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COLLEGE OF HEALTH SCIENCES

ANTICONVULSANT EFFECT OF ETHANOLIC LEAF EXTRACT OF EHRETIA CYMOSA THONN (BORAGINACEAE) IN MURINE MODELS

BY

LARTEY RICHARD NELSON

(10552293)

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN PHARMACOLOGY DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

JULY, 2018

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DECLARATION

DECLARATION BY THE CANDIDATE

I hereby declare that this is the product of my research undertaken under supervision and has neither been presented in whole nor part for another degree elsewhere. I am solely responsible for any residual flaws in the work.

Signature…….. Date 03/ 05/2022

LARTEY RICHARD NELSON (10552293)

DECLARATION BY SUPERVISORS

We hereby declare that the principal work and presentation of the thesis were supervised by us by guidelines on the supervision of the thesis laid down by the University of Ghana.

Principal supervisor

Signature…… Date 03/ 05/2022

(DR. PATRICK AMOATENG)

Co-supervisor

Signature…… Date 03/ 05/2022

(DR. KENNEDY E. KUKUIA)

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ABSTRACT

Purpose: *Ehretia cymosa* is used locally in Ghana to treat epilepsy. To validate this anecdotal information with scientific data, the anticonvulsant effect of the ethanolic extract of the leaves of *Ehretia cymosa* was studied in murine models.

Materials and Methods: The potential anticonvulsant activity of an ethanol extract of *Ehretia cymosa* (ECE) (30, 100, and 300 mg kg^{-1}) was tested employing the acute pentylenetetrazole (PTZ)-, PTZ-induced kindling, picrotoxin-induced seizures and maximal electroshock (MES) in mice. The extract's effect on motor co-ordination and nociception was also tested using the rota-rod and the hot plate tests respectively. Acute and sub-chronic toxicity tests were also done to ascertain how safe the extract is in rodents.

Results: This study showed that, the ethanolic extract of ECE possesses anticonvulsant effects in the various seizure threshold models used, except in the maximal electroshock seizure model. The latencies to the first myoclonic jerks were increased by ECE while both the duration and frequencies of seizures reduced significantly. There was however no effect on motor coordination even when highest dose of 300 mg kg^{-1} was used. No mortalities were recorded in the animals used during the period of this study. The ECE also did not have any significant effect on any of the serum biochemical parameters after the study.

Conclusions: The ethanolic extract of the leaves of the *Ehretia cymosa* was found to possess anticonvulsant properties, and possibly acts through the GABAergic transmission pathway but has no muscle relaxant properties. The findings from this study, therefore, give scientific

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credence to the traditional use of *Ehretia cymosa* to manage epilepsy. The 10-week oral administration of the extract of *Ehretia cymosa* under the prevailing laboratory conditions was found to be relatively safe in male Sprague–Dawley rats.

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DEDICATION

I dedicate this work to my wonderful parents, Mr. John Attuah Lartey (of blessed memory), and Mrs. Attuah Gloria Lartey. You have been a blessing in my life.

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ACKNOWLEDGMENT

I wish to express my utmost gratitude to God for His continuous blessings and mercies throughout this study.

My profound appreciation goes to my mentor and supervisor Dr. Patrick Amoateng. Thank you for your support, expert direction, guidance, and great life lessons that you have taught me. For the mentorship I have received, I must say you have inspired me to aim far and beyond. I also say a hearty appreciation to Dr Kennedy Edem Kukuia, my co-supervisor for his mentorship and guidance as well. I say a very big thank you to all senior members, all lecturers, and technical support staff of the Department of Pharmacology and Toxicology, for the immense encouragement and emotional support.

To the Head of Department of Animal Experimentation, Noguchi Memorial Institute for Medical Research (NMIMR), Dr. Samuel Adjei, I am glad to have been allowed to work in such an august department. I have learned some technical skills that would be with me forever. I thank all the staff and technical workers, especially Mr. Ahedor Believe, for his selfless assistance and help during my work there. I am also grateful to the technical staff of the Pharmacognosy department of the School of Pharmacy of KNUST especially Mr. Osafo Asare for his help in the collection of my plant sample.

I am also very thankful to my work colleague Ms. Authentia Sokpe who pushed me to complete this work when I felt like giving up. May the Almighty God bless you abundantly.

To my parents, Mr. and Mrs. Lartey, may God continue to bless you. Thank you for your financial support and advice. A big thanks to my family, especially my sister Lily who has been a mother and friend to me throughout my work. To all my colleagues, thank you all for your encouragement and support. God bless you. To friends, family, strangers, and anyone who has contributed to the successful completion of this work, I say may the Lord richly bless you.

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ABBREVIATIONS

AED	Antiepileptic drugs
CBZ	Carbamazepine
CNS	Central Nervous System
DZP	Diazepam
ECE	*Ehretia cymosa* Extract
ED_{50}	Effective dose for 50%
EEG	Electroencephalography
GABA	γ- aminobutyric acid
GABA-T	GABA Transaminase
GAT1,	GABA transporter 1
HLTE	Hind limb Tonic extensions
ILAE	International League Against Epilepsy
LD_{50}	Lethal dose which causes the death of 50%
MEST	Maximal Electroshock Threshold test

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MRI	Magnetic resonance imaging
NMDA	N-Methyl-D-aspartate
PIC	Picrotoxin
PTZ	Pentylenetetrazole
SE	*Status epilepticus*
SV2A	Synaptic vesicle protein 2A
TLE	Temporal lobe epilepsy
VPA	Valproic acid

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CHAPTER 1 INTRODUCTION

1.0 GENERAL INTRODUCTION

Epilepsy is a disease condition that dates back to the very existence of mankind with references in ancient writings including the Bible (WHO, 2019; King James Version, Matthew 17:14-15). In the pre-Hippocratic era, the condition was however thought of as a retribution for offences committed. Thus, its treatment was sought in the temples (York, 2005). This can be seen where one man pleaded with the Messiah to cure his son who usually exhibited the symptoms of epilepsy (King James Version, Matthew 17:14-15). By the man's description, it can be inferred that he believed the disease had a spiritual cause and as such needed spiritual healing. The Messiah went ahead and healed the said boy who was described by the father as a lunatic; sorely vexed and often times fell into the fire (King James Version, Matthew 17:17 -18). However, Hippocrates, the father of medicine, disputed the mystical cause of epilepsy. To this, he said that the only reason men thought epilepsy was divine was that there was no understanding of the disease (York, 2005) Modern physicians also agree with this view. It is therefore incumbent on the scientist to explore the scientific dimension to the disease epilepsy.

The International League Against Epilepsy (ILAE) defines epilepsy as a disease of the central nervous system (CNS) which is also characterized by seizures. The seizures are transitory, unprovoked, sudden, and recurrent episodes of abnormal hypersynchronous neuronal discharge. In other words, it is a brain condition which is associated with the occurrence of more than one epileptic seizure. There is also an increased susceptibility to generate more

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seizures in addition to psychological, cognitive, neurobiological and social disturbance (R S. Fisher et al., 2005). Epilepsies are classified into four groups namely: partial (focal) seizures, generalized seizures, combined generalized and focal, and the unclassified (unknown) seizures. Categorization of the condition is determined by the type of seizure. The partial type of seizures stem from networks linked to one hemisphere of the brain and this is usually accompanied by an underlying structural disease. On the other hand, generalized seizures originate from bilaterally distributed networks which lead to the concurrent start of extensive electrical discharge with no localizing characteristics referable to a single hemisphere (Wilkinson et al., 2017). The combined generalized and focal type are seizures which have characteristics of both focal and generalized types of seizures. The unknown epilepsy refers to one with little information on the clinical condition. The unknown type of epilepsy may be re-classified as focal, generalised or combined generalized and focal types of epilepsy if later enough clinical information becomes available (Chang, Leung, Ho, & Yung, 2017).

Though epilepsy is one of the oldest documented conditions dating as far back as the year 4000 BC **(**WHO, 2019), successful cure has not been achieved. Thus, an estimated sixty-five (65) million still live with the condition and out of this number, the active epilepsy cases with recurrent seizures requiring regular treatment at any given time is estimated to be around 4 to 10 per 1000 people (in developed countries) and 7 to 14 in developing countries. In addition to this, the number of people that are estimated to be diagnosed with epilepsy each year from these countries is said to be around 2.4 million relative to 30 to 50 per every 100 000 people (Vogel, Franz, Jochen, & Dieter, 2015).

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To compound this worrying statistics, there is fear, discrimination, and social stigma attached to the disease and this can even be used as grounds to annul or prohibit marriages in some countries (Vogel et al., 2015). Aside from these, the treatment and management of epilepsy with various anticonvulsant drugs have a lot of side effects. Some of these unwanted effects such as aberrant gum growth (gum hyperplasia), hardening of the face, and excessive hair growth or hirsutism affect the physical appearance or the beauty of the patients. There are also recorded cases of cognitive impairment, hypersensitivity reactions, behavioural changes, and peripheral neuropathy. There are also issues like Dupuytren's contractures, gastrointestinal problems, hyponatremia, leukopenia, weight gain, and endocrine changes reported as side effects of taking some of these antiepileptic drugs. There is also the issue of tolerance and dependence associated with the use of antiepileptic drugs (Löscher & Schmidt, 2006). These effects are experienced depending on the type of medication being used to manage the condition (Vogel et al., 2015). The numerous adverse effects associated with anti-seizure drugs have negatively impacted the clinical effectiveness of the medications.

Also, the socio-economical differences between the developed and developing world impact clinical outcomes largely. Epilepsy places a heavy economic burden on both patients and their countries. Consequently, it is pertinent to assess the treatment and management of epilepsy so as not to only to increase quality of life, but also consider the financial or economic burden of the prescribed treatments for this disease condition (Liu, Liu, & Meng, 2013). For these reasons, there should be a pragmatic research into novel plant-based medicines that are readily available and safer to use for the control of convulsions.

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Ehretia cymosa, known as *Okusua* in the Twi, is a shrub or small tree with drooping branches and can be found in the savannah, forest thickets, and cultivated lands (Mshana, 2000). The infusion of the leaves is used as a bath to manage fevers and convulsions. The roots are used to cure tetanus and dysentery, whereas the leaves are employed in the treatment of headaches, constipation in children, and fractures (Mshana, 2000). The hydroethanolic extract of the leaves has been reported to have antidiabetic, antimicrobial, and antioxidant properties (H. M. Burkill, 1985). Despite its folkloric use for convulsions, there is no scientific data confirming its anti-convulsant or antiepileptic properties. There is therefore the need to investigate its antiepileptic and related neuropharmacological properties as well as its safety.

1.1 PROBLEM STATEMENT

An estimated 65 million people suffer from epilepsy worldwide and it is the most predominant cause of chronic neurological diseases. Even more frightening is that, about 30 percent of the people who live with epilepsy are unresponsive to clinical management (Łukawski et al., 2018). Unfortunately, a third of these people in this category live in Africa, and this accounts for nearly 16% of people afflicted globally. Furthermore, the problem is compounded by a wide treatment gap that can even be as high as 100 percent in some suburbs in the African region (Mugumbate & Zimba, 2018). People living with epilepsy have, throughout history, been associated with the divine, demonic, and supernatural attributes (Devinsky & Lai, 2008). The mystical associations with the disease coupled with poor scientific understanding of the disease have resulted in serious social implications for patients,

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especially for women due to the stigma attached to the condition (Kpobi, Swartz, & Keikelame, 2018). In addition to these, antiepileptic drugs (AEDs) have side effects and poor tolerability which greatly affects patients' compliance. Patients on antiepileptic drugs go through a strained social and emotional wellbeing due to physical changes like acne, alopecia, hirsutism and weight gain which in turn lead to a low quality of life (Chen et al., 2015). These side effects lead to increased drug non-adherence which in turn lead to reduced effectiveness of AED regimen leading to higher rates of road traffic accidents, injuries and fractures (Faught, Duh, Weiner, Guerin, & Cunnington, 2008). The consequence of this AED non-adherence is increased mortality rates and adverse clinical outcomes. This can lead to patients being incorrectly classified as having a refractory epilepsy (O'Rourke & O'Brien, 2017). In addition to these, non-adherent adults with epilepsy (AWE) are known to run the risk of developing convulsive *status epilepticus* (Skinner et al., 2010). The most serious outcome of AED non-adherence is the increased risk of sudden unexplained death in AWE (Lathers, Koehler, Wecht, & Schraeder, 2011). It is therefore imperative to identify the barriers to AED and remove those hurdles to enable a better management of this disease condition.

Aside from the cosmetic side effects, some of the currently available anti-seizure drugs like felbamate, phenytoin and valproic acid have been implicated in liver toxicity (Vidaurre, Gedela, & Yarosz, 2017). With regards to primary health care, an estimated 80 percent of the worldwide population depend on herbal medicines (Sam, 2019) hence herbal medicines can be a good source of antiepileptic drugs that are easily accessible, able to prevent epileptogenesis and with few side effects.

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AIM

This research seeks to investigate the anti-epileptic effect of the ethanolic extract of *Ehretia cymosa.*

OBJECTIVES

1. To determine the effects of *Ehretia cymosa* extract on motor co-ordination and nociception.
2. Investigate the effect of the extract of *Ehretia cymosa* extract on convulsive threshold tests using the acute and chronic models of convulsion.
3. Perform acute, sub-acute, and sub-chronic toxicity tests.

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CHAPTER 2 LITERATURE REVIEW

1.2 EPILEPTIC SEIZURES

Epileptic seizures are defined as transient occurrences of signs and/or symptoms as a result of excessive, aberrant or hypersynchronous neuronal brain activity (Falco-Walter, Scheffer, & Fisher, 2018). Approximately 65 million people in the world are affected by this condition (Ngugi, Bottomley, Kleinschmidt, Sander, & Newton, 2010) and the prevalence of this condition is around 6.4 cases per 1000 people according to data available (Fiest et al., 2017). Even though it is one of the most predominant and disabling neurologic diseases, there is still not a complete comprehension of the pathophysiology and proven treatment regimen to combat the condition (Stafstrom & Carmant, 2015). Case management of the condition is usually by the use of antiepileptic drugs. However, there are about one-third of such patients who do not attain seizure control with the available drugs (Devinsky et al., 2018).

One of the most effective ways to help eliminate these seizures is through surgeries but only a small percentage of these patients are qualified for such surgeries (Wiebe, Blume, Girvin, & Eliasziw, 2001). A lot of the patients whose conditions have become drug-resistant are not eligible and the options available for such patients are the use of neurostimulation devices, adherence to the good dietary regimen, or partaking in clinical trials for new anti-seizure drugs (ASDs) (Wiebe et al., 2001) patients may relapse. An estimated 10 percent of the people in the world will experience a seizure at least once in their life (Hauser & Beghi, 2008). Epilepsy can lead to death directly through events like head injuries from falls, burns, drowning, vehicular accidents, sudden death that occurs in epilepsy and *status epilepticus*. There can be indirect causes like suicide, pneumonia, or through the adverse drug reactions of

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anti-seizure drugs like obesity and cardiovascular reactions (Devinsky, Spruill, Thurman, & Friedman, 2016).

1.3 SEIZURES AND EPILEPSIES

Seizures are defined as paroxysmal change of neurologic function due to excessive, hypersynchronous neuronal discharge in the brain. It is usually unprovoked and recurrent and the cause can be attributed to an underlying brain disorder (Shorvon, Andermann, & Guerrini, 2011). When the cause of seizures is through reversible condition like fever or hypoglycemia, it is not considered epilepsy (Shorvon *et al.*, 2011). Epilepsy syndrome on the other hand refers to a pattern of clinical manifestations that consistently occur together with seizures as a primary manifestation. Some of the features of an epilepsy syndrome include similar age of onset electroencephalogram (EEG) findings, etiology, inheritance pattern and response to particular antiepileptic drugs (Stafstrom & Carmant, 2015).

Seizures are classified into 3 groups: generalized type seizures, focal type seizures which was formerly called partial, and epileptic spasms. Focal seizures stem from neuronal networks specific to one area of the cerebral hemisphere. Generalized seizures originate in bilateral distributed neuronal networks. A focal seizure can later become a generalized seizure. For an appropriate diagnosis to be made, a physician must combine a comprehensive history in addition to results from an electroencephalogram (EEG) and response to anti-seizure medications (Stafstrom & Carmant, 2015).

Generalized seizures are classified as absence, generalized tonic-clonic, myoclonic and atonic seizures. Absence seizures which were formerly named *petit mal* is a type of seizure in which

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the patient stares into space for a few seconds and also becomes unresponsive to external verbal stimuli but blinks the eye or nods the head occasionally. Generalized tonic-clonic (GTC) seizures which were previously named *grand mal* comprise of bilateral symmetric convulsive movements in addition to the body becoming stiff. After this, jerking of all the limbs take place. There is no loss of consciousness during such an episode. In myoclonic seizures, there is an abrupt, short very fast movement that is not associated with any loss of consciousness. The muscle contractions associated with this kind of seizure are usually brief and involuntary and may impair one or several other muscles. For this reason, myoclonic seizures can either be classified focal type seizures or generalized type seizures. On the other hand, atonic seizures are characterized by body tone loss, which lead to a head drop or fall (Stafstrom & Carmant, 2015).

When the brain has an imbalance between excitation (E) and inhibition (I), then, seizures can be said to be occurring (Rho, Sankar, & Stafstrom, 2010). The excitation/inhibition variation can be as a result of a change at various stages of the function of the brain from subcellular to genetic signaling cascades to general neuronal circuits. This imbalance can either have a genetic cause or can be acquired. The genetic pathological causes of this disease can be ubiquitous in nature. Again, cerebral injuries that are acquired are able to alter the circuit function, for example, change in the structure of the hippocampal circuitry due to persistent head injury or febrile seizures (Berkovic, 2015). Synaptic excitatory role matures before inhibitory function in a young brain leading to increased excitation and seizure generation. More so, due to the propensity of the neurotransmitter GABA to cause excitation instead of inhibition early in life (Ben-Ari, 2002; Pitkänen, Lukasiuk, Dudek, & Staley, 2015) it can be

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partly deduced the brains of the very young people are easily prone to having seizures (G. L. Holmes & Ben-Ari, 1998).

Focal seizures can be classified based on a level of awareness. The term awareness is used as a substitute for consciousness because consciousness comprises many aspects that can be difficult to fully evaluate. The level of consciousness and awareness are therefore two of many possible features of a seizure, but they play an important role with regards to seizures (Robert S. Fisher, 2017). Awareness is basically, the knowledge of the environment and of self. The ability to determine awareness is a practical tool utilized to assess if consciousness level is compromised or not. It is therefore the awareness during a seizure, and not awareness of whether or not a seizure has occurred (Robert S. Fisher, 2017).

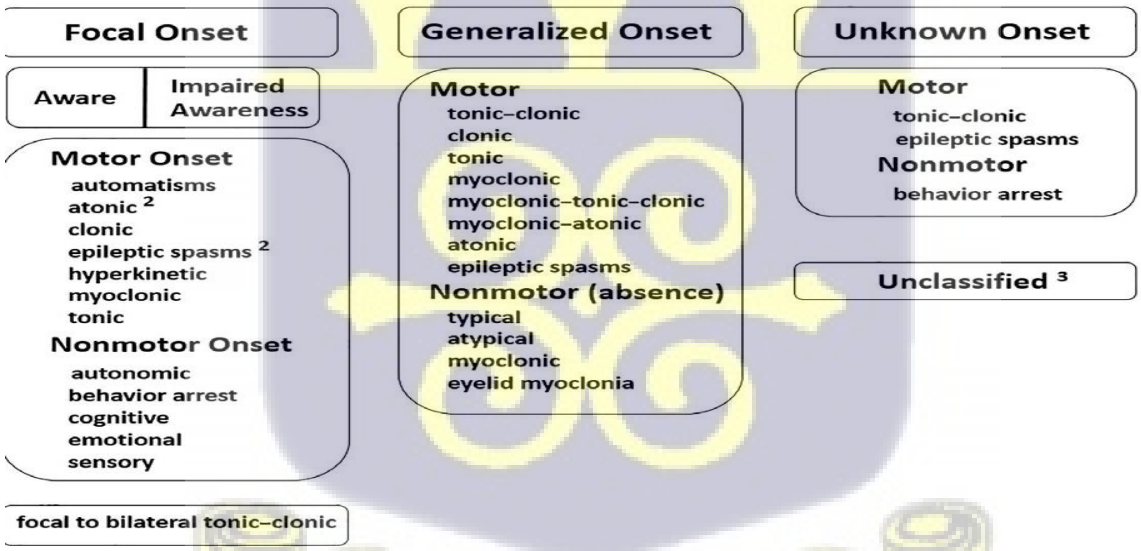

Figure 2.1: The ILAE 2017 classification of types of seizures (Robert S. Fisher, 2017)

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1.4 MECHANISMS OF EPILEPTOGENESIS

Epileptogenesis is the process through which a formerly functional normal brain changes such that it becomes biased towards the production of the aberrant electrical events which in turn result in chronic seizures. In other words, epileptogenesis is the process whereby there is growth and extension of tissue which is able to generate seizures that are spontaneous in nature, which ultimately result in an epileptic condition (Łukawski et al., 2018). Various mechanisms have been proposed to be the mode of initiation of the disease. The ability to prevent chronic epileptic disorder by using the necessary procedures would be touted as the most ideal goal in the clinical management of an epileptic condition but this, has not been successful to this day. Due to this, various clinical trials aimed at preventing chronic epilepsy have often yielded bleak, discouraging results (Di Maio, 2014). There are various antiepileptogenic drugs like eslicarbazepine and diazepam but the connection between inhibition of epileptogenesis and neuroprotection is not apparent (Łukawski et al., 2018). The ability to prevent epileptogenesis after brain injury is currently a challenge that is still unmet (Pitkänen & Lukasiuk, 2011). Research in animal models is currently the best source of information in the research into epileptogenesis. As a result, a lot of animal models are being researched into, to better understand the pharmacological and pathophysiological causes of the disease (Russo & Citraro, 2018). The process of epileptogenesis happens in three phases. It first of all starts with, causative injury or event; after which there is a latent period during which the alterations that were caused by the said injury also change the formerly normal brain into an epileptic brain. Lastly, the third phase follows and this is the point where the epilepsy becomes fully established and chronic. The best point in the epileptogenesis whereby

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various medical management can be utilized to stop the ultimate occurrence of a chronic epilepsy is at the latent period during which an acquired epileptogenesis is believed to coalesce (Goldberg & Coulter, 2013).

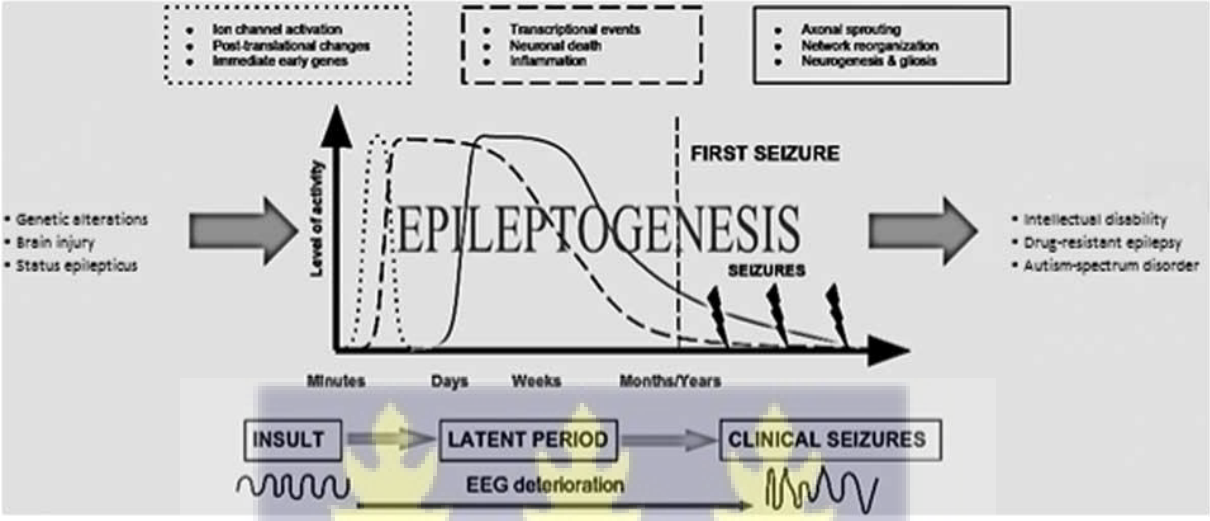

Figure 2.2: A figure showing the process of epileptogenesis (Alyu & Dikmen, 2017)

2.3.1 THE EMERGING TARGETS AND NOVEL STRATEGIES FOR FUTURE TREATMENT

The discovery of novel antiepileptic drugs which would help to better control patients' seizures may require a fundamental change in the strategies of drug development. Instead of randomly screening compound libraries, rational drug design could rather be based on scientific understanding of the pathophysiological mechanisms of ictogenesis of epilepsies. After this is achieved, new compounds can be made to target specific established epileptic brain defects, which can lead to the direct control of causative pathways without causing harm

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to the normal neuronal function. Aside this, there could also be the use of more innovative ways that mimic one or more characteristics of pharmacoresistance in humans and integrate them into epileptogenesis models thereby aiding in characterizing possible disease-modifying therapies (Brodie et al., 2011; Walker, White, & Sander, 2002).

1.4.1 VOLTAGE-GATED SODIUM CHANNEL SUBTYPES AND EPILEPSY

Voltage-gated sodium channels (VGSCs) are necessary for the production and propagation of coordinated action potentials in the whole nervous system. For this reason, they are believed to have a vital part in alleviation and occurrence of epilepsy. There have been several genetic studies on people living with epilepsy and these have helped characterized several mutations of more than 700 among the genes that code for voltage-gated sodium channels validating the role they play in pathogenesis. Again, a lot of anti-seizure medications are known to act on VGSCs to reduce seizures (Czuczwar & Patsalos, 2001). VGSCs are imperatively necessary for moderating neuronal excitability and subsequently, network activity. They can be found all over the central neuron compartments and their population is increased at the nodes of Ranvier and axon initial segments (AIS). In myelinated neurons, the collection of voltage-gated sodium channels at the nodes of Ranvier is important, the increase of the speed of action potential transmission by saltatory conduction (Conti, Hille, Neumcke, Nonner, & Stämpfli, 1976; Kaplan et al., 2001). Research into the genetics of epileptic patients has found a vast number of mutations out of the genes known to encode for these ion channels. A significant amount of these mutations are in VGSC genes with most discovered in *SCN1A*, and fewer in

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SCN2A, *SCN8A*, and *SCN1B* (Herlenius et al., 2007; Mulley et al., 2005; Striano et al., 2006; Wallace et al., 1998). VGSCs are very important mediators of intrinsic neuronal and muscle excitability. Any aberrant activity is key to the pathophysiology of seizures, and a lot of widely used antiepileptic medications, like carbamazepine and phenytoin are known inhibitors of VGSC activity. Many of the known antiepileptic medications block the VGSC but the drugs currently available are mostly non-selective and as such do not utilize the discrete attributes of the brain channels. VGSC are thought to be found at various regions on the neuronal membranes and populations. For this reason, other compounds can be produced that can target only sodium channel conformations and its subtypes studied to be over-expressed in epileptic tissue, in so doing, sparing normal neuronal function. Lacosamide is one example of a blocker of sodium channel with distinct attributes with regards to selectivity for a specific biophysical state or subunit but unfortunately have a lot of side effects (Brodie et al., 2011; Errington, Stöhr, Heers, & Lees, 2008; Remy et al., 2003).

1.4.2 $GABA_A$ RECEPTOR SUBUNITS AND EPILEPSY

This is a ligand-gated ion channel receptor that has a centrally placed anion pore around which five separate protein subunits are arranged. The anion pore is permeable to bicarbonate and chloride ions. There are 19 $GABA_A$ receptor subunits discovered so far and it has been shown that the composition of the subunit can affect the pharmacology and physiology of the receptor (Garcia, Kolesky, & Jenkins, 2010). In the brain, the receptors of $GABA_A$ possess an age-adapted role. In its early development, they moderate excitatory activities which result in the trigger of signalling processes that are calcium-sensitive necessary for brain

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differentiation. They however transmit inhibitory signals during later phases of their development (ILAE, 2018). These inhibitory effects have been significantly utilized in the management of diseases where there is the need of silencing neuronal activity. The function and physiology in the brain of epileptic patient or a *status epilepticus* subject change according to literature available (ILAE, 2018) and as such may have an influence on the propensity to seizures. For this reason, many available anticonvulsants have $GABA_A$ receptors as their primary or secondary targets (Chang et al., 2017). Some of these drugs act by increasing $GABA_A$ receptor activity through direct interaction with the receptor for example barbiturates, carbamazepine and benzodiazepines. Others indirectly increase the available GABA such as valproate and vigabatrin (Czuczwar & Patsalos, 2001; Granger et al., 1995; P. Kumar, A. Jhanjee, & M. Bhatia, 2010; Meldrum & Rogawski, 2007; Quilichini, Chiron, Ben-Ari, & Gozlan, 2006). Furthermore, anticonvulsants such as topiramate, zonisamide, acetazolamide can inhibit carbonic anhydrase through the reduction of the depolarizing outcome of $GABA_A$ receptors (Davis, Penschuck, Fritschy, & McCarthy, 2000; Dodgson, Shank, & Maryanoff, 2000; Nishimori et al., 2005; Reiss & Oles, 1996). Barbiturates which are part of the examples of available $GABA_A$ receptor medications are relatively non-selective (Walker et al., 2002). The ability to produce drugs that are of subunit specificity could help avoid unwanted side effects which are related to non-selective modulation of $GABA_A$. There are records for subunit-specific AED: like stiripentol which has been shown to preferentially activate α3-β3-γ2-containing receptors. Hence, the ability to have drugs that are $GABA_A$ selective and can specifically act on subunits studied to be upregulated in epileptic brain tissue, may demonstrate being efficacious against seizures with less unwanted drug reactions

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(Brodie et al., 2011; Brooks-Kayal, Shumate, Jin, Rikhter, & Coulter, 1998; Johnston, 2005; Stell, Brickley, Tang, Farrant, & Mody, 2003).

1.5 DISEASE MODIFICATION AND ANTIEPILEPTOGENESIS

The recent antiepileptic medications generally manage ictogenesis, or the commencement of paroxysmal activity (Reiss & Oles, 1996). Due to these, various classes of AEDs act by suppressing excitability of neuronal activity by blocking sodium channels or increasing inhibitory GABAergic activity (Czuczwar & Patsalos, 2001; White, 1999). Both cognition and ictogenesis are moderated by excitability of neuronal activity. For this reason, using the normal methods of AEDs screening may be difficult to find drugs that are non-impairing. However, employing chronic animal models of epilepsy may be used to improve drug screening. Thus, by using kindled or genetically modified animals (Matagne & Klitgaard, 1998) it may be possible to find novel AEDs that prevent the neuronal hypersynchronization which leads to an ictal event, without obstructing normal neuronal excitability (Margineanu & Klitgaard, 2000). When a normal brain undergoes multiphase events which end up producing alterations that enhance the development of spontaneous seizures, we term it as epileptogenesis. This can be as a result of brain damage caused by things like head trauma (Golarai, Greenwood, Feeney, & Connor, 2001), growth (Goldberg *et al.*, 2013), stroke (Margineanu & Klitgaard, 2000), infection (Golarai et al., 2001), or *status epilepticus*. Usually, brain damage does not immediately lead to seizures and this is a latency period that may last for weeks or even years. In these latency periods, the normal seizure thresholds are lowered due to continuous alterations to the brain which also subsequently lead to

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spontaneous seizures (Golarai et al., 2001; Santhakumar, Ratzliff, Jeng, Toth, & Soltesz, 2001). Immediately seizures start occurring, the epileptic disease state worsens. Each seizure then has the propensity to produce extra neuronal changes that may additionally reduce seizure threshold (Goddard, McIntyre, & Leech, 1969). Most anticonvulsants act by either reducing seizure frequency or duration of seizure by reducing neuronal excitation, but ideal antiepileptogenic agents would either block the first epileptogenic development or alter the epileptic disease state after the onset of seizure (McNamara, 1984). The ideal screening for antiepileptogenic drug action would be studies that have the ability to reduce changes in cellular network and molecular properties that happen during the process of epileptogenesis. The animal model that is mostly employed for assessing the anti-convulsive characteristics of AEDs is the focal kindling. In this model, initial exposure to repeated sub-convulsive stimulus ultimately evokes seizures (Albright & Burnham, 1980; Goddard et al., 1969; McNamara, 1984). Initially, stimuli in the kindling process only elicit short-duration after-discharges as a result of a synchronous neuronal discharge near the stimulation. Further kindling stimulation produces prolonged after-discharges involving larger brain compartments with the quick involvement of the limbic system. With longer and repeated stimuli, behavioural seizures accompany the after-discharges and become more complex. It takes a number of days or weeks before there can be an increased sensitivity to a formerly sub-convulsant stimuli which subsequently reach a peak during which the kindling stimuli result in seizures and after-discharges (McIntosh & Levy, 2021). Since kindled seizures are inducible and have durations and behavioural and electrographic characteristics, kindling can be employed as a screening method for anti-seizure effects that are characterized easily. Potential AEDs can be

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administered to animals that have been fully kindled and the consequence on electrographic and behavioural seizures measured (Teskey, 2020). Kindling is progressive in nature and for this reason, repeated seizures over time can cause a reduction in seizure thresholds. This may be similar to the process of human epileptogenesis. The long interval between trauma and seizure manifestation in posttraumatic epilepsy may be a reflection of a slow kindling process (Skinner et al., 2010). This idea is buttressed by the generation of generalized seizures in a patient undergoing electrical stimulation of the thalamus (Lathers et al., 2011). There is data showing that the current antiepileptic drugs have antiseizure properties but not antiepileptogenic ones (Suchomelova et al., 2006). In other words, the symptoms can be managed but the ultimate development of epilepsy is not affected. Contrary to this, the priority of management of epilepsy is the discovery of therapeutic agents that can prevent or correct the neuropsychological and neurological deterioration accompanied with chronic seizures or, even stop epilepsy in individuals who are at risk (Brodie et al., 2011; Walker et al., 2002). FIG 2.3 illustrates some of the mechanisms of some the antiepileptic medications.

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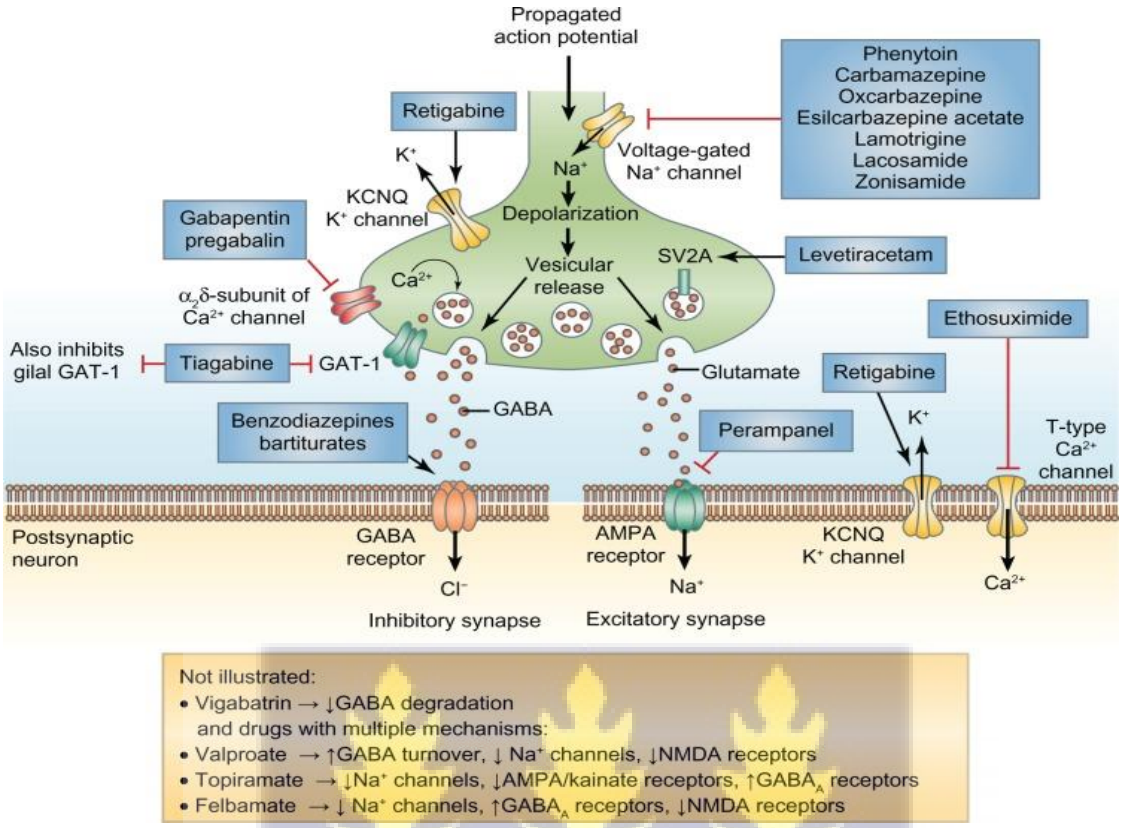

Figure 2.3: A figure showing the mechanism of anti-epileptic drugs *(Shih, Tatum, & A Rudzinski, 2013)*

1.6 SIDE EFFECTS OF ANTIEPILEPTIC DRUGS

The core aim of the management of people with epilepsy is to completely abolish or significantly reduce frequency of seizures, reduce the unwanted side-effects of drug therapy and greatly enhance medical and neuropsychiatric comorbidities (Pitkänen, 2010) Anti-seizure drugs (ASDs) aim to subdue the development, severity and the propagation of epileptic seizures but these medications are to be taken up to 4 times a day for years, and sometimes for throughout one's lifetime (Faught et al., 2008; Ryvlin, Cucherat, & Rheims, 2011). Drug noncompliance to ASDs treatment is not uncommon and this usually results in

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the seizure relapse which in turn leads to increased mortality, injuries, and frequent hospital visits and admissions with added costs (Faught et al., 2008). Unlike other disorders, such as hypertension in which an 80% compliance is accompanied with good disease management, a single missed dose of an ASD can result in a fatal seizure (Devinsky et al., 2018). Antiepileptic drugs (AEDs) can effectively manage patients with epilepsy but there is usually treatment failure and poor drug compliance due to the numerous side-effects experienced by taking these AEDs. These side-effects can lead to about 25% of the patients discontinuing treatment (Kwan & Brodie, 2000; Perucca, Carter, Vahle, & Gilliam, 2009; Uijl et al., 2009) and this lead to a significant reduction in their quality of life (Luoni et al., 2011; Uijl et al., 2009). Some of the frequently reported adverse drug reactions of AEDs are fatigue, tremors, gastrointestinal symptoms and memory problems. While others too experience dizziness, osteoporosis, drowsiness, depression, changes to their weight, and nausea (Carpay, Aldenkamp, & Van Donselaar, 2005). The impact of these adverse effects may further necessitate the need for specialist care which brings additional financial burden to the patient (de Kinderen et al., 2014). A lot of adverse drug effects are reported with AEDs but the most prevalent is CNS effects. When the regimen of an antiepileptic drug fails, it can lead to untoward effects which may be due to drug intolerance or to inadequate seizure control also due to drug inefficacy or a combination of both (Kwan & Brodie, 2000). The AEDs are either a first-generation or a second generation. Examples of the first-generation are valproate, phenobarbital, phenytoin, carbamazepine and primidone. The second-generation AEDs offer better response over first-generation drugs nonetheless, either of them can cause significant adverse effects (Cramer, Fisher, Ben-Menachem, French, & Mattson, 1999). Poorly managed

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or controlled seizures may lead to a combination of drug therapy which further leads to potential pharmacokinetic or pharmacodynamic interactions leading to greater side-effects than when monotherapy is used (Hirsch et al., 2004). There are situations where seizures are not adequately controlled or there can be an imbalance between the control of seizures and adverse effects. In such instances, there may have to be a discontinuation of the drug which leads the patient being put on a new drug with their unique side effects (Lawal, Ogunwande, Salvador, Sanni, & Opoku, 2014). Some of the cognitive effects experienced by patients include loss of memory, diminished intelligence, attention and language skills. Even though most AEDs can cause cognitive impairment, phenobarbital and topiramate have been reported to have the most effects on cognitive abilities. Furthermore, the same phenobarbital has been shown to cause mental slowing which studies have shown that this diminished cognitive ability improves once the medication is stopped. Exposure of children to phenobarbital leads to intelligence scores less than those taking valproate (Lawal, Opoku, & Ogunwande, 2015). Anxiety and depression are common among epileptic patients (Blumer, Montouris, & Hermann, 1995). When these conditions exist before the diagnosis of epilepsy, the said conditions may worsen subtly with treatment thereby making it difficult to recognize (Ohemu et al., 2014).

Carbamazepine has known side effects such as impaired balance, leucopenia, diplopia, blurred vision and drowsiness (Wilkinson et al., 2017). There is also been vertigo and loss of coordination, aplastic anemia and agranulocytosis have also been reported (Pellock, 1987). Patients on lamotrigine can develop Stevens-Johnson syndrome or toxic epidermal necrolysis

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especially those on valproic acid. Other unwanted effects include photosensitivity, diplopia, blurred vision, agitation, tremors, aplastic anemia and vomiting. There are reports indicating patients on levetiracetam to develop psychiatric side effects such as depression and agitation and other side effects like blood dyscrasias, dyspepsia, drowsiness, diplopia whiles the teratogenic effects of sodium valproate has also been reported (Alyu & Dikmen, 2017) and can also cause tremors, thrombocytopenia, nausea, pancreatitis, hair loss, edema, and ataxia (McIntosh & Levy, 2021). Phenytoin has been removed as a first-line drug because of its numerous toxicity issues which include gum hyperplasia (fig. 2.4), nystagmus, diplopia, tremor, dysarthria, and ataxia (Teskey, 2020). It can also lead to a decrease in intellect, depression, acne, and gum hypertrophy. Patients taking this drug can also develop coarse facial features polyneuropathy and blood dyscrasias (Wilkinson et al., 2017). Aside from these, lacosamide has been found to cause dizziness and ataxia and may even increase the risk of suicidal thoughts in patients taking these medications for any indication (P. Kumar, A. Jhanjee, & M. S. Bhatia, 2010).

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Figure 2.4: A clinical side effect of phenytoin manifesting as gingival hypertrophy (Khorsand & Saaveh, 2007)

1.7 ANIMAL MODELS OF EPILEPSY

Temporal lobe epilepsy (TLE) is mostly accompanied by variable and recurrent seizures and this make this neurological disorder very difficult to understand. More often than not, seizures in TLE are often resistant to anti-epileptic medications. There is the option of resection of the epileptogenic tissue surgically but it is expensive and at times unfeasible. For these reasons, animal models that mimic the neuropathological, behavioural and electroencephalographic attributes of epilepsy have been developed and researched into in the past decades to better understand the pathophysiology of TLE and to also help develop better AEDs (York, 2005).

Understanding epileptogenesis and seizure generation as well as their complex mechanisms in temporal lobe epilepsy and other forms of epilepsy is not completely possible in human clinical studies. Because of this, there has to be the use of appropriate animal models which will aim to mimic the symptoms of focal epilepsy with an epileptogenic injury or insult. The

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research into seizure mechanisms can give a better knowledge about the general functions and consciousness of the brain. Thus animal models can help with the advancement of the research into both neurophysiology and epilepsy (Löscher & Schmidt, 1988). FIG. 2.5 shows some of the various animal models employed in the discovery of antiepileptic medications.

1.7.1 CHEMOCONVULSANT MODELS

Research into the chemoconvulsant pentylenetetrazole (PTZ) started in the later part of 1940s and continued through the early 1950s. During this period, properties of PTZ seizure threshold model were described by various publications, and this helped to make a comparison between the anticonvulsant attributes of antiepileptic medications (AEDs) in the PTZ and other animal models. This lead to the PTZ model being used in addition to other various animal models to discover most of the drugs currently utilized in epileptic seizure management (Yadeta, 2016). PTZ is believed to employ an antagonistic mechanism at the picrotoxinin-sensitive site at the $GABA_A$ receptor complex (Stegaroiu, 2016). Furthermore, (Bourgeois, 2014) has shown that PTZ and picrotoxin interact with distinct overlapping regions of the $GABA_A$ receptor.

Chemoconvulsants are compounds that activate seizures and they can be employed in assessing various AEDs that act on different types of seizures. For example, compounds like N-methyl-D, L-aspartate and strychnine have been shown to trigger generalized tonic-clonic seizures, whereas PTZ generates non-convulsive myoclonic or absence seizures (Porter et al., 1984). Through these models, drugs like trimethadione, ethosuximide, valproate, and other efficacious medications have been discovered (Liu et al., 2013). Administrations of several

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doses of flurothyl or PTZ have also been employed to imitate recurrent generalized tonic-clonic seizures in young rodents (Pitkänen, Buckmaster, Galanopoulou, & Moshé, 2017). A single administration of PTZ can induce seizures and when it is given in sufficient amounts, status epilepticus can even be induced (Gregory L Holmes, Sarkisian, Ben-Ari, & Chevassus-Au-Louis, 1999; Pitkänen et al., 2017).

1.7.2 ELECTRICAL STIMULATION

To better understand the mechanisms underlying epileptogenesis and ictogenesis, various animal models of seizures and epilepsy have contributed immensely to this effect. Furthermore, these models have played a significant part in the preclinical development and discovery of new antiepileptic drugs (AEDs) (Löscher, 2011). A lot of animal models of chronic brain disorders that are known to elucidate the pathophysiology of epilepsy and some of these chronic models of epilepsy are the kindling model of temporal lobe epilepsy (TLE), post-status models of TLE and genetic models of different types of epilepsy. Furthermore, the results from these models can be used to assess the effects of antiepileptic medications. A differentiation of the study of chronic models with models of acute seizures in previously healthy animals is the maximal electroshock seizure test. This can aid in testing of drugs in chronic models of epilepsy to provide data which is more predictive of clinical efficacy and unwanted drug reactions. Given this, chronic models can be employed early in the development and discovery of drugs to help reduce false positives (Loscher, 2002).

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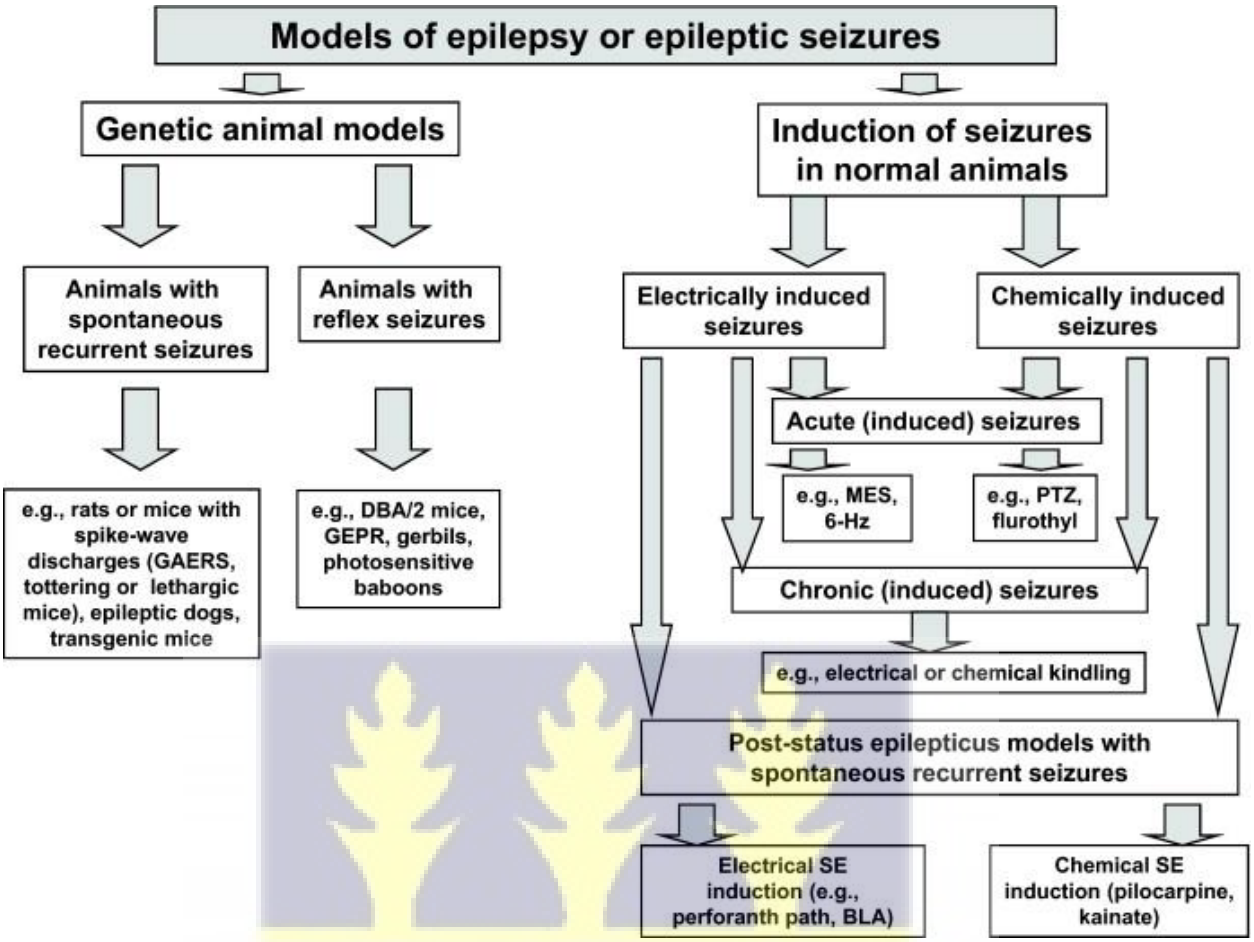

Figure 2.5: An overview of models for specific types of epilepsy or epileptic seizures (Löscher, 2011)

1.8 *EHRETIA CYMOSA* THONN BORAGINACEAE

1.8.1 PLANT DESCRIPTION

Ehretia cymose (see fig. 2.6) is a small tree that belongs to the Boraginaceae or borage family. It is found over a wide area of habitat throughout central, eastern, and western Africa, including Ghana, Côte d'Ivoire, Benin, Cameroon, Ethiopia and Kenya (Wikipedia Contributors, 2018, June 19). The plant is referred to as *Okosua* by the Akan-Twi speaking

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tribes from Ghana (Humphrey Morrison Burkill, 1985). The leaves of the plant are used among the people of south western Nigeria for the treatment of measles (Oladunmoye & Kehinde, 2011). The leaves have also been reported to be used for the management of epilepsy, spasms and convulsions, as a laxative, febrifuge, analgesic and for paralysis (H. M. Burkill, 1985). It is a small to medium deciduous tree or shrub that can grow up to 20 to 25 m tall. It has low and crooked branches that can grow up to up to 30 cm in diameter. The surface of the bark is grey to pale brown, with protruding lenticels. The leaves are spirally arranged and are simple and entire without any stipules present. The petiole is 1 to 3.5 cm long with an elliptical blade that is slightly grooved (Lemmens, 2009).

It grows up to 7 m tall in the western parts but can grow as tall as 20 to 25 m as recorded in some parts of Southern Nigeria and Guinea. The fruit is normally black in colour, ovoid to globose drupe which is about 2-6 mm long (Harris & Harris, 2002). It has greyish brown wood with alternate lighter and darker bands (Conti et al., 1976).

The main types of compounds identified in its volatile oils are fatty acids, sesquiterpenes, monoterpenes, , alcohols, phenylpropanoids and esters, (Jeruto, Mutai, Lukhoba, & Ouma, 2011). Its volatile oils have been demonstrated to have biological properties like cytotoxicity, insecticidal and antimicrobial activities (Lawal et al., 2014; Lawal et al., 2015).

2.6.2 TRADITIONAL USES

The leaf of *Ehretia cymosa* is used in Ghana to treat fractures and to also enhance bone modelling. The leaves of the plant are used for treating headaches and fevers and also used for its mild laxative effects (Dalziel, 1937; Lewis & Avioli, 1991). Chewing sticks are made out

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of the branches for maintaining good gum and teeth hygiene (H. M. Burkill, 1985; Lewis & Avioli, 1991). An infusion of the leaf is used as a wash to treat convulsions, muscle spasms and fever (Borokini & Omotayo, 2012). The sap of the leaf is reported to be a mild laxative and can also be used as a hemostatic. Decoctions from the leaves are used to treat muscle stiffness, fevers and toothache. The twigs of the leaves are used in combination with other parts of plants to treat gastric ulcers whiles the decoctions of the leaf are used to cure dysentery and tetanus. The roots and leaves have been reported to be used as aphrodisiacs (Jeruto et al., 2011). Decoctions of the roots and bark are used to manage menstrual problems while a decoction of the bark alone is externally used to cure skin conditions. The people from Maasai treat brucellosis with the roots which are crushed in water and used against stomach diseases. *Ehretia cymosa* also serves locally as an important feed for livestock and in agroforestry in Ethiopia, it is sometimes planted as ornamental trees (Lemmens, 2009).

The tree has several uses, providing food, wood and medicines for the local population of South Western Nigeria (Mulley et al., 2005). There is reported use of the leaves for the management of viral conditions like measles among the Southern Western people of Nigeria (Mulley et al., 2005). The decoction of the bark is used to regularize menstrual cycle and also treat pneumonia (Bankole et al., 2016; Ohemu et al., 2014). There is also reported use of the plant for the management of mental problems, venereal diseases, dry cough, malaria and tonsillitis, (Jeruto, Tooa, Mwamburia, & Amuka, 2015).

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Figure 2.6 **An image of *Ehretia cymosa* Thonn (Boraginaceae) http://tropical.theferns.info**

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CHAPTER 3 MATERIALS AND METHODS

1.9 DRUGS AND CHEMICALS

This study employed the use of various chemicals including pentylenetetrazole, picrotoxin, phenobarbitone sodium, carbamazepine (Sigma-Aldrich Inc., St. Louis, MO, USA);

1.10 PLANT COLLECTION

The leaves of *Ehretia cymosa* were harvested from Kwahu Asakraka (6.62942N6˚37'45.9048", -0.68647W0˚41'11.30253") in the Eastern Region of Ghana, and authenticated at the Pharmacognosy Department, KNUST and a specimen with voucher number, KNUST/2020/MN1/L009, was stored at the herbarium of the Faculty of Pharmacy, KNUST.

1.11 PREPARATION OF EXTRACT

The leaves of the plant were powdered and serially extracted with 70% ethanol over 48 hours using a Soxhlet apparatus and concentrated under reduced pressure at 40-60 ^{o}C to a dark brown syrupy mass in a rotary evaporator. A water bath was used to dry resulting syrup mass and stored in a desiccator. The resulting extract was named *Ehretia cymosa* extract or ECE.

1.12 QUALITATIVE PHYTOCHEMICAL ANALYSIS ON CRUDE EXTRACT

Screening of the extract to detect the presence of phytochemical constituents such as glycosides, tannins, sterols, alkaloids, terpenoids, saponins and flavonoids was done using procedures described by Evans and Trease (2009).

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1.12.1 TEST FOR TANNINS

This was done by taking a quantity of 0.2 g of the extract which was dissolved in 25 mL of water and the resulting volume was topped up to 25 mL. A 1 mL aliquot of the extract was then added to 10 mL of water and 2 drops of 1% ferric chloride which produced the appearance of blue-black or green precipitate (ppt).

1.12.2 TEST FOR REDUCING SUGARS

Twenty milligrams of the extract were warmed with 5 mL dilute H_2SO4 on a water bath for 2 minutes and allowed to cool and filtered. Four drops of 20 % NaOH were added to the resulting filtrate after which 1mL volume of Fehling`s A and B solutions were added to the filtrate. It was then warmed and a red-brown ppt was then observed.

1.12.3 TEST FOR SAPONINS

About 0.2 g of each extract was taken and shaken vigorously with about 10 mL of water for about one minute in a stoppered test tube and later the presence of a persistent froth was then observed.

1.12.4 TEST FOR ALKALOIDS

Ten milliliter of dilute HCl was boiled with 0.2 g of the extract for 5 minutes. The resulting supernatant was then filtered into a separate test tube. About 3 drops of Dragendorff`s reagent was added to one milliliter of the filtrate and shaken together the appearance an orange spot ppt, was then observed.

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1.12.5 TEST FOR FLAVONOIDS

A volume of 10 mL of 98% ethanol was added to 0.2 g of the extract. A small amount of zinc metal was then added followed by the drop-wise addition of concentrated HCl. The filtrate was then examined for the appearance of colors ranging from orange to red representing the presence of flavones, orange to crimson indicating flavonols, and crimson to magenta indicating presence of flavanones.

1.12.6 TEST FOR STEROLS

Two milliliter of chloroform was added to 0.2 g and filtered. An amount of 0.2 g of the extract was added to 2 mL of chloroform and filtered. A volume of 2 mL of acetic anhydride was added to 1 mL of the filtrate after which few drops of concentrated H_2SO4 was carefully added along the sides of the test tube. Violet to blue coloration which was produced indicated the presence of sterols.

1.12.7 TEST FOR TERPENOIDS

An amount of 0.2 g of the extract was added to 2 mL of chloroform in a test tube followed by the addition of 1 mL of concentrated H_2SO4. A reddish-brown coloration at interface indicated the presence of terpenoids.

1.13 HANDLING OF ANIMALS

Sprague-Dawley rats (150-200 g) and Institute of Cancer Research (ICR) mice (20 – 25 g) were obtained from the Animal Department of Noguchi Memorial Institute for Medical

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Research, University of Ghana, Legon and kept in their Animal Experimentation labs. The animals were housed in cages with wood shavings as bedding and fed with standard mice chow, given water ad libitum. All animal procedures and techniques used in these studies were done under the Noguchi Institute of Animal Care and Use Committee (NIACUC) guidelines with a reference number 2017-05-2R**.**

1.14 IRWIN OBSERVATION TEST

Irwin observation test is usually employed to evaluate the effects of a new substance on the physiological and behavioural function of experimental animals. The results obtained from the this test gives an insight into potential toxicity and enables doses to be selected for specific neuropharmacological investigation (Vogel et al., 2015). In this study, mice were grouped (n=5) and made to acclimatize for 24 hours before the start of the experiment. The laboratory mice were given the extract and compared with a control group given distilled water 10 mL kg^{-1}, *p.o.* The effect of the extract was evaluated at 6 doses; 10, 30, 100, 300, 1000, and 3000 mg kg^{-1} administered *p.o.* immediately before the test. Observations were performed 15, 30, 60, 120, and 180 minutes after administration of the extract and 24 and 48 hours later. Changes in physiological, behavioural and neurotoxicity symptoms, pupil diameter and rectal temperature were observed and recorded according to a standardized observation grid derived from that of Irwin (Biney, Mante, Boakye-Gyasi, Kukuia, & Woode, 2014; Irwin, 1968).

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1.15 HOT PLATE TEST

The mice were weighed individually and made to acclimatize for 24 hours before the commencement of the experiment during which they were not given any food except for water. This is due to the fact that some food substances might have analgesic effect. The animals were put into 4 groups (n=5). These mice were pre-treated with the extract of *Ehretia cymosa* at 3 doses 30, 100 and 300 mg kg^{-1} *p.o.*, and control of normal saline 10 mL kg^{-1}. After 60 minutes of pre-treatment, the animals were placed on a hot plate and the latency time which is the time for which mouse remains on the hot plate at (55˚C ± 0.1˚C) without licking or flicking of the hind limb or jumping was then recorded. A cut-off time of 30 seconds was the time limit for all the animals and this was done to prevent damage to tissues. The readings were taken after 0, 30, 60, 90, and 120 min post-administration of the test drug. The percentage analgesia was calculated using the following formula (Naveed, 2014).;

$$\%\ Analgesia = \frac{(\text{Test latency} - \text{control latency})}{(\text{Cut} - \text{off time} - \text{control latency})} * 100$$

1.16 SKELETAL MUSCLE EFFECTS OF ECE

1.16.1 ROTAROD TEST

Some test substances with anticonvulsant effect may impair motor coordination. This can be an important factor in the testing for anticonvulsant activity. The experiment was done to ascertain the effect of ECE motor coordination. Mice were randomly put into four groups (n=5) and trained to remain on a rotating rod (Ugo-Basile model 7600, Comerio, VA, Italy), rotating at 25 revolutions per min for 180 s over three days. This is referred to as habituation. . The actual study took place 24 hours after the last habituation during which the animals were

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given ECE 30, 100 and 300 mg kg^{-1} and distilled water 10 mL kg^{-1} *p.o.* after which they were put on the rotating rod and the latency of the mice to fall off the rotating rod within the stipulated time of 180 s was determined one-hour post-treatment.

1.17 ANTICONVULSANT EFFECT OF *EHRETIA CYMOSA*

1.17.1 PENTYLENETETRAZOLE (PTZ)-INDUCED SEIZURES

Clonic-tonic seizures were induced in drug/vehicle pre-treated male Institute of Cancer Research (ICR) mice (20-30 g) by subcutaneous injection of 75 mg kg^{-1} pentylenetetrazole (PTZ) into the loose skin fold on the back of the neck of the mice. The animals were pre-treated with ECE (30, 100, and 300 mg kg^{-1}) or phenobarbitone sodium (3, 10, 30 mg kg^{-1}) thirty minutes before the injection of PTZ. The control animals received 0.9 % saline solution (10 mL kg^{-1}). After the PTZ injection, the animals were placed in a testing chamber (made of perspex of dimensions 15×15×15 cm). A mirror angled at 45˚ below the floor of the chamber allowed a complete view of the convulsive event of PTZ. The behavior of the animals was captured with a camcorder (EverioTM model GZ-MG 130U, JVC, Tokyo, Japan) placed directly opposite to the mirror. The video recordings were later analyzed by tracking parameters including latencies to myoclonic jerks and clonic-tonic seizures and the duration of clonic-tonic seizures using Behavior Tracker Version 4.0 for Windows. The ED_{50} (a measure of anticonvulsant potency) was calculated by plotting the percent seizure inhibition of the drug to the vehicle-treated group. The ability of the drug or extract to prevent the

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seizures or prolong the latency of onset of the hind-limb tonic extensions was used as an indication for anticonvulsant activity .

1.17.2 PICROTOXIN-INDUCED SEIZURES

Clonic-tonic seizures were induced in drug or vehicle pre-treated male ICR mice (20-30 g) by an intraperitoneal injection of 3 mg kg^{-1} picrotoxin (PIC). The mice were pre-treated with ECE (30, 100, 300 mg kg^{-1}), or phenobarbitone sodium (3, 10, and 30 mg kg^{-1}) thirty minutes before the injection of PIC. The control animals received distilled water (10 mL kg^{-1}, *p.o*). After the PIC injection, the animals were put in a testing chamber and a video recording of the event made. The video recordings were also later analyzed by tracking parameters including latency to myoclonic jerks and clonic-tonic seizures and the duration of clonic-tonic seizures using Behavior Tracker version 4.0 for Windows. The ED_{50} was calculated as indicated in the PTZ test. The mortality rate was also determined for each drug treatment group. The ability of a drug/extract to prevent the seizures or delay/prolong the latency of onset of the hind-limb tonic extensions was considered as an indication of anticonvulsant activity.

1.17.3 PENTYLENETETRAZOLE-INDUCED KINDLING

To kindle the mice to spontaneous seizures, 35 mg kg^{-1} of PTZ was injected *i.p*. every 48 h into saline-, ECE- or phenobarbitone-treated male ICR mice (20-30 g). After the PTZ injection, the rats were placed in a testing chamber (made of perspex of dimensions 15×15×15 cm^3). A mirror angled at 45° below the floor of the chamber allowed a complete view of the convulsive event of PTZ. The behavior of the animals was captured with a camcorder (JVC

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Hard Disk Camcorder, GZ-MG 130U) placed directly opposite to the mirror. Seizure intensities were classified according to the Racine score (Racine, 1972) as follows:

Stage 0: no response

Stage 1: ear and facial twitching

Stage 2: convulsive waves throughout the body

Stage 3: myoclonic jerks, rearing

Stage 4: turning over onto one side

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

Also, the latency to the onset of myoclonic jerks was measured and analyzed. Each rat was considered fully kindled after showing stage 4 or 5 after two consecutive PTZ administrations. On day seven after kindling had been achieved, the rats were challenged with 35 mg kg^{-1} of PTZ and the entire event was also recorded. The ED_{50} was then calculated.

1.17.4 MAXIMAL ELECTROSHOCK TEST

The experiment employed the use of male ICR mice put into seven groups (n=10). Out of these, three of the groups were treated with the extract (10, 30, and 100 mg/kg, *p.o.*). Three other groups were treated with carbamazepine (10, 30, and 100 mg/kg, *p.o.*). The last group served as the control and they were administered distilled water (10 mL/kg, *p.o.*). The convulsions were then induced in the mice after 1 hour of drug pre-treatment. This was done by passing alternating electrical current (50 Hz, 60 mA, and 0.2 s) through the electrodes

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attached to the earlobe of the mice. After this, the duration of tonic hind limb extension seizures was determined in each dose group (K. E. Kukuia et al., 2012).

1.18 TOXICITY STUDIES

1.18.1 ANIMAL GROUPINGS AND EXTRACT ADMINISTRATION

The Sprague-Dawley rats were randomly put into four groups (n=10); namely; control group (normal saline 10 mL kg^{-1}), ECE 30, 100, and ECE 300 mg kg^{-1} doses and made to acclimatize for 5 days. The animals were subsequently given a daily dose of the test substances for 72 days by oral gavage. This was done to mimic the traditional folkloric route of administration. All drug administrations were given at 8:00 GMT each day and the blood collection and post-mortem examinations were done before 15:00 GMT.

1.18.2 HISTOPATHOLOGY AND BIOCHEMICAL ANALYSIS

1.18.2.1 PREPARATION OF SPECIMEN

All animals were sacrificed and their organs (liver, kidney, lungs, spleen, heart, and brain) were harvested and placed in labelled containers containing 10% neutral buffered formalin. The organs were transported to the pathology laboratory of School of Dentistry, Korle-bu for processing and examination.

Blood was collected from the jugular vein into serum separating gel tubes (BD Vacutainer® blood collection Tube Product, USA) and ethylene diamine-tetracetate (EDTA) tubes (Mediplus vacutainer K3, Sunphoria Co. Ltd., Taiwan) for serum preparation and haematological analysis respectively. Values for the level liver transaminases (AST, ALT,

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ALP), total protein (TP), direct bilirubin (D. Bilirubin) indirect bilirubin (Indi. Bilirubin), total bilirubin (T. Bilirubin), triglycerides and albumin in the serum were measure. Also, the levels of kidney urea (Ur), creatinine (Cr), lipid profile (HDL, LDL, total cholesterol) were calculated. These were done using an automated clinical chemistry analyzer (ABX Pentra C200, Horiba Medical, USA).

1.18.2.2 PROCESSING OF TISSUES

Portions of the organs were selected into labelled tissue processing cassettes and processed into paraffin blocks. Each was passed through ascending grades of alcohol (70%, 80%, 90%, and absolute) and further two changes of absolute alcohol for dehydration, cleared in three changes of xylene, and finally infiltrated and embedded in paraffin wax. Five-micron sections were cut from each block, mounted on microscope slides and stained using the haematoxylin and eosin.

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CHAPTER 4 RESULTS

1.19 PHYTOCHEMISTRY SCREENING

Phytochemical analysis of the leaf extract of *Ehretia cymosa* (ECE) (Table 4.1) revealed the presence of these plant secondary metabolites: tannins, saponins, terpenes, triterpenes, phenolic compounds, flavanoids, volatile oils, steroids, and glycosides. Alkaloids were however absent.

Table 4.1: Primary phytochemical screening of ECE

PHYTOCHEMICAL	REMARK
Tannins	+
Glycosides	+
Alkaloids	-
Flavonoids	+
Terpenoids	+
Saponins	+
Volatile Oils	+
Phenolic compounds	+

(+) Present (-) Absent

1.20 IRWIN TEST

No death was recorded after 48 h, thus LD_{50} was estimated to be greater than 3000 mg kg^{-1} (Table 4.2). This test provided the dose ranges (30, 100, and 300 mg kg^{-1}) for the actual work.

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Table 4.2: IRWIN TEST RESULTS

Dose (mg/kg)	Mortality	Latency (min)	Observed drug effects
10	0	15-120	Sedation, urination, defecation,
30	0	15-120	Sedation, urination, defecation, analgesia
100	0	15-180	Sedation, urination, defecation, analgesia
300	0	15-180	Sedation, urination, defecation, analgesia
1000	0	15-120	Sedation, urination, defecation, analgesia, altered respiration, tremors
3000	0	15-120	Sedation, urination, defecation, analgesia, altered respiration
Saline	0	15-30	urination, defecation

1.21 EFFECT OF EXTRACT ON NEUROMUSCULAR ACTIVITY

1.21.1 ROTAROD

The extract, *Ehretia cymosa* (30, 100, 300 mg kg^{-1}), did not have any significant effect ($F_{3, 16}$=0.3460 P=0.7925) on time spent on the rotating rod when compared to saline group (Figure 4.1).

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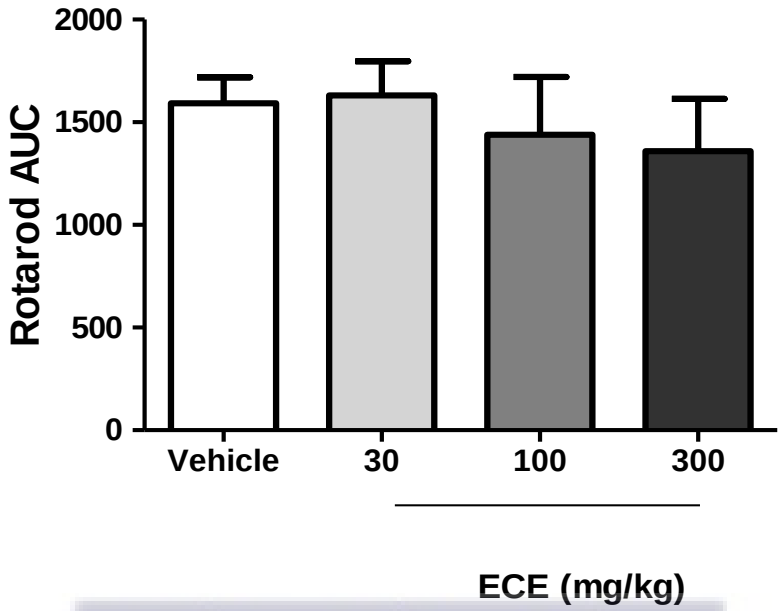

Figure 4.1: Effect of *Ehretia cymosa* extract ECE 30, 100, and 300 mg kg^{-1} on neuromuscular coordination in mice in the rotarod test. Data are Mean ± SEM (n=5)

1.21.2 HOT PLATE TEST

The latency time (time for which mouse remained on the hot plate (55˚C ± 0.1˚C) without licking or flicking of the hind limb or jumping) was recorded. The ECE extract could not significantly increase the latency ($F_{3, 8}$ =2.907 P=0.1011) as compared to the control (Figure 4.2).

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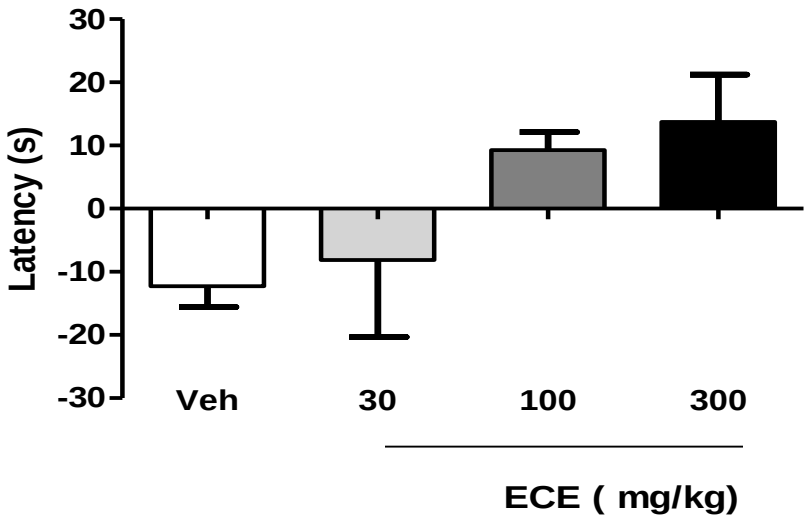

Figure 4.2: Effect of ECE (30, 100, 300 mg kg^{-1}) on latency [time for which mouse remained on the hot plate (55˚C ± 0.1˚C) without licking or flicking of the hind limb or jumping in seconds. Each column represents the mean ± SEM (n = 5).

1.22 ANTICONVULSANT THRESHOLD TESTS

1.22.1 PTZ-INDUCED SEIZURE TEST

The test employed pentylenetetrazole (75 mg kg^{-1}*s.c*) which induced myoclonic convulsions in all animals pre-treated with normal saline (10 mL kg^{-1}). *Ehretia cymosa* 30, 100 and 300 mg kg^{-1} reduced the duration of tonic convulsions significantly; fig 4.3C (P= 0.0024; $F_{3, 16}$ = 7.491). In the ECE treated animals, the frequency of convulsions was progressively and significantly reduced at all three doses 30, 100 and 300 mg kg^{-1} fig. 4.3B (P= 0.0031; $F_{3, 16}$ = 7.069). There was an observed significant (P<0.0001, $F_{3, 16}$ = 2.797) increase in latency to the first myoclonic jerk fig. 4.3A, with statistical significance at doses 30 mg kg^{-1}(P= 0.0031; $F_{3, 16}$ = 2.797). Phenobarbitone on the other hand which was used as a reference anticonvulsant produced a dose-dependent increase in latency to the first myoclonic jerk for the 3 and 30. It significantly

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delayed the onset ($F_{3, 16}$=5.285, P=0.0100) and reduced the frequency ($F_{3, 16}$=7.069, P=0.0031) and duration ($F_{3, 23}$=4.177, P=0.0231) of PTZ-induced convulsions (Figure 4.3).

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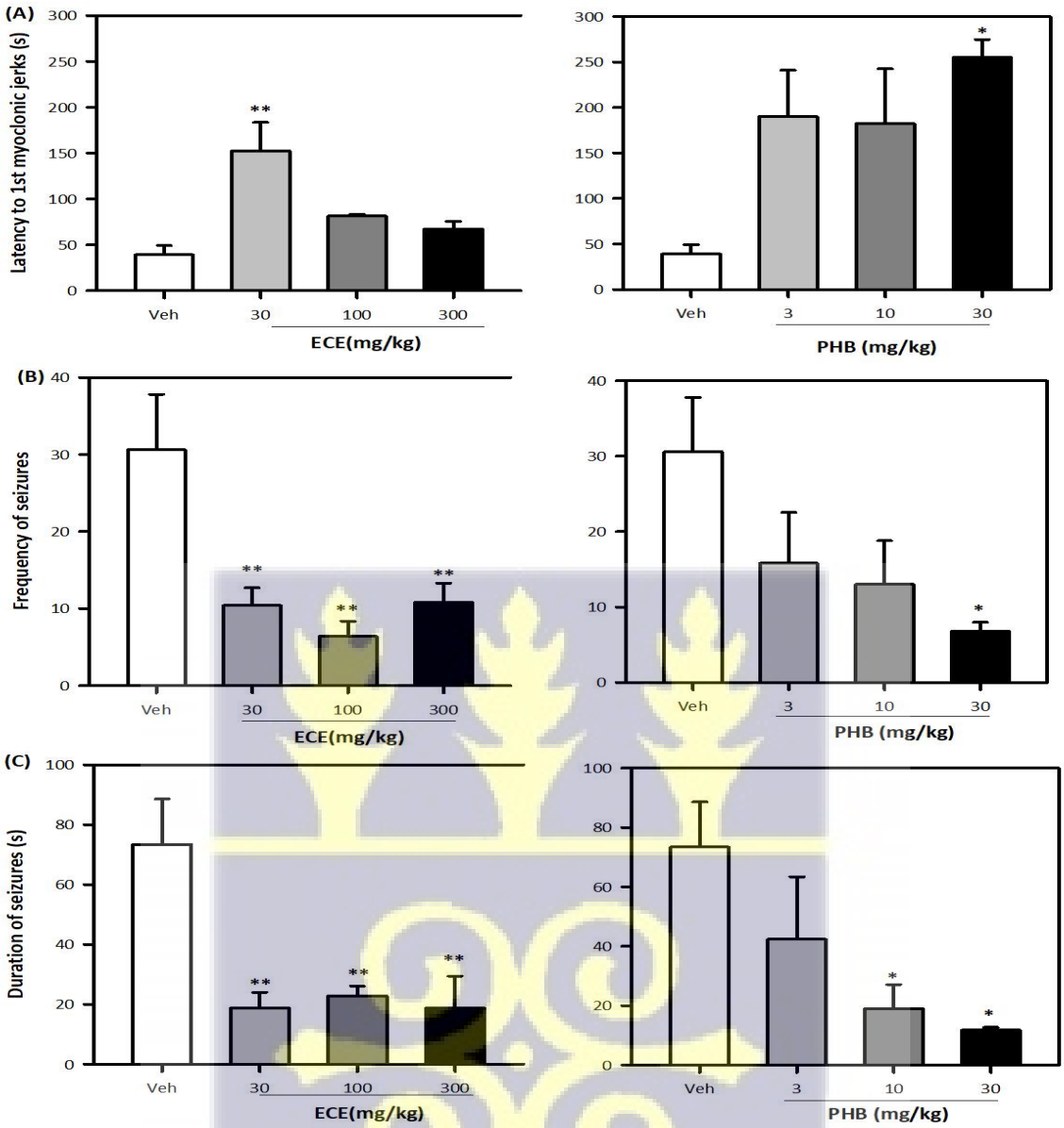

Figure 4.3: Effect of ECE 30, 100, 300 mg kg^{-1} and phenobarbitone 3, 10, 30 mg kg^{-1} on the latency to first myoclonic jerks, the total frequency of the seizures, and total frequency of the seizures induced by PTZ. Each column represents the mean ± SEM (n = 5). *P < 0.05, **P < 0.01compared with vehicle-treated group (one-way analysis of variance followed by Newman–Keuls post hoc test).

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1.22.2 PICROTOXIN INDUCED SEIZURE TEST

The picrotoxin (3 mg kg^{-1}) experiment induced generalized tonic-clonic convulsions in all animals treated. ECE 30, 100, 300 significantly; fig. 4.4C ($F_{3, 16}$=12.57, P=0. 0. 0.0002) reduced the duration of tonic-clonic convulsions in the test animals. It (30 mg kg^{-1} 100 mg kg^{-1} and 300 mg kg^{-1}) also significantly (P=0.0.0004: $F_{3, 24}$ =8.422) increased the latency fig. 4.4A to the first myoclonic jerk . For the frequency of convulsions, only 100 mg kg^{-1} reduced the frequency but not significantly (P=0.0779: $F_{3, 16}$ =2.735); fig 4.4B. The standard control phenobarbitone, reduced the frequency but not significantly ($F_{3, 16}$=0.0779, P=0. 2.735) and significantly reduced both the duration (P=*0.0.0403:* $F_{3, 16}$ =3.491) of picrotoxin-induced convulsions and also increased significantly (P=0.0007: $F_{3, 16}$ =49.811) the latency to convulsions in the 3, 10, and 30 mg kg^{-1} groups (Figure 4.4).

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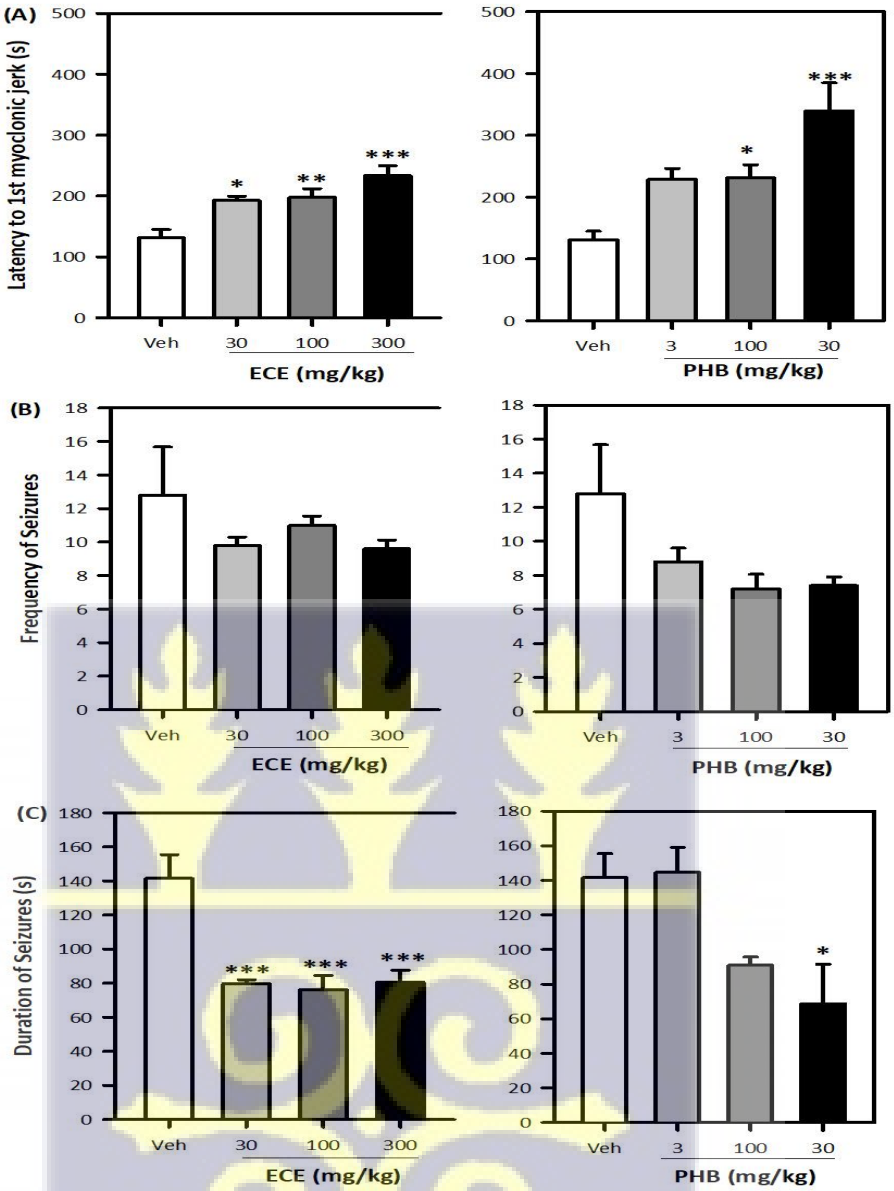

Figure 4.4:Effect of ECE 30, 100, 300 mg kg-1 and phenobarbitone 3, 10, 30 mg kg-1 on the latencies to first myoclonic jerks, the total frequency of the seizures, and total frequency of the seizures induced by picrotoxin. Each column represents the mean ± SEM (n = 5). *P < 0.05, **P < 0.01compared with vehicle-treated group (one-way analysis of variance followed by Newman–Keuls post hoc test).

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1.22.3 MAXIMAL ELECTROSHOCK TEST

The maximal electroshock model administered electric current (50 Hz, 60 mA, and 0.2 s) which induced hind limb tonic extensions in all animals pre-treated. For *Ehretia cymosa*, only the highest dose 300 mg kg^{-1} significantly (P<=0.0167: $F_{3, 16}$ = 4.151) reduced the duration of the first hind limb tonic extensions (HLTE); fig. 4.5A. The extract also delayed the total duration of the HLTE but was not statistically significant ($F_{3, 16}$= 0.3300, P=0. 0.8037); fig. 4.6C. The standard drug carbamazepine did not reduce the duration of first hind limb tonic extensions (HLTE) (P=0.0791: $F_{3, 16}$ =2.554) nor the total duration of the tonic hind limb convulsions (Fig. 4.5B and Fig. 4.6D).

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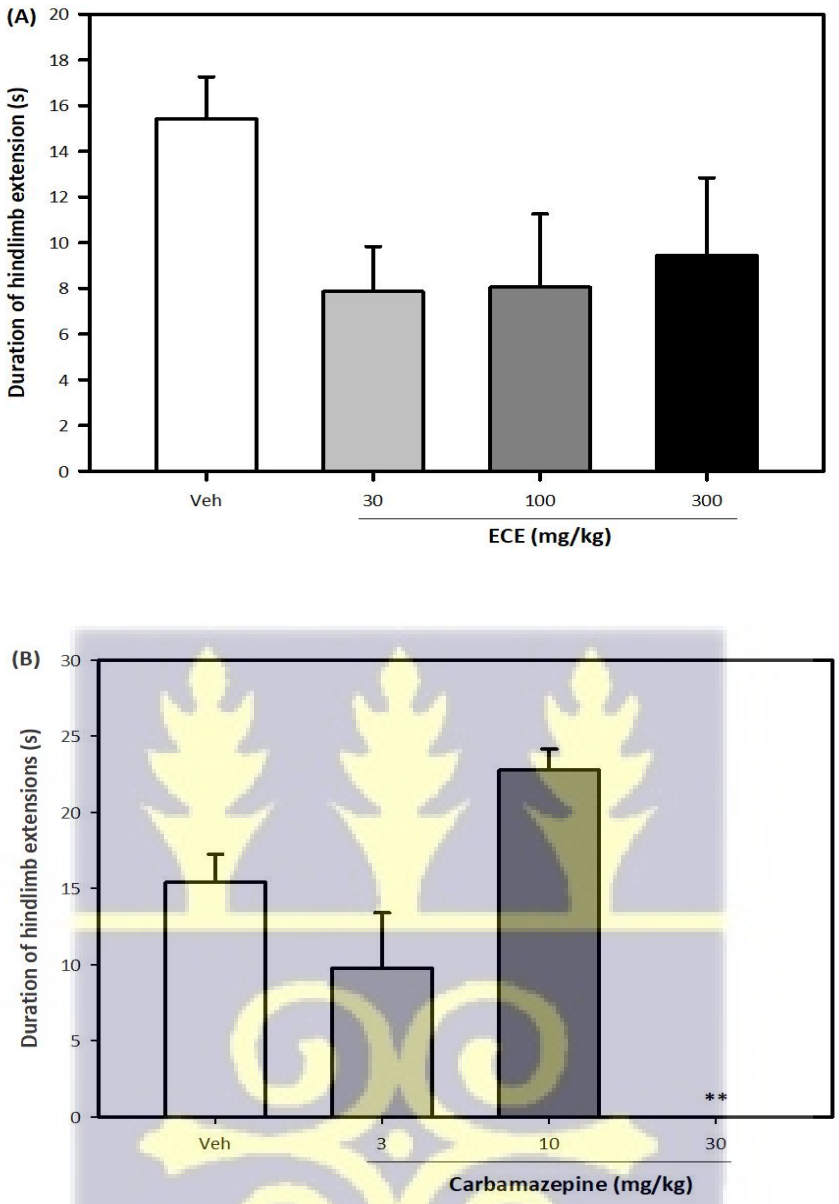

Figure 4.5: Effect of ECE 30, 100 and 300 mg kg^{-1} and carbamazepine 3, 10 and 30 mg kg^{-1} on the duration of First HLE induced by Maximal Electroshock Test (MEST).
Each column represents the mean ± SEM (n = 7). *P < 0.05, **P < 0.01 ***P < 0.001 compared with vehicle-treated group (one-way analysis of variance followed by Newman–Keuls post hoc test).

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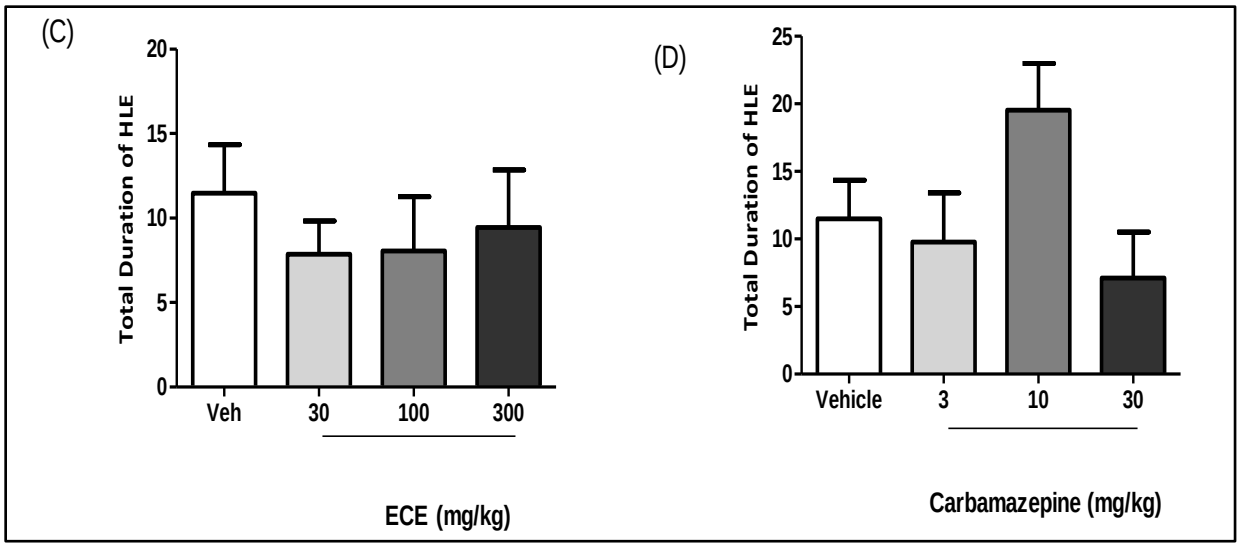

Figure 4.6: Effect of ECE 30, 100 and 300 mg kg^{-1} and carbamazepine 3, 10 and 30 mg kg^{-1} on the total duration of Hind Limb Extensions (HLE) induced by Maximal Electroshock Test (MEST).
Each column represents the mean ± SEM (n = 5). *P < 0.05, **P < 0.01 ***P < 0.001 compared with vehicle-treated group (one-way analysis of variance followed by Newman–Keuls post hoc test).

1.22.4 PTZ KINDLING TEST

The experiment involved giving the vehicle-treated group, a repeated administration of 35 mg kg^{-1} of PTZ on alternate days which caused a gradual increase in the convulsant's responses as scored using the Racine scale. The score had increased from 0 to 3 by the 9th day and maintained a Racine score of 4 from the 27th to the 39th day and reached a peak severity of Racine score of 5 by the 41st day which was maintained for 2 weeks (Fig. 4.7A). ECE significantly depressed the kindled seizures at all the dose levels tested ($F_{3, 84}$ = 24.97, P< 0.0001); fig. 4.7B. None of the animals in the extract-treated groups achieved seizure score 5, even after 22 injections of PTZ 35 mg kg^{-1}. The percentage severity of seizures (calculated from the AUC) shows that ECE attenuated PTZ kindled seizure activity by reducing the severity of seizures by 50%--70%. Phenobarbitone also produced a significant dose-dependent

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depression of the kindled seizure activity ($F_{3, 84}$ = 38.09, P < 0.0001); fig. 4.7D, and the percent severity of seizures was significantly reduced by 70% (Figure 4.7).

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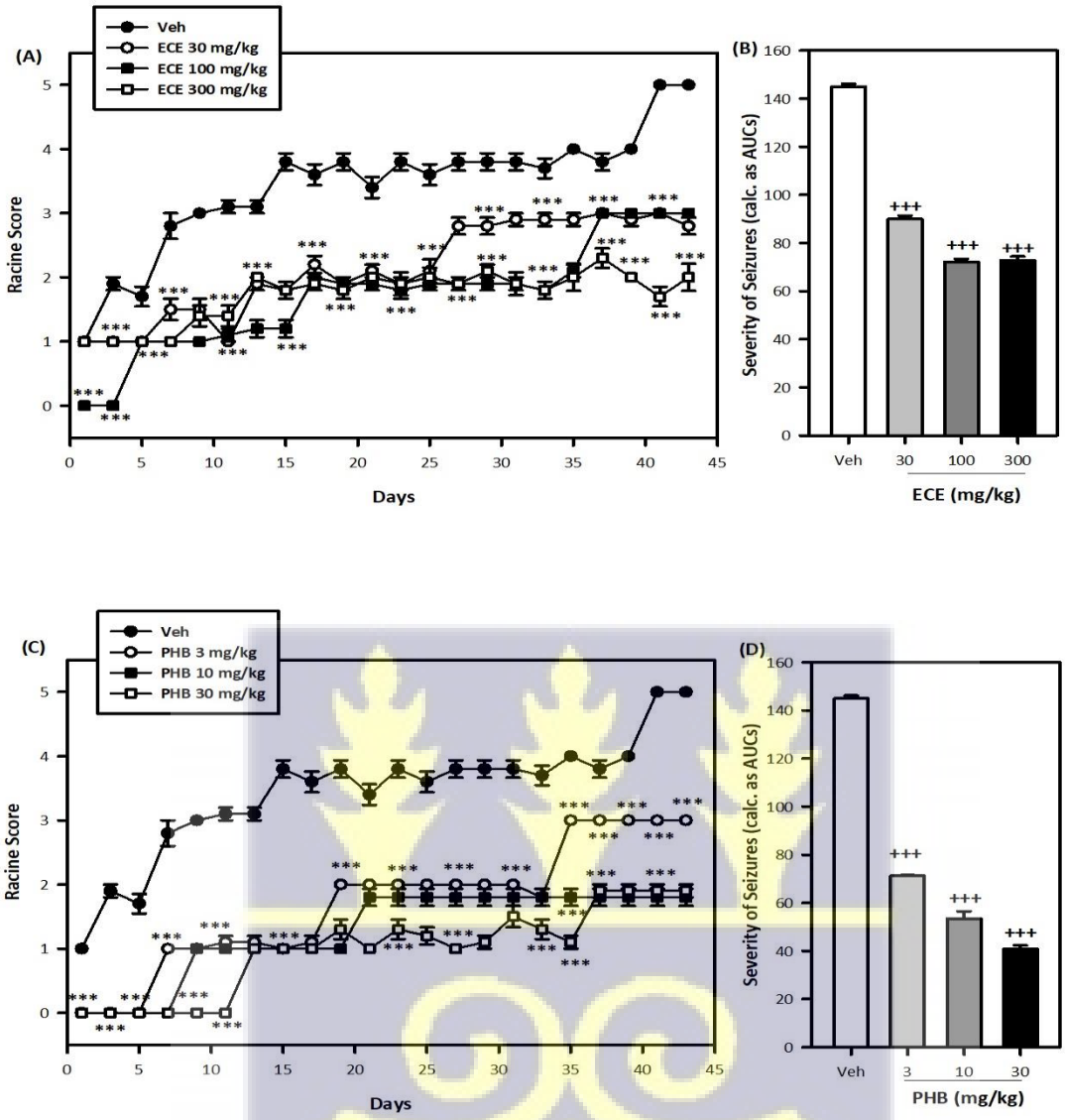

Figure 4.7:The dose-response effects of ECE 30, 100, and 300 mg kg^{-1} (A and B) and phenobarbitone 3, 10, and 30 mg kg^{-1} (C and D) on the PTZ-kindled mice.
The left panels show the time course of effects over the 32 days and the right panels show the percent severity of seizures calculated from the AUCs for the test duration. Values are means ± SEM (n=10). *P < 0.05, ** P < 0.01, ***P < 0.001 compared with vehicle-treated group (two-way analysis of variance followed by Bonferroni'spost hoc test). †P<0.05, †††P<0.001 compared with the vehicle-treated group (one-way analysis of variance followed by Newman–Keul'spost hoc test).

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1.23 TOXICITY STUDIES

1.23.1 BIOCHEMICAL PARAMETERS

The study evaluated the lipid profile, kidney and liver functions of the animals. There was no significant difference (P=0.08-0.99) between the vehicle-treated and the ECE (30, 100, and 300 mg kg^{-1})-treated rats regarding the serum concentrations of urea or creatinine (kidney function) of the rat subjects. There was also no significant difference between the treatment groups with regards to HDL (P=0.99), LDL (P=0.92), total cholesterol (P=0.51), and triglycerides (P=0.25), when compared to the control. The other parameters measured for the lipid profile were not significantly different (P=0.25-0.99) for the ECE-treated animals and the vehicle. However, ECE-treated rats (30 mg kg^{-1}) showed significant difference in direct bilirubin (P=0.02) when compared to vehicle-treated rats (Table 4.3).

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Table 4.3: A table showing the biochemical analysis of a single administration of ECE (30, 100 and 300 mg kg^{-1}) after a 72-day study period in Sprague-Dawley male rats

Parameters	Vehicle	ECE 30	ECE 100	ECE 300	P-value
Renal function test (mmol L^{-1})					
Urea	8.41 ± 1.46	8.14 ± 0.78	8.34 ± 0.92	8.34 ± 0.36	0.99
Creatinine	67.04± 12.06	38.38 ± 2.37	42.72 ± 6.24	46.66 ± 4.30	0.08
Lipid Profile (mmol L^{-1})					
Total Cholesterol	2.04 ± 0.10	1.85± 0.14	2.0 ± 0.16	2.25 ± 0.11	0.51
Triglycerides	1.41 ± 0.21	1.30 ± 0.06	1.21 ± 0.28	1.77 ± 0.16	0.25
HDL	0.92 ± 0.11	0.86 ± 0.058	1.13 ± 0.09	1.19 ± 0.11	0.99
LDL	0.48 ± 0.12	0.48 ± 00.11	0.43 ± 0.072	0.51 ± 0.11	0.92
Liver function test					
Total Protein (g L-1)	88.98± 2.21	81.13±1.713	81.64±4.647	85.46±1.617	0.24

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Albumin (gL-1)	39.44± 1.247	35.70±1.369	37.64±2.157	37.56± 0.96	0.44
D.Bilirubin (µmolL-1)	1.33 ± 0.024	1.003±0.059	1.22 ± 0.089	1.23± 0.05	0.02*
Ind.Bilirubin (µmolL-1)	0.54 ± 0.44	0.43 ± 0.13	0.52 ± 0.17	1.30±0.1897	0.13
T.Bilirubin (µmolL-1)	1.86 ± 0.44	1.45± 0.15	1.76± 0.20	2.53 ± 0.16	0.09
ALT (UL-1)	91.50 ± 27.92	67.73±5.663	88.76±19.23	87.90±6.624	0.82
AST (IUL-1)	269.6 ± 42.09	183.4±18.09	276.5±55.94	205.5±27.65	0.32

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1.23.2 HISTOLOGICAL EXAMINATION OF ISOLATED TISSUES

. The liver of extract-treated rats showed normal liver sections with a characteristic hexagonal arrangement of hepatocytes in lobules surrounding a central vein (Figure 4.9). The examination of the heart sections showed a characteristic branching arrangement of myocardial fibres with centrally placed nuclei (Figure 4.11). The kidney sections revealed normal tubules, glomeruli, and renal capsules (Figure 4.10). The histology of the lungs showed normal alveolar sacs, alveoli as well as alveolar ducts, bronchus, and bronchioles. Lastly, the sectioned brain revealed normal pia mater, molecular layer, granular layer, and white matter as well as dura mater and with normal Purkinje and pyramidal cells (Figure 4.8). There were no distortions of the normal architecture of tissues of any of the organs with immigrant cell types which may have constituted a form of pathology in all the doses levels as compared to that of the control.

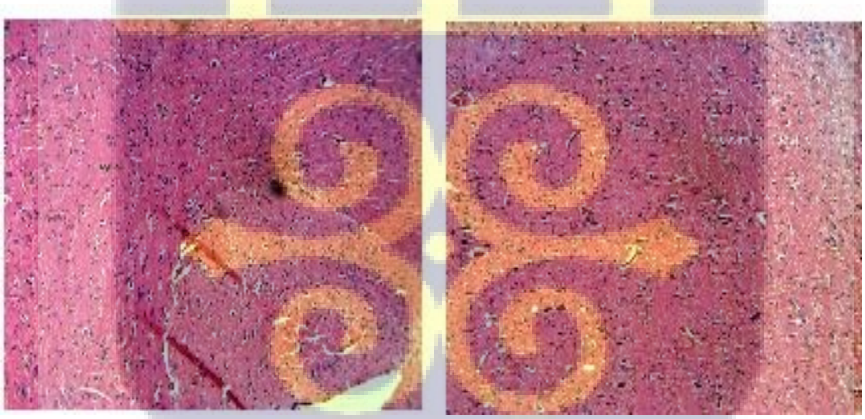

Figure 4.8: The Photomicrographs of Brain isolated from rats after 72-day continuous administration of (A) Vehicle, (B) ECE (30, 100 and 300 mg kg^{-1}), (H&E staining, 40×).

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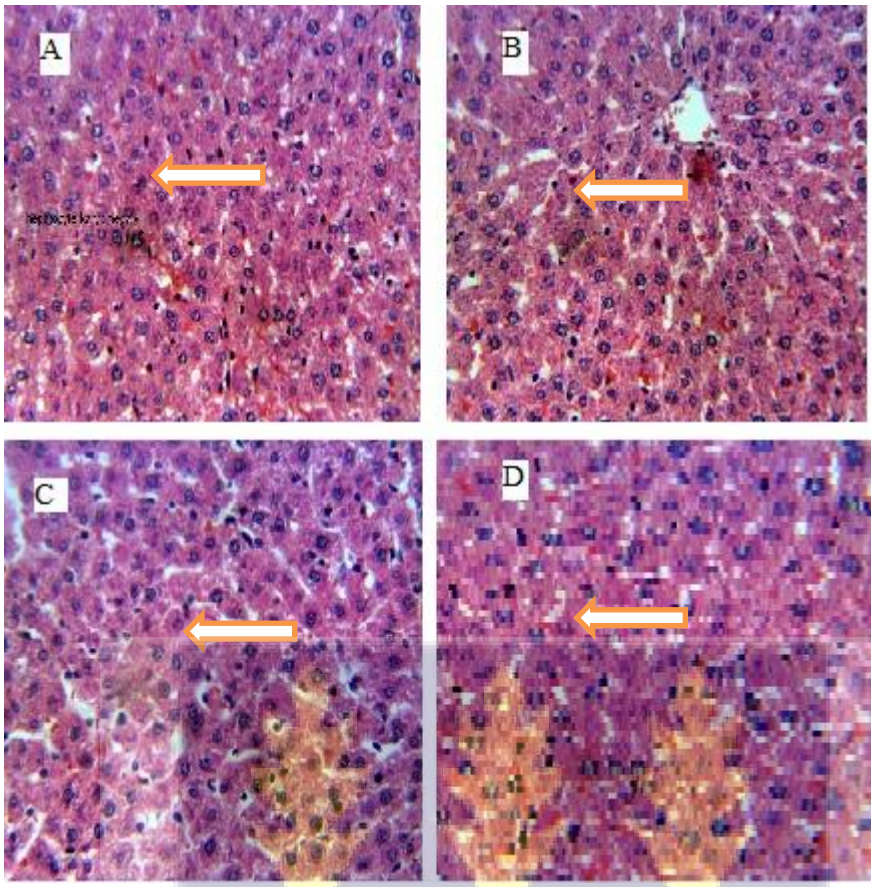

Figure 4.9:The Photomicrographs of livers isolated from rats after 72-day continuous administration of (A) Vehicle, (B) 30 (C) 100 and (D) 300 mg kg^{-1}) (H&E staining, 40×). The arrow shows the evenly distributed hepatocytes.

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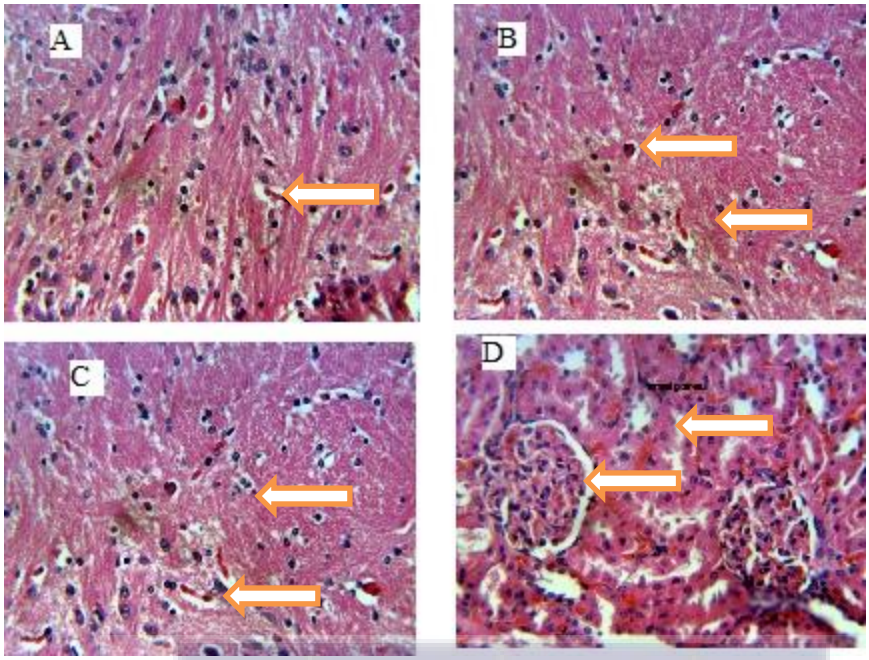

Figure 4.10:Photomicrographs of kidneys isolated from rats after 72-day continuous administration of (A) Vehicle, (B) ECE 30, (C) 100 and (D) 300 mg kg^{-1} showing normal renal tubule and glomeruli (H&E staining, 40×).

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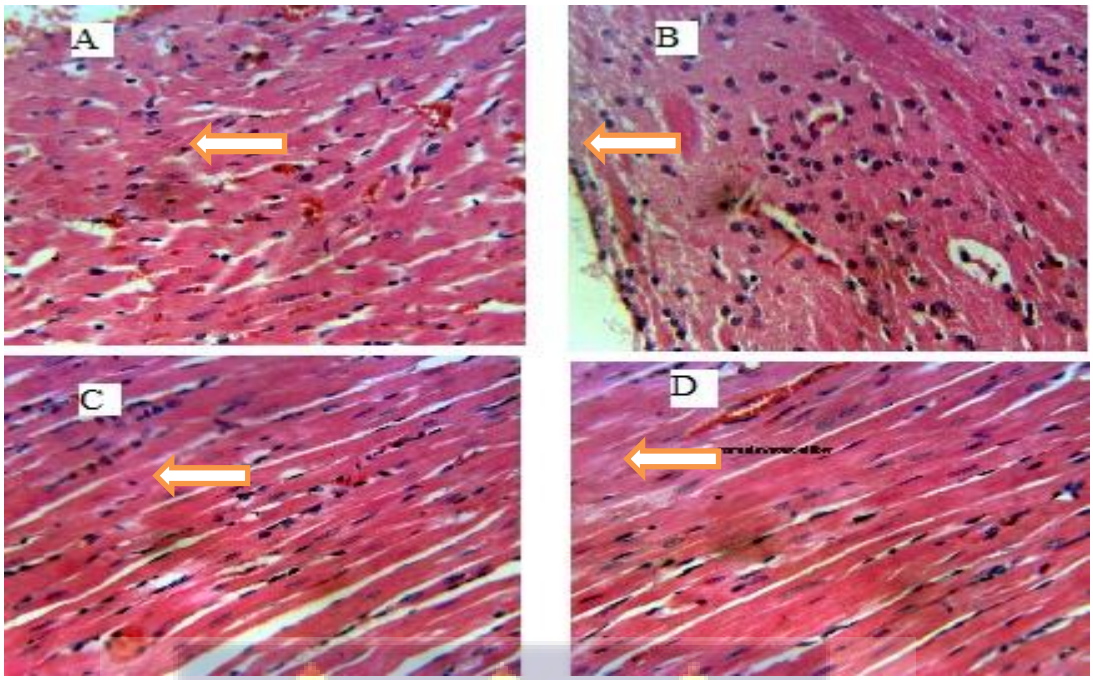

Figure 4.11:Photomicrographs of hearts isolated from rats after 72-day continuous administration of (A) Vehicle, (B) 30, (C) 100, (D) ECE 300 mg kg^{-1}) showing normal myocardial fibers with characteristic central nuclei and branching arrangement as indicated by the arrows.

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CHAPTER 5 DISCUSSION

The present work demonstrated that ECE has anticonvulsant potential. The extract reduced seizure activity in mice in various models at doses that were considered non-toxic. Animal models used for screening the pharmacological effect of ECE include Irwin's test, PTZ-kindling, picrotoxin induced seizure test, rotarod test, maximal electroshock test, the hotplate test and acute and sub-chronic toxicity studies.

Irwin observation test is employed to assess the effects of an investigative drug on physiological and behavioural activity. The results obtained from this test are used to assess the potential toxicity and to help in the selection of doses for a particular neuropharmacological investigation (Vogel et al., 2015). It provides an understanding of the possible toxicity or otherwise of the test substance. From this, novel therapeutic agents may be discovered through a systematic way of assessing behavioural and physiological functions of the test substance qualitatively (Biney et al., 2014; Irwin, 1968). After the observation test employing an oral gavage of 6 different doses of the extract and control, mortality was zero after 48 hours and the LD_{50} was found to be greater than 3000 mg kg^{-1}. Irwin test revealed reduced sensitivity to tail pinch touch indicating a potential analgesic effect, and reduced activity which also indicated potential sedative and neuromuscular effects. There was however altered respiration and slight tremors elicited at higher doses of 1000 and 3000 mg kg^{-1} but mortality was zero in these groups too. Most of the CNS effect was elicited at the 30, 100 and 300 mg kg^{-1} dose groups and no tremors or altered respiration was also seen in those groups. The CNS effects were mostly elicited between

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30 minutes to one hour after the oral administration. Based on these findings, the 30, 100, and 300 mg kg^{-1} dose groups were selected for this work, and a one hour waiting time was also selected as pre-treatment time for this work.

From Irwin test, a potential neuromuscular effect of the extract was indicated so the rotarod apparatus test was used to validate that effect. The rotarod test is used to assess the behavioural effect of drugs on motor coordination (Vogel et al., 2015). The present study showed that ECE could cause a reduction in time spent on the rotating rod but this was not statistically significant at all doses tested i.e. 30, 100, 300 mg kg^{-1}. This means that the doses that can cause a potential reduction in neuromuscular activity with regards to the effects seen or elicited during Irwin's test can be said to be above the doses 30, 100, and 300 mg kg^{-1} that were used for this work.

The hot plate test is a simple behavioural test used for assessing the consequence of investigative drugs on the pain threshold sensitivity. The test is based on the logic that rodents will lick their paws or attempt escaping by jumping when they are put on a hot surface,. Investigative drugs that can alter the nociception threshold will either increase the latency to jumping or licking indicating an analgesic effect while those with hyperalgesic effect decrease the latency to licking/jumping (Vogel et al., 2015). The test, however, could not significantly increase the latency ($F_{3, 8}$ =2.907 P=0.1011 fig 4.1) as compared to the control. It increased the latency to paw licking of the 100 and 300 mg kg^{-1} but not significantly when compared to the control. But again, drugs that have a low analgesic effects, specifically, those that are essentially anti-inflammatory agents like ibuprofen,

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aspirin and paracetamol, exhibit less pronounced outcome than well-known analgesics such as the opioids (Vogel et al., 2015). This means that the extract's possible analgesic effect seen during Irwin test could be due to the extract's anti-inflammatory effects and not necessarily an analgesic effect.

This experimental work employed various models of seizures and provided evidence that the ethanolic extract of the leaves of *Ehretia cymosa* has anticonvulsant activity and that the extract is also safe in murine models. The anticonvulsant activity was evident in both the acute and chronic models of seizures and no toxicity was recorded in both models of toxicity that were used.

The study employed convulsive threshold tests that are used in estimating the probability of an investigative drug to induce alterations in the propensity to the occurrence of seizures either spontaneously or, more importantly, in the company with other treatments (Vogel et al., 2015). In the acute models, PTZ, picrotoxin, and the maximal electroshock tests were employed whereas the PTZ kindling model was used for the chronic model.

The PTZ acute model is a model that is thought to have an antagonistic action on $GABA_A$ receptors (Auvin & Nehlig, 2017) and represents a well-founded model for human absence and generalized seizures (Patrick Amoateng, Eric Woode, & Samuel B Kombian, 2012; Löscher & Schmidt, 1988). The extract elicited anticonvulsant activity against seizures induced by PTZ by increasing the latency to the first myoclonic jerks and clonic seizures in mice as well as reducingthe duration and frequency of the clonic seizures. Thus, the ability of the extract to inhibit seizures induced by PTZ by ECE

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postulates that ECE may produce this effect by increasing GABAergic neurotransmission. With regards to the PTZ procedure, pro- or anti-convulsant property is validated by both the frequency of the convulsant events such as tonic seizures, clonic seizures as well as deaths and by their latency of occurrence. Latency to seizures can be used to evaluate pro-convulsant activity, whereas determination of frequency is most useful for identifying anticonvulsant activity (K. K. E. Kukuia, Ameyaw, Woode, Mante, & Adongo, 2016). Since the extract was able to both reduce the frequency and also increase the latency to the first myoclonic jerk and the total duration, it can be postulated that the extract may be efficacious in either absence or myoclonic generalized seizures just like drugs such as benzodiazepines, phenobarbitone and valproate which can inhibit seizures induced by PTZ (Castel-Branco, Alves, Figueiredo, Falcão, & Caramona, 2009).

One of the most important causes of neuronal hyperexcitability in epilepsy are disturbances in the equilibrium between excitatory and inhibitory systems. It is possible that a defective GABAergic system, which plays an important part in inhibiting neuronal activity, may ultimately reduce seizure thresholds (Szyndler et al., 2018). PIC is an antagonist of the $GABA_A$-receptor that stimulates seizures by blocking the chloride-ion channels linked to $GABA_A$ receptors, thereby inhibiting the transmission of chloride ions into neurons. This subsequently leads to reduced $GABA_A$ transmission and activity in the brain. Thus, seizures arising from picrotoxin are as a result of the decreased $GABA_A$ receptor-mediated inhibition tips the balance in favour of the glutamate-mediated excitatory transmission (P. Amoateng, E. Woode, & S. B. Kombian, 2012). The study

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demonstrated the ability of the extract to attenuate or reduce the seizures caused by picrotoxin. The extract was able to significantly increase the latency to the first myoclonic jerk thereby increasing the seizure threshold. It also greatly reduced both the frequency and the total duration of seizures. The ability of ECE to reduce picrotoxin-induced seizures may be due to an interaction with $GABA_A$ receptors and/ or GABA neurotransmission. This is because the extract worked similarly as the reference drug phenobarbitone which is a known γ-aminobutyric acid (GABA)–enhancing drug (Castel-Branco et al., 2009) which works through GABAergic neurotransmission by enhancing chloride ion flux through the chloride channels of $GABA_A$ receptors (P. Amoateng et al., 2012).

The maximal electroshock (MES) test is considered the best validated preclinical test that predicts anticonvulsant activity against generalized seizures of the tonic-clonic (grand mal) type (Castel-Branco et al., 2009). It allows the determination of the propensity of an investigative drug to stop the spread of seizures through the neural tissue (Castel-Branco et al., 2009). The ability of a test substance to abolish tonic hindlimb extension which is the blanket attribute of maximal electroshock in mice is a tool to predict the ability of investigative substance to stop the seizure spread from the epileptic focus. Furthermore, the effectiveness of a test in this model correlates well with inhibiting generalized tonic-clonic seizures and shows the investigative substance can inhibit or prevent discharge of seizures within the substrate of the brainstem (K. K. E. Kukuia et al., 2016; Porter et al., 1984). For *Ehretia cymosa*, only the highest dose 300 mg kg^{-1} significantly ($P <= 0.0167$: $F_{3, 16} = 4.151$ fig 4.5) reduced the duration of the first hind limb tonic extensions (HLTE).

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The extract also reduced the total duration of the HLTE however, it was not statistically significant. A primary drawback of the MES test with supramaximal stimulation is its inability to detect the anticonvulsant medications that increase the threshold of seizures but are not potent enough to raise the threshold above 150 mA in rats or 50 mA in mice, even though such drugs, for example, ethosuximide could be of clinical importance (Castel-Branco et al., 2009). This was seen in the study when the reference drug carbamazepine was neither able to significantly (P= 0.1394: $F_{3, 16}$ = 2.010; fig. 4.5B) reduce the duration of first hind limb tonic extensions (HLTE) (P= 0.0791: $F_{3, 16}$ = 2.554 fig 4.6D) nor reduce total duration of the tonic hindlimb seizures. Even though the ECE did not abolish tonic hind limb extension in the MES test, it caused a significant reduction in the duration of first hind limb tonic extensions (HLTE) at the highest dose 300 mg kg^{-1}. For this reason, the extract may not be effective in completely abolishing generalized tonic-clonic seizures but may reduce the duration of these seizures.

The PTZ kindling is a process that involves an increasing intensity of convulsion activity due to repetitive administration of chemical or electrical sub-convulsive stimulators (Ozlem & Okan, 2015). In animal models, it involves the generation of repeated, short, focal seizures with chemical or electrical or stimulation, which ultimately leads to permanently increased sensitivity to such stimuli. This results in longer, more severe seizures, and subsequently to chronic epilepsy (Goldberg & Coulter, 2013). The extract showed potential for preventing epileptogenesis or chronic epilepsy even after 42 days during which the animals were given 22 injections of sub-convulsive does of

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pentylenetetrazole (35 mg kg^{-1}). The reference drug phenobarbitone which is a barbiturate and known to prevent epileptogenesis (Goldberg & Coulter, 2013), also gave similar results. With regards to the reference drug, the Racine score peaked at 3 around the 20th day which was maintained until the 42nd day. The ability of the extract to prevent full-blown spontaneous seizures implies the extract can prevent chronic epilepsies.

This study also assessed the toxicity of ethanolic extract of *Ehretia cymosa* Thonn following a 72-day continuous administration of the selected doses 30, 100, and 300 mg kg^{-1} doses in male Sprague-Dawley rats. This was performed to give added information with regards to the toxicological properties of the ethanolic extract of the leaves rodents. The extract was generally safe since the biochemical parameters and histological exminations did not show any overt abnormalities. Concerning the renal system, no significant changes were found in their urea and creatinine measurements. The histological analysis of the kidney also revealed normal tubules, glomeruli, and renal corpuscles compared to the control group. This shows that the extract may be devoid of any potential constituent that can induce renal impairment or damage. The continuous administration of the extract also did not have any significant effect on the lipid profile. Total cholesterol, HDL, LDL, and triglycerides were analysed and no significant difference was found between the test groups and the control. Furthermore, histological analysis of the liver revealed a normal architecture of sections with a characteristic hexagonal arrangement of hepatocytes in lobules surrounding a central vein. This means the extract may be devoid of any potential constituent that can induce liver damage or impairment. However, low doses

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of the extract reduced direct biluribin. Though not demonstrated clearly, the extract's ability to reduce total direct bilirubin might be useful in reducing jaundice (Arthur, Woode, Terlabi, & Larbie, 2012). Future work on the plant can research more into bilirubin lowering potential or ability of the plant extract.

Mesial temporal lobe epilepsy (TLE) refers to an electro-clinical syndrome in which seizures emerge from the temporal lobe. This is usually accompanied by mesial temporal sclerosis which is a pattern of hippocampal damage, including atrophy and gliosis, seen in humans with TLE and in animal models of chronic TLE (Goldberg & Coulter, 2013). From the histological analysis of the brain, the sections revealed normal microscopic structures. There were no distortions of the normal architecture of tissues of any of the organs with immigrant cell types which may have constituted a form of pathology in all the doses levels as compared to that of the control. Epileptogenesis refers to a dynamic phenomenon that increasingly changes neuronal excitability, establishes critical interconnections, and perhaps requires intricate structural changes before the initial spontaneous seizure occurs (Pitkänen & Lukasiuk, 2011). These alterations can include gliosis, axonal damage or sprouting, neurodegeneration, neurogenesis, dendritic plasticity, and reorganization of the molecular architecture of individual neuronal cells (Pitkänen & Lukasiuk, 2011) There were no indications of gliosis and this was absent in all the doses used.

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CHAPTER 6 CONCLUSIOS AND RECOMMENDATIONS

1.24 CONCLUSION

This study showed that the ethanolic leaf extract of *Ehretia cymosa* (ECE) possesses anticonvulsant activity in models that identify drugs effective against generalized and partial seizures. The extract, however, may not possess neuromuscular and analgesic effects even though more work has to be done to validate that. The study also showed that the extract generally doesn't have any significant effects on the biochemical parameters and histological analysis after a 72-day continuous administration of the extract. Together with the biochemical and histological analysis, it can there be said that the extract may be relatively safe in murine models

This study, therefore, gives evidence to support the use of the leaves of *Ehretia cymosa* as a remedy for epilepsy and also validates its safety in African Traditional Medicine.

1.25 RECOMMENDATIONS

This study recommends:

- Future experiments should separate, purify, and attempt to identify the chemical entity or entities in the extract that are responsible for the CNS effects of *Ehretia cymosa*.
- Chronic toxicity should be done on the extract and evaluated to establish its safety.

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- Other models like the zebra fish models of anti-convulsion should be used to assess/confirm the anticonvulsant properties of the secondary metabolites and investigate other possible mechanisms of action.

- More work should be done to assess its analgesic, neuromuscular, and other related CNS activity of the extract.

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