most abundant excitatory neurotransmitter in the vertebrate nervous system (Omote *et al*., 2011). It is involved in every major excitatory function in the vertebrate brain and accounts for over 90% of the synaptic connections in the human brain (Heath & Shaw, 2002). It also serves as the primary neurotransmitter for some localized brain regions, such as granule cells in the cerebellum (Hisano, 2003).

Biochemical receptors for glutamate fall into three major classes, known as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, N-methyl-D-aspartate (NMDA) receptors, and metabotropic glutamate receptors (Fu *et al*., 2018; Sherman, 2014). Many synapses use multiple types of glutamate receptors. AMPA receptors are ionotropic receptors specialized for fast excitation. In many synapses they produce excitatory electrical responses in their targets a fraction of a millisecond after being stimulated (Reiner & Levitz, 2018; Kullmann & Lamsa, 2007). NMDA receptors are also ionotropic, but they differ from AMPA receptors in being permeable, when activated, to calcium (Cull-Candy *et al.*, 2006). Their properties make them particularly important for learning and memory (Riedel *et al.*, 2003). Metabotropic receptors act through second messenger systems to create slow, sustained effects on their targets (Reiner & Levitz, 2018).

GABA on the other hand mediates fast inhibitory transmission via the activation of ionotropic receptors. Its actions are thought to be primarily excitatory in the developing brain (Ben-Ari, 2014). Activation of a $GABA_A$ receptor thus leads to efflux of Cl^- ions from the cell and this manifest as depolarizing current (Nawafleh *et al.,* 2022). GABAergic interneurons mature faster in the hippocampus and the GABA machinery appears earlier than glutamatergic transmission. Thus, GABA is considered the major excitatory neurotransmitter in many regions of the brain before the maturation of glutamatergic synapses (Ben-Ari *et al.*, 2007; Luján *et al.*, 2005; Herlenius & Lagercrantz, 2004).

One approach to understanding the physiological role of GABAergic inhibition is to record from synaptically-coupled pairs of neurons. In the hippocampus, dual electrophysiological recordings of GABAergic interneurons and their postsynaptic target cells, pyramidal neurons, have shown that single interneurons exert powerful inhibitory actions on the excitability of individual pyramidal cells (Isaacson, 2000).

2.2 Cerebral cortical functions of neurons

Cerebral and cortical functions of neurons are integral components of the brain's overall function and are responsible for various cognitive and sensory processes (Maldonado, 2018). These functions involve complex interactions among different types of neurons, neurotransmitters, and neural networks within the cerebral cortex, which is the outermost layer of the brain responsible for higher-order cognitive functions (Molnár *et al.*, 2019). Some of these complex interactions include information processing, sensory perception, motor control, memory formation among others.

Neurons in the cerebral cortex are primarily responsible for processing and integrating information from various sensory modalities, such as sight, sound, touch, and taste (Molnár *et al.*, 2019). They

also play a crucial role in higher cognitive processes like thinking, reasoning, and decision-making. The cerebral cortex is divided into different regions, each with specialized functions, such as the visual cortex for processing visual information and the prefrontal cortex for executive functions like planning and problem-solving (Carpenter *et al.*, 2000).

Also, the cerebral cortex contains regions dedicated to each of the senses. For example, the somatosensory cortex is responsible for processing tactile sensations like touch and pressure, while the auditory cortex processes sound, and the olfactory cortex is involved in the sense of smell (Rupini & Nandagopal, 2015). Neurons in these areas receive and process sensory input, allowing us to perceive and interpret the world around us.

Neurons in the cerebral cortex also control voluntary movements (Isomura *et al.*, 2009). The primary motor cortex, for instance, is responsible for planning and executing motor actions. When you decide to move a limb or perform a specific action, neurons in this region send signals to the muscles to carry out those movements (Koch & Rothwell, 2009).

Adding up, the cerebral cortex is vital for memory processing (Mizuno-Matsumoto *et al.*, 2020). Different regions, such as the hippocampus and various parts of the temporal and frontal lobes, are involved in encoding, storing, and retrieving memories (Simons & Spiers, 2003). Neurons play a crucial role in forming and strengthening synaptic connections associated with memory traces (Lisman *et al.*, 2018).

In summary, cerebral and cortical functions of neurons are diverse and intricate, underpinning the brain's ability to process sensory information, control movements, form memories, regulate emotions, communicate, and engage in complex cognitive processes. These functions result from

the coordinated activity of billions of neurons working together within the cerebral cortex, making it the most sophisticated part of the human brain.

2.3 The hippocampus of the brain

The hippocampus is a crucial region in the brain associated with various functions related to learning and memory, especially spatial memory and the formation of new long-term memories (Deng *et al*., 2010). Neural connections in the hippocampus play a significant role in these processes. There are several key regions and connections within the hippocampus, and each has its specific function.

The dentate gyrus is the first region of the hippocampus that receives input from the entorhinal cortex (Jonas & Lisman, 2014). It serves as a gateway to the hippocampal circuitry. One of its primary functions is pattern separation, which helps in distinguishing similar inputs and forming distinct representations of memories (Vivar *et al.*, 2012).

The CA3 (Cornu Ammonis 3) is a region of the hippocampus that receives input from the dentate gyrus (Chauhan *et al.*, 2021). It is known for its recurrent connections, which are thought to be crucial for memory consolidation and the rapid encoding of new information. CA3 is also involved in associating different elements of a memory, which can contribute to the formation of complex memories (Cherubini & Miles, 2015).

Another region within the hippocampus is the CA1 (Cornu Ammonis 1), and it receives input from CA3. CA1 plays a critical role in the retrieval of stored memories and their integration into existing knowledge (Schlichting & Preston, 2015). It also sends information back to the entorhinal cortex and other brain regions, allowing for the transfer of information between the hippocampus and the neocortex (Schlichting *et al.*, 2014).

Also, the subiculum which is another region of the hippocampus is located at the output end of the hippocampus and is responsible for transmitting information from the hippocampus to other brain regions, including the cortex (O'Mara, 2006). It plays a role in spatial memory and navigation (Buzsáki, 2015).

Finally, the entorhinal cortex is a key interface between the hippocampus and the neocortex (Rozov *et al.*, 2020). It provides input to the hippocampus, conveying information about spatial and contextual aspects of the environment. It is also involved in the formation of cognitive maps, which are essential for spatial memory (Steffenach *et al.*, 2005).

These neural connections and their functions within the hippocampus are essential for the formation, storage, and retrieval of memories, as well as for spatial orientation and navigation in the environment (Vismer *et al.*, 2015). Damage to the hippocampus, as seen in conditions like Epileptic states and Alzheimer's disease, can severely impair these functions, leading to memory deficits and spatial disorientation (Van Hoesen *et al.*, 2000).

2.4 Seizure Mechanisms

An electrical disturbance in the brain known as a seizure is caused by brain cells that fire very rhythmically. It is defined by the temporary development of symptoms and signs brought on by abnormally synchronized neuronal activity in the brain (Bowman *et al.*, 2001; Nye & Thadani, 2015).

Physiologically, excitatory neurons are subjected to refractoriness after it has fired. Some of the factors contributing to this refractoriness includes; inhibitory neurons' impact, electrical changes inside excitatory neurons, and the detrimental effects of adenosine (Neske *et al.*, 2015).

Over 1,600 ion-channel mutations and 41 ion-channel genes have been linked to the onset of epileptic seizures (Chen *et al.,* 2017; Wei *et al.,* 2017). An influx of Ca^{2+} can lead to a prolonged depolarization in individual neurons, which results in prolonged activation of Na^{+} channels and recurrent action potentials.

2.4.1 Generalized Seizures

A generalized seizure happens when the aberrant electrical activity that causes a seizure starts at synchronously in both hemispheres of the brain (Englot & Blumenfeld, 2009). This usually shoots from a localized region and quickly fires through the two hemispheres of the brain. Different types of Generalized Seizures include; Absence seizures, atonic, myoclonic, tonic and clonic seizures.

2.4.1.1 Absence Seizures

Absence seizure is also known as Petit mal seizure. It is characterized by abrupt onset of staring spells. A person experiencing an absence seizure will often stop moving or may blink rapidly or stare into space than 15 seconds in one place (Chen et al., 2005; Tononi & Koch, 2008). Even though the individual may not recall what occurred during the seizure, their normal level of attentiveness returns right afterward.

2.4.1.2 Atonic Seizures

An Atonic seizure An Atonic seizure is characterized by loss of muscle tone (atonia) which leads to limping and slumping and may cause to injury to the individual (Thijs *et al.*, 2009). Certain epilepsy syndromes, including Lennox-Gastaut syndrome, are characterized by atonic seizures (Crumrine, 2002).

2.4.1.3 Tonic - Clonic Seizures

A person experiences a tonic seizure, when the muscles undergo spasm and the individual loses consciousness (Tononi & Koch, 2008). It presents with arching of the trunk as a result of spasm of muscles of the spine, chest and limb and rolling of the eyes (Jankovic & Lang, 2004). Breathing is interrupted as a result of spasm of the muscles of the chest, and the person's lips and face may turn gray or blue (Devinsky, 2007).

Clonic seizures cause a person's muscles to contract and relax leading to jerky movements of the body. Elbow, leg, and neck muscles all quickly flex and then relax (Rudzinski & Shih, 2010). As the seizure passes, the jerking motion becomes less severe until it eventually stops. The individual usually lets out a long sigh as the jerking ceases before starting to breathe normally again. When tonic and clonic movements occur synchronously, tonic-clonic seizures, also known as "grand mal" or "convulsive" seizures, take place (Brodie *et al.*, 2018). Depending on the severity of the seizure, a seizure usually lasts a few minutes or less, after which the person is likely to stay unconscious for another few minutes.

2.4.2 Focal Seizures

Focal seizures which could be olfactory, motor, sensory, cognitive, autonomic, or autonomic-autonomic phenomena can make a person confused or dazed during a complicated partial seizure and be unable to reply to questions or instructions (Wang *et al.,* 2017). This is usually coupled with a Jacksonian march, which occurs when jerking activity spreads from one muscle group to another (Unterberger *et al.,* 2018).

Focal seizures develop from networks that are confined to one hemisphere (Burman & Parrish, 2018) with Subcortical structures being the source. Seizure semiology can be used to pinpoint the specific region of the brain, lobe, or hemisphere that is responsible for the development and spread of the condition (McGonigal & Chauvel, 2004).

2.5 Neurochemical mechanisms underlying epilepsy

2.5.1 GABA

Several studies have shown that GABA is involved in the pathophysiology of epilepsy in both animal models and patients suffering from epilepsy (Bozzi *et al.,* 2018; Engelborghs *et al.,* 2000; Yin *et al.,* 2013). The GABA hypothesis of epilepsy implies that a reduction of GABAergic inhibition results in epilepsy whereas an enhancement of GABAergic inhibition results in an anti-epileptic effect (Catterall *et al.,* 2010). Inhibitory postsynaptic potentials (IPSPs) gradually decrease in amplitude during repetitive activation of cortical circuits. This phenomenon might be caused by decreases in GABA release from terminals, desensitization of GABA receptors that are coupled to increases in Cl conductance or alterations in the ionic gradient because of intracellular accumulation of Cl^- (Engelborghs *et al.,* 2000). Moreover, Cl^--K^+ co-transport becomes less effective during seizures as it depends on the K^+ gradient. As Cl^--K^+ co-transport depends on

metabolic processes, its effectiveness may be affected by hypoxia or ischemia as well (Pedersen *et al.*, 2006).

GABA levels and glutamic acid decarboxylase (GAD) activity were shown to be reduced in epileptic foci surgically excised from patients with intractable epilepsy and in CSF of patients with certain types of epilepsy (Badawy *et al.*, 2009). Several endogenous (guanidino compounds) and exogenous (e.g., bicuculline, picrotoxin, penicillin, pilocarpine, pentylenetetrazol) convulsant inhibit GABAergic transmission through inhibition of GABA synthesis or through interaction with distinct sites at the postsynaptic GABA(A) receptor (De Risio & Platt, 2014).

2.5.2 Glutamate

Glutamatergic synapses play a critical role in all epileptic phenomena (Engelborghs et al., 2000). Activation of both ionotropic and metabotropic postsynaptic glutamate receptors serve as proconvulsant (Alexander & Godwin, 2006).

An increased sensitivity to the action of glutamate at NMDA receptors is seen in hippocampal slices from kindled rats and in cortical slices from cortical foci in human epilepsy (Alexander & Godwin, 2006). This results in an enhanced entry of Ca2+ into neurons during synaptic activity. Changes in metabotropic glutamate receptor function may also play a key role in onset of seizures (Chapman, 2000).

2.6 Epileptic seizures and Human Genetics

An estimated 40% of patients who experience epileptic seizures have genetic predispositions that play a role in the etiology of seizures (Anwar *et al.*, 2020). The majority of familial epilepsies, including juvenile myoclonic epilepsy, childhood absence epilepsy, and benign childhood epilepsy, have a complex pattern of inheritance caused by the interplay of several loci with environmental factors (Berkovic & Scheffer, 2001; Helbig *et al.*, 2008). Biochemical alterations (such as elevated plasma glutamate levels) that can be connected to a widespread increase in

cortical excitability have been seen in absence seizure sufferers (and their first-degree relatives) (Brambilla *et al.*, 2003; Palmieri *et al.*, 2010).

Genetic data from research on absence epilepsy in animals reveals a very straightforward inheritance factor of one gene that decides whether an individual is epileptic or not, while other genes regulate the frequency and length of epileptic fits (Grone & Baraban, 2015; Winawer, 2002).

A rare autosomal dominant condition called episodic ataxia type 1 is characterized by transient ataxic episodes accompanied by myokymia. Partial epileptic episodes are also present in patients with this disease (Jen *et al.*, 2007; Rajakulendran *et al.*, 2007). Point mutations in the human voltage-gated potassium channel gene on chromosome 12p13 are related to the syndrome (Eunson *et al.*, 2000).

2.7 Electrophysiology of Neurons During Seizure States

Neurons communicate through electrical impulses, which are generated and propagated by changes in membrane potential (Fields, 2008). During a seizure, there is a significant increase in neuronal excitability, making neurons more prone to firing action potentials (Misonou, 2010). This heightened excitability is often a result of an imbalance between excitatory and inhibitory neurotransmitters, such as glutamate and GABA (Uzunova *et al.*, 2016).

In a healthy brain, neurons fire in a coordinated but asynchronous manner. During a seizure, however, groups of neurons become excessively synchronized, leading to the generation of hypersynchronous electrical activity (Margineanu, 2010). This synchronization can occur in specific brain regions or even propagate throughout the entire brain, depending on the seizure type (Schwaller *et al.*, 2004).

One other effect of neurons during seizure states includes ionic imbalance which can cause abnormal neuronal firing (Scharfman, 2007). Neurons maintain their resting membrane potential through the selective movement of ions, primarily sodium (Na^+), potassium (K^+), and chloride (Cl^-) (Clausen & Poulsen, 2013). During seizures, there is often an imbalance in these ions. For example, an excessive influx of sodium or calcium ions can lead to depolarization and hyperexcitability, triggering seizure activity (Johnkennedy, 2021).

Also, neurotransmitter Alterations plays a key role in abnormal firing of neurons during seizure states. Dysregulation of neurotransmitters is a key factor in seizure generation. Glutamate, the brain's primary excitatory neurotransmitter, can be excessively released during seizures, further promoting excitatory signaling (Alcoreza *et al.*, 2021). Conversely, the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) may be impaired, reducing its ability to counterbalance excitatory activity.

All these abnormal activities of neurons during seizure states can be monitored using electrophysiological tests (Englot *et al.*, 2008; Klaassen *et al.*, 2006).

Electrophysiological tests like electroencephalography (EEG) are commonly used to diagnose seizures and identify their location in the brain (Bowyer, 2016). EEG records the electrical activity of the brain through electrodes placed on the scalp, and abnormal electrical patterns detected during seizures help doctors diagnose and classify different types of seizures (Siuly *et al.*, 2016).

Also, Electrophysiology is used to investigate the mechanisms underlying seizures (Staba *et al.*, 2014). Techniques such as patch-clamp recordings or extracellular field potential recordings to study the electrical properties of neurons and how they contribute to the generation and propagation of seizures (Jung *et al.*, 2014). In a nutshell, seizures are complex events that involve

alterations in the electrophysiology of neurons. Understanding these changes is essential for both the diagnosis and treatment of epilepsy

2.8. Biochemical Tests

2.8.1 Liver Function Test

2.8.1.1 Alanine transaminases

Serum alanine transaminases (ALT) is not only measured to determine a possible liver damage, but also useful in monitoring the general health status. This is an enzyme that is mainly found in the cytosol of the hepatocytes, and plays a role in gluconeogenesis. Typically, it catalyzes the transfer of amino acid from L-alanine to alpha ketoglutarate to produce L-glutamate and pyruvate in the liver. ALT is released as a result of liver injury from the injured liver cells into the serum, which causes the levels to increase significantly in the serum. While serum levels of ALT have been used almost extensively as a marker for liver dysfunction. Gender related ALT levels studied showed that, men have slightly higher serum levels of ALT compared with their female counterparts (Parti *et al.*, 2002). Gender however did not affect the levels of serum ALT in another study (Kotila *et al.*, 2005).

2.8.1.2 Aspartate transaminases

Elevated levels of Aspartate transaminases may be as a result of several conditions such as increased hemolysis, and not hepatic damage (Nsiah *et al.*, 2011). Nonetheless, serum levels of aspartate can be used in combination with other specific enzymes that assess liver damage to make a very sound conclusion regarding its (liver) integrity. Aspartate transaminases and alanine transaminases are however, considered as the two common tests when measuring liver enzymes. Thus, it plays a very important role in the assessment of normal function of the liver. Aspartate transaminases has been known as an enzyme that catalyzes the formation of glucose from other substrates. Thus, they play a

role in gluconeogenesis, which is essential for the body's metabolic activities. Patients whose biochemical test for AST showed marked elevation are usually present with acute liver injury (Giannini et al., 2005).

2.8.1.3 Gamma-glutamyl transferase

Gamma-glutamyl transferase (GGT) is a cell-surface protein, which is mainly produced in the liver and present in serum. It contributes to the extracellular catabolism of glutathione (an antioxidant that protects the cell against oxidative stress) (Emdin *et al.,* 2005; Whitfield, 2001). Thus, the higher the levels of GGT in the serum, the more the oxidative stress. It is important to bring to light that, significant increase in GGT levels may reflect a possible increased exposure to certain xenobiotics of organic sources that are metabolized in the liver through the process of glutathionylation. GGT serves as a serum biomarker of hepatobiliary damage. The serum activity of GGT has been used as early marker of inflammation and oxidative stress (due to its catabolic activity on the antioxidant glutathione) (Nothlings *et al.,* 2012; Schmelzer *et al.,* 2011; Menke *et al.,* 2000). In most cases, GGT levels in the serum has been used as a biomarker of alcohol-related liver disease (Whitfield, 2001).

2.8.1.4 Bilirubin

Bilirubin is formed as a result of the breakdown of a component that is mostly derived from the haemoglobin of erythrocytes, called haeme. It could also be produced from the breakdown of haeme derived from other haemoproteins including catalase, myoglobin as well as cytochrome. The haeme is broken down to form biliverdin, by haeme oxygenase (HO), a rate-limiting enzymes in haeme catabolism. The biliverdin is consequently converted to bilirubin by biliverdin reductase (Rochette *et al.,* 2018; O'Brien *et al.,* 2015). The formed bilirubin then binds to albumin in the circulation, where it is transported to the liver. In the liver, it is conjugated by uridine diphosphate-glucuronyl transferase 1A1 with glucuronic acid. (Keppler *et al.,* 2014).

2.8.1.5 Albumin

Although studies have documented the presence of albumin mRNA in extra-hepatic places of the body including the brain, pancreas and kidney, albumin is predominantly synthesized in the liver (Yoshida *et al.,* 1997). Among the several functions of albumin are its anti-inflammatory and anti-oxidant properties, through a number of mechanisms, as well as a binding capacity. Albumin can bind to a number of substances including drugs and bilirubin. In conditions such as liver cirrhosis, the ongoing systemic inflammation leads to imbalanced redox state. Thus, there is the formation of more oxidized forms of albumin. The increase in the percentages of the oxidized forms of the albumin has been associated with the severity of the liver cirrhosis and mortality (Domenicali *et al.,* 2014: Oetti *et al.,* 2013). Albumin has been documented in a large study as a major prognostic factor that can significantly predict death in patients with cirrhosis (D'Amico *et al.,* 2013). Albumin synthesis is invariably reduced in conditions such as liver dysfunction, as a result of at least in part, decreased synthetic capacity. Thus, hypoalbuminemia could reflect a destruction in liver synthetic function, and the possibility of developing hepatic deterioration (Carvalho and Machado, 2018).

Reduced levels of albumin lead to decreased oncotic pressure, which will allow the leakage of fluids from the interstitial spaces into the peritoneal cavity, leading to conditions such as ascites. A longitudinal study previously conducted has documented a strong association of albumin with liver fibrosis (Pinto *et al.*, 2003).

2.8.1.6 Lactate dehydrogenase

The major cytoplasmic enzyme in the glycolytic anaerobic pathway that catalyzes the reduction of pyruvate to L-lactate is lactate hydrogenase (LDH) with oxidation of NADH is to NAD+. LDH is present in all tissues, with its isoenzymes in specific tissues. LDH 1 is mostly found in heart, erythrocytes, and the kidney. While LDH 3 is found predominantly in the lungs, LDH 4 is mainly present in the kidneys, placenta, and the pancreas. The predominant isozyme found in the liver and the skeletal muscle is LDH 5 (Panteginini *et al.*, 2014). Lactate dehydrogenase (LDH) has been measured in haemoglobinopathies, and used as a surrogate marker for intravascular hemolysis. Although LDH levels in the serum has been used invariably as a marker of hemolysis, the levels in combination with other blood tests may also reflect tissue damage in conditions such as liver disease.

2.8.2 Kidney Function Test

2.8.2.1 Creatinine

Creatinine is the waste product of creatine, which the muscles use to produce energy, (Kumar & Gill, 2018). The amino acids arginine, glycine, and methionine are trans-aminated to form creatine in the liver, pancreas, and kidneys. Then, when creatine circulates throughout the body, it is phosphorylated in the brain and skeletal muscle to become phosphocreatine (Heim, 2010). It is measured in milligrams per deciliter (mg/dL) or millimoles per liter (mmol/L) (Schwartz & Furth, 2007). Normal ranges are

0.6 to 1.2 mg/dL or 60 -120 µmol/L. Creatinine levels greater than 2.0 or more in babies and 5.0 or more in adults indicate severe kidney impairment.

Kidney obstruction caused by an enlarged prostate or kidney stones, is one disorder that might affect the kidneys' ability to remove creatinine from the body (Gulmi & Felsen, 2012).

Increased protein intake can potentially be a deciding factor, however studies over a longer time frame have indicated that high-protein diets have no appreciable effect on blood creatinine levels after two years (Ikizler *et al.*, 2013). Reduced or loss of muscle mass, rapid weight loss, and pregnancy all lower creatinine levels (Cartin-Ceba *et al.*, 2007; Naylor *et al.*, 2000).

2.8.2.2 Urea

Urea is a byproduct of protein metabolism and is often referred to as blood urea nitrogen (Salazar, 2014). Protein-derived amino acids are converted to ammonia via the process of deamination. The liver's enzymes then change ammonia into urea (Agnelli, 2016). As a result, the amount of urea in the blood depends on the amount of protein consumed, how well the body can break down protein, and how well the kidneys can remove urea from the body. Urea is transported by the blood to the kidneys, where it is filtered and expelled from the body through the urine (Rawitch & Baynes, 2022, Rawitch et al., 2014). About 90% of the body's urea production is eliminated if the kidneys are operating normally (Amin et al., 2014; Dobre et al., 2013). Thus, the blood urea levels can indicate how effectively the kidneys are functioning. Since it is almost entirely eliminated from the body by the kidneys, measuring it in the blood and urine can help detect kidney illness (Yakubu et al., 2003).

The quantity of urea in the blood can be impacted by a variety of liver and renal illnesses (Makris & Spanou, 2016). Urea concentrations will be high if the liver produces more of it or the kidneys are less effective at removing it.

3.0 RESEARCH METHODOLOGY

3.1 Plant Collection

The Ghana Herbarium, Department of Plant and Environmental Biology, University of Ghana, Legon, Accra was the place where whole plant of *Synedrella nodiflora* was gathered, identified, and validated.

3.2 Preparation of Extract of *Synedrella nodiflora.*

The stem and the leaves were air-dried for seven days, pulverized into powder, and cold-macerated in water containing 70% v/v ethanol. To remove traces of ethanol, the hydro-ethanolic content was evaporated under decreased pressure using a rotary evaporator (Buchi rotavapor® r-300, Flawil, Switzerland). The aqueous fraction was lyophilized after being frozen at -200°C (bench-top freeze dryer, Labfreez Instruments Co. Ltd. Beijing, China). The extract was labeled (as SNE) and stored in a refrigerator at 4-8°c after calculating the percentage yield of dried SNE (10% w/w) (Amoateng *et al.*, 2012).

3.3 Animals

The Animal Experimentation Department of the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, was where ICR mice (4-5 weeks old) weighing 20-30g were procured and kept. The animals were housed in stainless steel cages (34 cm long, 47cm wide and 18 cm high), fed a normal commercial pellet diet (Gafco, Tema), water *ad libitum*, and kept under laboratory temperature of 24–28°C, relative humidity 60–70%.

All of the procedures and techniques utilized in these studies followed the Standards of the National Institute for the care and use of laboratory animals (NIH, Department of Health Services publication no. 83-23, revised 1985). The Ethical and Protocol Review Committee of the University of Ghana's College of Health Sciences approved the study.

3.4 Determining the Sample Size for an Animal Study

The resource equation approach (E= Total number of animals – Total Number of groups) was used to estimate sample size for the various tests (Charan & Biswas, 2013). Based on the sample size, a value "E" is calculated in this approach. “E” equals the total number of animals minus the total number of groups. The value of “E” should be between 10 and 20 for an optimum sample size. If “E” is less than 10, more animals should be added; if it is greater than 20, the sample size should be reduced. A sample size for the following tests was determined as listed below:

a. PTZ kindling (n = 10) in three groups

b. Acute PTZ (8 groups, n=5)

Where “n” represents number of mice in each group.

3.5 Chemicals and Drugs

PTZ, Carbamazipine, Diazepam, Phenobarbitone sodium, and Flumazenil were all purchased at different periods of the study.

3.6 Pentylenetetrazol (PTZ) seizure Induction.

3.6.1 Acute PTZ Seizure Induction

The anticonvulsant testing method described by (Amoateng *et al.*, 2012) was used. For the acute PTZ behavioral experiments, the following groups were used;

Group 1: vehicle (0.9% saline, 0.01 ml/kg) + PTZ (75 mg/kg) – Control group

Group 2: SNE (100 mg/kg) + PTZ (75 mg/kg)

Group 3: SNE (300 mg/kg) + PTZ (75 mg/kg)

Group 4: SNE (1000 mg/kg) + PTZ (75 mg/kg)

Group 5: Phenobarbitone sodium (30 mg/kg) + PTZ (75 mg/kg)

Group 6: Flumazinil (2 mg/kg) + PTZ (75 mg/kg)

Group 7: Flumazinil (2 mg/kg) + SNE (1000 mg/kg) + PTZ (75 mg/kg)

Group 8: Flumazinil (2 mg/kg) + Phenobarbitone sodium (30 mg/kg) + PTZ (75 mg/kg)

Acute seizures were generated in drug/vehicle-pretreated male ICR mice by administering 75 mg/kg PTZ intraperitoneally. SNE (100–1000 mg/kg, orally) or Phenobarbitone sodium (30 mg/kg) intraperitoneally 30 minutes before PTZ administration.

The control animals received 0.9% saline solution orally (0.01 ml/kg). After the intraperitoneal PTZ injection, the animals were placed in a testing chamber (made of Perspex of dimensions 15 cm×15 cm× 15 cm). The episodes of convulsion induced by PTZ could be observed in a mirror angled at 45 degrees below the floor of the chamber. A camcorder (EverioTM model GZ-mg 130u, JVC, Tokyo, Japan) was set directly opposite the mirror to record the animals. The latencies as well as the duration of the seizures were determined from the video recordings using the public domain software JwatcherTM version 1.0 (University of California, Los Angeles, USA, and Macquarie University, Sydney, Australia; available at http://www.jwatcher.ulca.edu. Anticonvulsant action was defined as the ability of a drug/extract to prevent seizures or to prolong the latency or onset of tonic hind-limb extensions

3.6.2 PTZ kindling.

ICR Male mice (4 to 5 weeks old) weighing 25-30g were used for this procedure. Animals were observed in transparent Plexiglas containers that were open on the top for proper aeration. 16 Plexiglas enclosures with enough space for mice to move freely were used. The containers were thoroughly rinsed using water followed by disinfection with 70% (v/v) alcohol after each group. Observations on determining the onset of different phases of convulsions were made using a tracker software.

Different groups of mice were pretreated with *Synedrella nodiflora* Extract (SNE), Phenobarbitone sodium (PHE) and Normal Saline orally and was allowed a time lapse of 30 minutes before Pentylenetetrazol (PTZ) was injected at a threshold convulsant dose (35 mg/kg, I.P.). Animals demonstrated different phases of convulsions as follows:

Stage 0: no response

Stage 1: twitching of the ears and face

Stage 2: Neck jerks

Stage 3: myoclonic jerks

Stage 4: rolling onto the side

Stage 5: generalized tonic-clonic seizures, flipping over onto the back

The mice were allowed to acclimatize for 2 hours in the testing experimental room at the Animal Experimentation Department at the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon before initiating the kindling procedures. Animals were marked and weighed and kept in a cage for proper identification by randomization.

For the PTZ kindling behavioral investigations, the following groups were used. (n = 10 where "n" represents the number of mice in each group).

Group 1: vehicle (0.9% saline) + PTZ (35 mg/kg) – Control group

Group 2: SNE (1000 mg/kg) + PTZ (35 mg/kg)

Group 3: Phenobarbitone sodium (30 mg/kg) + PTZ (35 mg/kg).

The dose volume for all the injections were kept constant (10 ml/kg). On day one, each mouse received either Normal Saline, SNE or PHE, the volume of which was based on their body weight, 30 minutes before PTZ injection. Mice were then observed for the stages of the PTZ kindling for 30 minutes. When all three groups had been treated, animals were placed in cages for overnight recovery. The kindling procedure repeated every other day over a period of 35 days and the mice were considered fully kindled if the mice demonstrated stage 4 or 5 on two consecutive PTZ doses. On every kindling day, mice were weighed and also observed for abnormalities. A week after the last PTZ injection, animals were challenged with same sub-convulsant dose of pentylenetetrazol (35 mg/kg, i.p.). After completion of the experiment, mice were euthanized using a carbon dioxide euthanasia chamber. Blood samples were immediately taken for biochemical analysis. The hippocampus of the brain was resected for histological studies as described below.

3.7 Histology of the Hippocampus

Brain tissue was taken and placed in a formaldehyde solution after the mice were quickly decapitated before being dried with ethanol (70 percent for 24 h, 90 percent for 1 h and 100 percent for 1 h). After that, it was cleaned in xylene before being embedded in paraffin. Coronal slices of the temporal lobe of the brain were cut at 5 µm thickness exposing the hippocampus with a microtome (Leica RM 2025, Germany). The slices were mounted on glass slides, and stained with hematoxylin and eosin. Hematoxylin is used to illustrate nuclear detail in cells whilst Eosin is the

most commonly used counterstain that distinguishes between the cytoplasm and nuclei of cells (Blumcke et al., 2017). The hippocampus neuronal cell count was subsequently determined using light microscopy.

3.8 Biochemical Analysis

Intraperitoneal blood sample was collected and stored in plain glass tubes. Serum was obtained after centrifugation of the clotted blood. The serum was for evaluation of liver and kidney functions. Albumin, Globulins, Alkaline Phosphate, ALT, AST, Direct Bilirubin, Indirect Bilirubin, Total Bilirubin, Total Protein, Creatinine, Urea, and Blood Urea Nitrogen were all measured using an automated multi-parameter blood analyzer (Selectra pro and Cobas 311). (Thapa & Walia, 2007). 5 milliliters of serum were pipetted into the micro titer, which was then placed in the blood analyzer and analyzed automatically.

3.9 Statistical Analysis

GraphPad Prism version 5.0 for Windows, (GraphPad software, La Jolla, California, USA, www.graphpad.com), was used to analyze quantitative data. The means of Racine scores, as well as the weight of mice during the PTZ induced kindling procedure, were compared using a two-way ANOVA followed by Bonferroni post-tests. One-way ANOVA was used to examine the area under the curve, latency, frequency, and duration of convulsions during acute PTZ. $P<0.05$ was considered statistically significant. The mean ± SEM was used to express all of these variables. The frequencies were used to present all category data.

CHAPTER FOUR

4.0 RESULTS

4.1 Stages of PTZ kindling procedure scored on the Racine scale.

Repeated administration of 35 mg/kg of PTZ every other day caused a gradual increase in the seizure intensity as scored by the Racine scale in the Control group as compared to SNE and PHE group. By the 11th day, the score had increased from 0 to 2, and attained a severity on the Racine score of 3 by day 35 It showed a steady rise to 4 by the 35th day through to the 42nd day (Figure 1A). SNE significantly depressed the kindled seizures ($F_{2, 20} = 12.55$, $P = 0.0003$; Figure 1A) and none of the animals in these treatment group attained seizure score 4 or 5. The percentage severity of seizures (calculated from the AUC) shows that SNE reduced PTZ kindled seizure activity by reducing the Score 4 on the Racine scale by 35%, Figure 1B). Phenobarbitone (30 mg/kg) also produced a significant depression of the kindled seizure activity ($P < 0.0003$; Figure 1A) and the percent severity of seizures was significantly reduced by 25%, p value of 0.001Figure 1B).

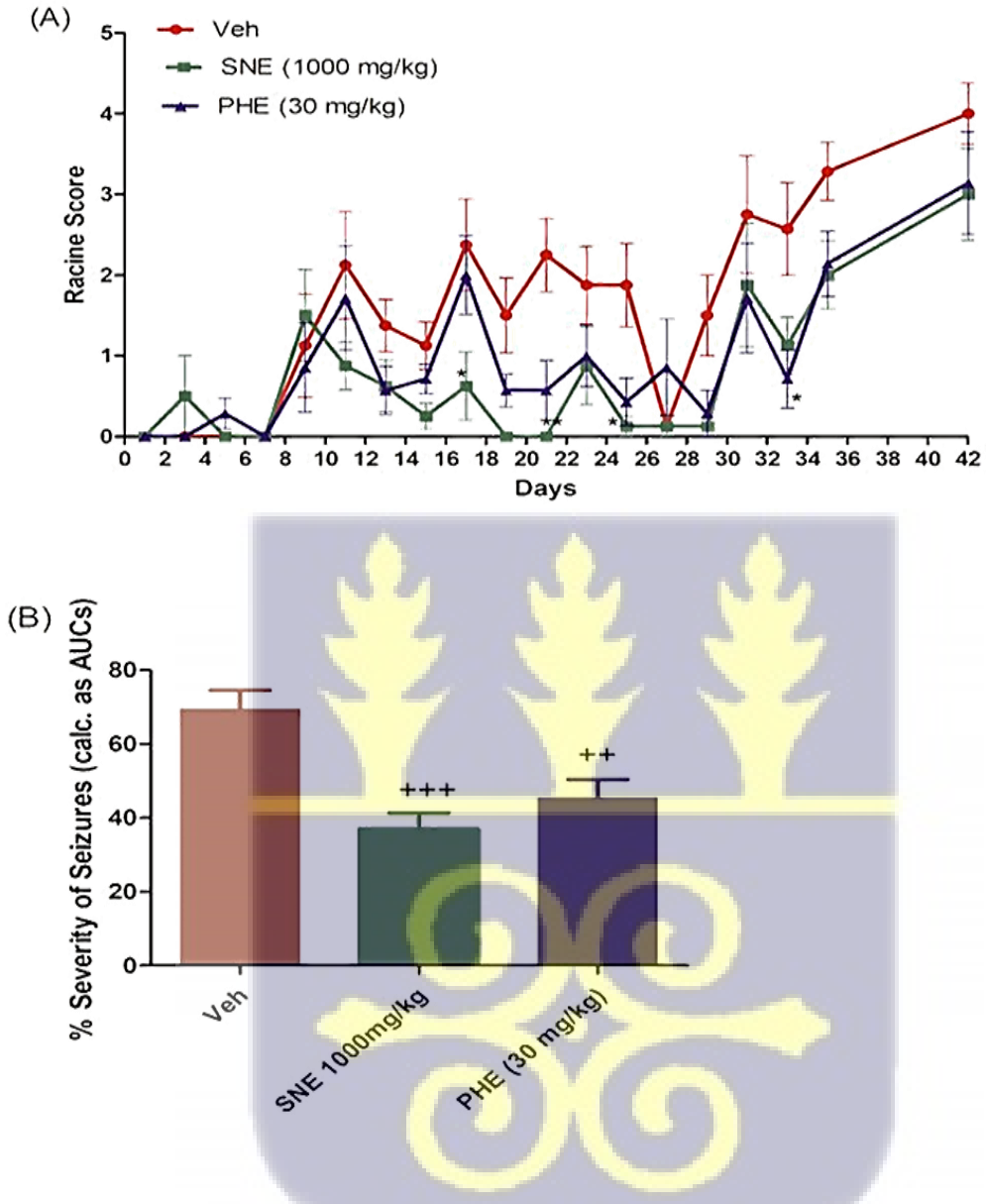

Fig 1.0 Seizure Intensity during PTZ-Induced Kindling

SNE (1000 mg/kg) and PHE (30 mg/kg) dose–response effects on PTZ-kindled mice.

(A) The time course of effects for all three groups (CTL- Red, SNE- Green, and PHE-blue) over a 42-day period was plotted using mean SEM (n = 10) and the values were displayed using Mean ± SEM. (n=10), P<0.05 when compared to the control group (2-way ANOVA followed by bonferroni post-tests)

(B) For the course of the test, the AUCS was used to compute the % severity of seizures. The Mean ± SEM (n = 10) was used to plot the data. (+++p=0.0003, One way ANOVA followed by Newman-Keuls Post Hoc test).

4.2 Latency, frequency and duration of convulsions following Acute PTZ induced seizures.

PTZ induced a sequence of events starting with myoclonic jerks which was then followed by an intense clonic convulsive phase. The SNE extract, showed significant anticonvulsant effect against seizures induced by PTZ. SNE increased the latency of the myoclonic jerks (F3, 20 = 4.111, P =0.0250; Figure 2A) and these effects were significant at dose levels of SNE 100, 300 and 1000 mg/kg. It equally reduced the frequency of convulsions significantly (F4, 20 =5.18, P=0.0051, Figure 3B). However, SNE produced no significant effect on the duration of the seizures ($F_{4, 18}$ = 2.396, P = 0.0885; Figure 2C).

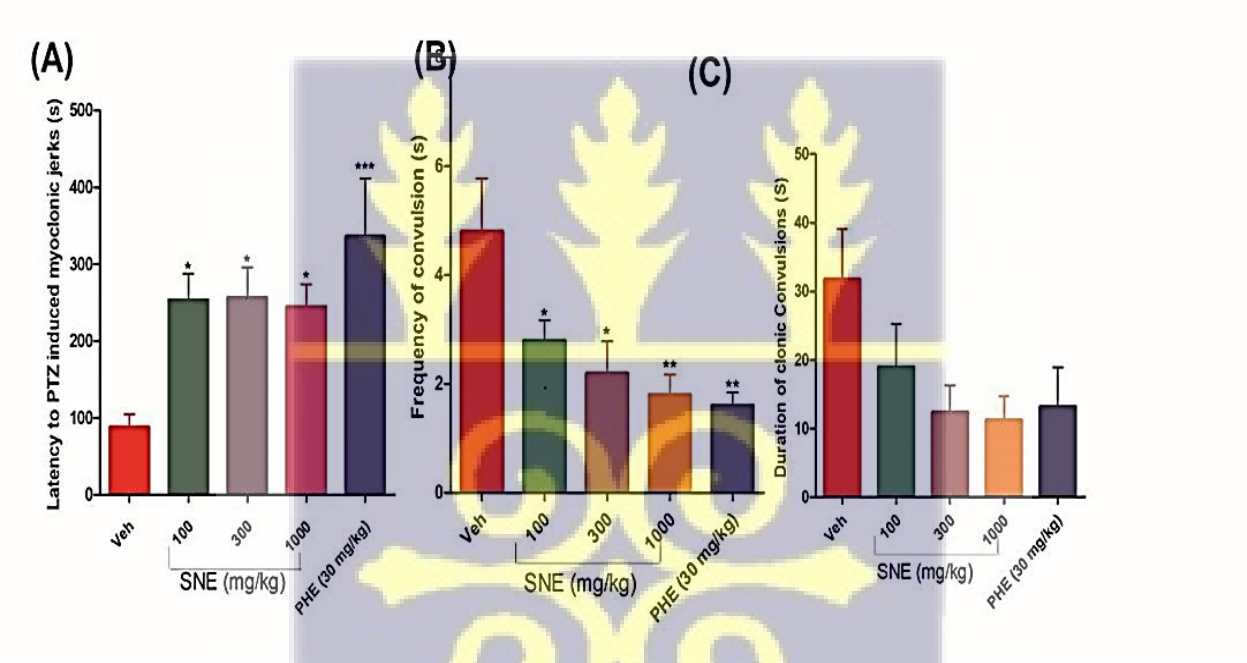

Figure 2: Effects of SNE (100, 300 1000 mg/kg) and PHE (30 mg/kg) on PTZ-induced seizure latencies, frequencies, and duration. The mean ± SEM (n = 5) is represented in each column compared to the Control group (one-way analysis of variance followed by Newman-Keuls post hoc test) (P=0.0250; figure 3a, p=0.0051, figure 3b, p = 0.0885; figure 3c).

4.3 Effect of Flumazenil (GABA receptor Blocker) on SNE and Phenobarbitone sodium following induced convulsions

SNE (1000 mg/kg) administered after Flumazenil (10ml, i.p) injection did not significantly affect latency, frequency and duration compared to control group. Diazepam, on the other hand, increased the latency of onset of seizures significantly ($p < 0.01$), but had no significant effect on the frequency and duration of seizures ($p<0.6$ and $p<0.065$ respectively) compared to control (table 1).

Table 1. Effect of Flumazenil (GABA receptor blocker) on SNE and Phenobarbitone Sodium following PTZ convulsions.

Drug (Dose)	Latency	Frequency	Duration
Flumazenil (10 ml) + CTL (0.9w/v saline)	155.8 ± 25.90	2.4 ± 0.7	29.25 ± 10.05
Flumazenil (10 ml) + SNE (1000 mg/kg)	96.00 ± 7.66	1.8 ± 0.2	29.25 ± 8.03
Flumazenil (10 ml) + PHE (30mg/kg)	242.2 ± 25.21*	2.8 ± 1.1	28.50 ± 5.07

Values are expressed as Mean ± SEM (n=5). *P<0.05 compared to vehicle treated control group. (One-way ANOVA followed by Newman-Keuls Post hoc test).

4.4 Biochemical Analysis after PTZ Kindling Procedure

The liver and the kidney parameters were examined after the PTZ procedure. Parameters such as albumin and Alkaline Phosphate (ALP) showed a significant difference comparing SNE (1000 mg/kg) to the control group (CTL) ($p<0.3$). Generally, there was a slight insignificant rise in liver enzymes ALT and AST across the three groups however. Creatinine levels were generally reported to be low across all the groups with Urea and Blood Urea nitrogen (BUN) been reported to be in normal ranges.

Table 2: Biochemical analysis after PTZ kindling Procedure.

LFT (Unit)	CTL	PHE (30 mg/kg)	SNE (1000 mg/kg)
Albumin (g/L)	20.8 ± 1.3	29.7 ± 1.5**	35.0 ± 4.3***
Globulins (g/L)	39.9 ± 2.5	40.2 ± 3.4	41.8 ± 8.3
ALP (U/L)	35.3 ± 2.5	23.0 ± 3.5	60 ± 12.2*
ALT (U/L)	70.8 ± 6.2	64.0 ± 9.1	103.3 ± 21
AST (U/L)	407 ± 70.9	296. 5 ± 45	403 ± 128
Direct Bilirubin (umol/L)	10.9 ± 1.3	16.5 ± 6.7	27.6 ± 9.0
Indirect Bilirubin (Umol/L)	94.6 ± 18.5	150.2 ± 45	167.0 ± 52
Total Bilirubin (Umol/L)	112.0 ± 17.7	199 ± 73.4	245 ± 99.5
Total Protein (g/L)	61.3 ± 1.9	69.5 ± 4.0	77.5 ± 14.6
KFT			
Creatinine (Umol/L)	24.8 ± 2.2	27.9 ± 1.6	30.4 ± 8.8
Urea (mmol/L)	5.7 ± 0.5	6.2 ± 0.4	6.9 ± 0.6
BUN (mmol/L)	2.8 ± 0.2	2.9 ± 0.1	3.2 ± 0.27

Values are expressed as Mean ± SEM (n=5). *P<0.05 compared to vehicle treated control group. (One-way ANOVA followed by Newman-Keuls test).

4.5 Histology of the Hippocampus following PTZ kindling.

Fig 4 shows the stained sections of the hippocampus as well as the neuronal cell density count (in thousands of cells per mm^3) after chronic PTZ induced kindling procedure. There was a slight hippocampal neuronal cell increment of SNE (1000 mg/kg) and PHE (30mg/kg) compared to the control group, however they were not statistically significant (P= 0.0631).

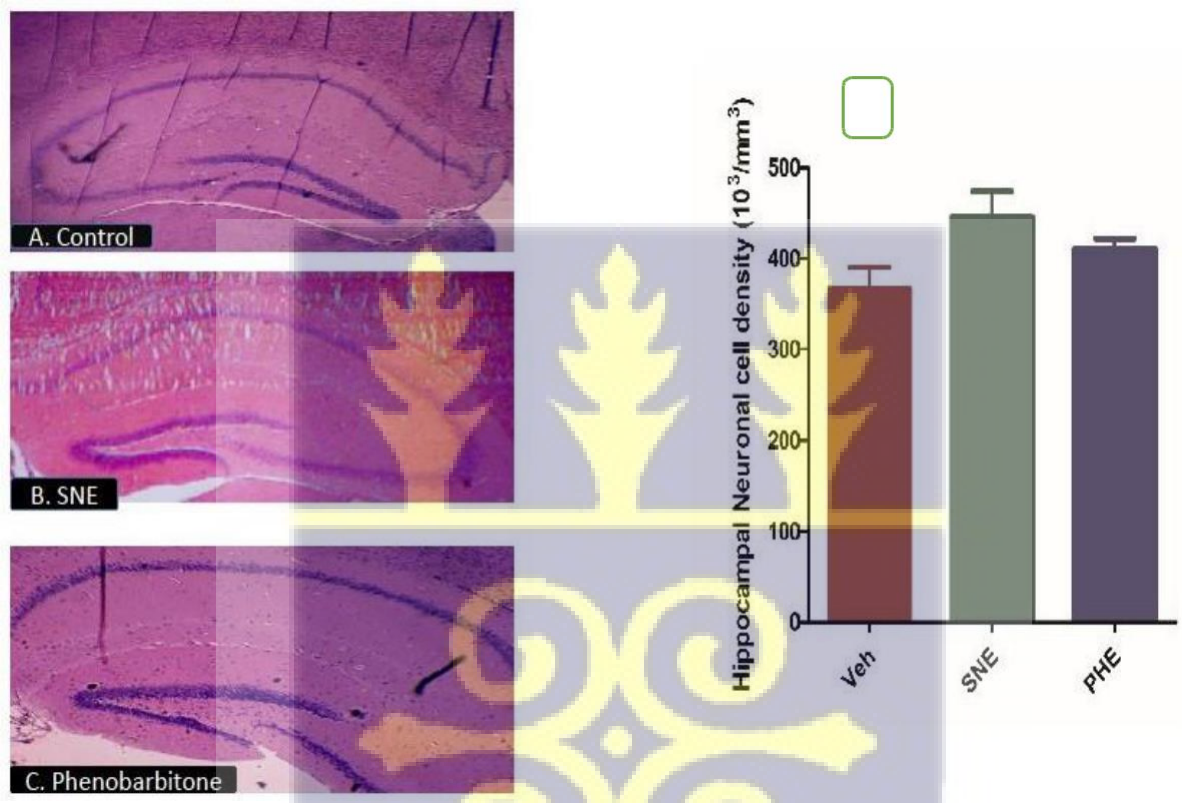

Fig 3. Photomicrograph of the hippocampus of the brain following PTZ induced kindling.

A- Control group, B- SNE and C-PHE). A graph of the neuronal cells count has been plotted (D). Values were plotted using Mean ± SEM (One-way ANOVA followed by Newman-Keuls post hoc test).

4.6. Average Mice Body weight After PTZ kindling.

Fig. 5 shows the weight of the mice following the PTZ kindling procedure. In the control group, there was a steady rise in weight reaching a peak weight of 35g on day14. This was followed by a fall in weight till day 17 and started to rise again. This is slightly different in the case of SNE which achieved a peak weight of 34g on day 12 followed by a steady decline till day 19. It was steady after day 21. There were no statistical differences observed comparing the weight of SNE (1000 mg/kg) with the control group. Phenobarbitone Sodium (30 mg/kg), a reference drug did reach a peak weight of 32g on day 13 after which it was characterized with steady decline till the last day.

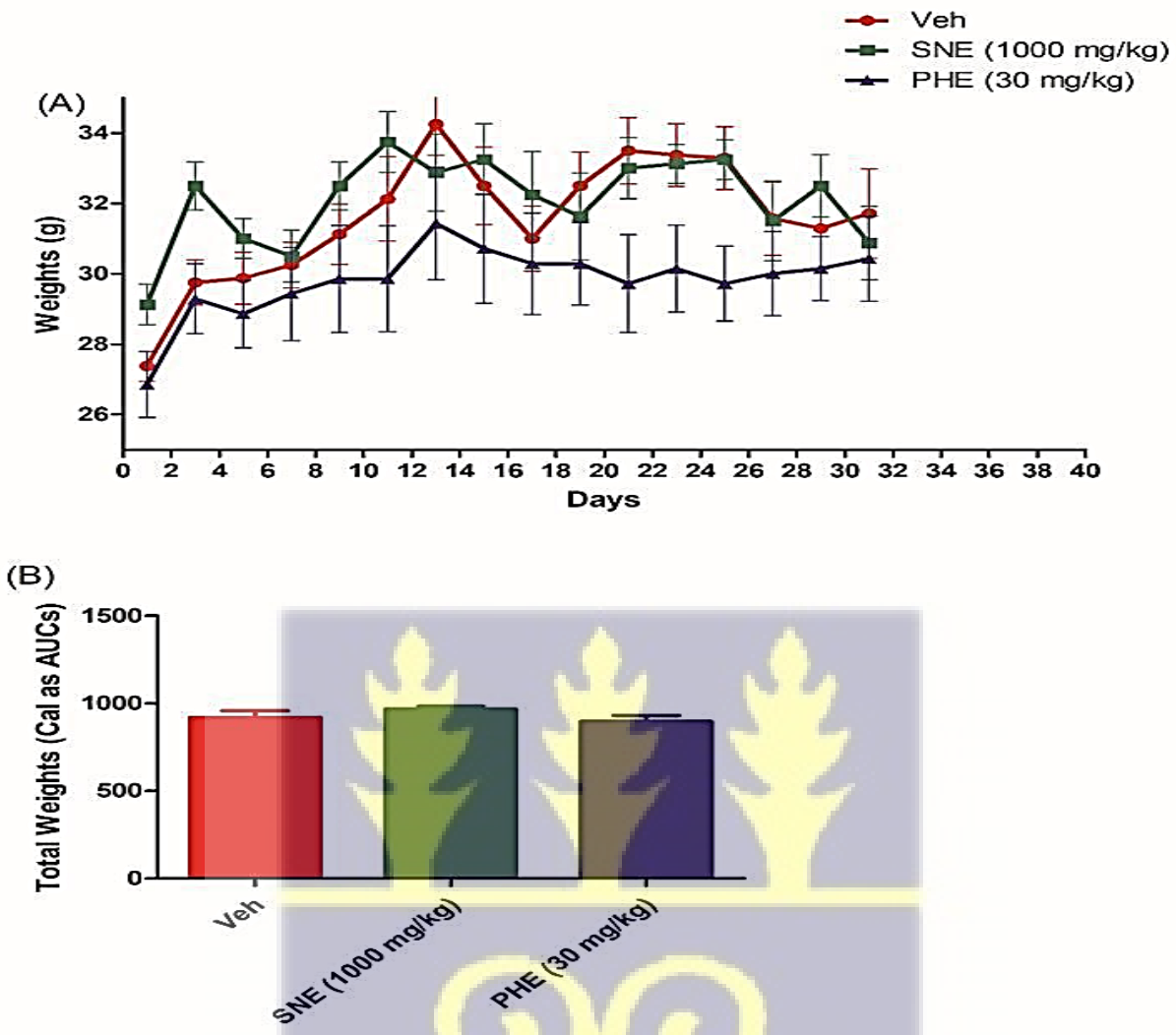

Fig.4 Effects of PTZ kindling on Body weight

The effect of SNE (1000 mg/kg) and PHE (30 mg/kg) on body weight following PTZ induced convulsions. Values were plotted using mean ± SEM (n = 10). (A- 2-way ANOVA followed by Bonferroni post-tests). (B- One-way ANOVA followed by Newman-Keuls Post Hoc Test).

CHAPTER FIVE

5.0 DISCUSSION

5.1 SNE Inhibited Both Acute and Chronic PTZ induced Seizures Suggestively via GABAergic Pathway.

The study revealed that SNE inhibited PTZ-induced seizures in chronic phases. The PTZ test represents a valid model for human generalized and absence seizures. PTZ has been used experimentally to study seizure phenomenon and to identify drugs that may control seizure susceptibility (Amoateng *et al.*, 2012; Hamed, 2019).

The neuronal basis underlining the mechanism of seizure action of PTZ still remains unclear.

In this study, pretreating the mice with flumazenil ($GABA_A$ receptor blocker) followed by SNE administration showed no significant difference after PTZ induced seizures compared to the control group. This somewhat suggest that the extract reduce epileptic seizures via the GABAergic Pathway.

Reference anticonvulsant drugs such as diazepam and phenobarbitone, inhibit PTZ-induced seizure by enhancing the action of $GABA_A$ receptors, possibly by facilitating the GABA-mediated opening of chloride channels (Mohler *et al.*, 2002). Postsynaptic $GABA_A$ receptors are multiunit complexes with binding sites for the endogenous ligand GABA, benzodiazepines, barbiturates, and other ligands with a central chloride ion channel (Ralvenius *et al.*, 2015; Rudolph & Knoflach, 2011). Hence for SNE to reduce both latency, frequency of convulsions when applied alone but not when administered after flumazenil during acute PTZ induced seizures supports the assertion that SNE acts via the GABAergic pathway.

5.2 SNE significantly reduced PTZ induced seizures

The outcome of this study also points to a significant progressive decrement of seizure induced by PTZ kindling measured using the Racine score. PTZ-induced kindling is an acknowledged

experimental model of human seizures and useful for the study of seizure mechanisms (Samokhina & Samokhin 2018; Dhir 2012). It is characterized by cerebral deficit, changes in emotional behavior, and neuronal cell loss in hippocampal CA1, CA3, CA4 structures and dentate gyrus (Zhu *et al.*, 2016; Dhir 2012). This in contrast with the result of this study wherein there was some neuronal cell lost in the control group compared to the extract group even though it was not statistically significant.

SNE's ability to prevent full kindling by PTZ in mice may be as a result of its ability to inhibit lipid peroxidation and/or scavenge free radicals. This is supported by the fact that free radical generation due to increased activity of the glutamate plays a fundamental role in neuronal cell death associated with PTZ kindling in mice (Kutluhan *et al.*, 2009; Bashkatova *et al.*, 2003). When the production of free radicals increases or the defense mechanism of the body decreases, lipid peroxidation at polyunsaturated sites on biological membranes occurs leading to cell dysfunction (Akbas *et al.*, 2004).

5.3 Effect of SNE on Biochemical Indices.

The study as well investigated the effects of chronic PTZ induced kindling on Renal and Liver indices of the ICR mice. Although over all, there was a general increment in the serum concentrations for the liver enzymes, however, it was not significant comparing treatment groups to the control. The observed increment in the enzyme levels associated with liver function (ALT, AST & ALP) following the PTZ induce kindling procedure suggests organ damage especially the liver. This may not be extensive and as such does not raise cause for concern. However, further studies will be recommended to ensure the full safety profile of SNE should these studies proceed to clinical stages.

Serum alanine transaminases (ALT) is not only measured to determine a possible liver damage, but also useful in monitoring the general health status (Vazquez *et al.*, 2020; Joni *et al.*, 2020).

This enzyme is mainly found in the cytosol of the hepatocytes, and plays a role in gluconeogenesis (Moriles and Azer 2020). ALT as well as the other enzymes are released as a result of liver injury from the injured liver cells into the serum, which causes the levels to increase significantly in the serum (Ghosh *et al.*, 2020; Smith *et al.*, 2020).

Also, low creatinine levels observed and it can be associated with less muscle metabolism in the mice. This correlates with a study by Rodrigues *et al.*, (2014) whose study sought to establish standards for renal functions in mice through body size adjusted creatinine and urea levels. In their study they found out body size and muscle mass had influence on creatinine levels. As there was treatment induced weight changes in this study, changes in the creatinine levels would not have been strange. Nonetheless, one can only conclude that the weight changes were not drastic enough to be reflected in the creatinine levels.

Overall, the biochemical parameters (Kidney and Liver functions) assessed did not show any deteriorating effect following the application of the extract SNE.

CHAPTER SIX

6.0 Conclusion and Recommendation

6.1 Conclusion

The result of this study provides evidence that the ethanolic extract of the whole plant of *S. nodiflora* possesses antiseizure activity and suggested to be via the GABAergic pathway in murine experimental models. Also, the biochemical analysis which assesses the overall health of the kidney and the liver was normal in the dose dependent SNE group. Therefore, the effectiveness of the plant's extract used suggests that the herb can be used in treating seizures.

6.2 Recommendations

Future studies that will seek to explore the other pathways (eg. Glutamate pathway) is highly recommended. Also, an electrophysiological study to measure electrical changes in the entire brain specifically the hippocampus of the brain following the extract application would yield a better understanding of the mechanism of action of the extract in the brain. And lastly future studies focusing on Calcium Imaging Technique to check the impact of the extract on calcium ions at the synapse is greatly recommended.

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