PHARMACOLOGICAL RATIONALE FOR THE USE OF SOME MEDICINAL PLANTS AS ANTIEPILEPTIC AGENTS IN ZARIA, NIGERIA

BY

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DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACY

FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

JULY, 2008

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BY

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JULY, 2008

DECLARATION

I declare that the work in the dissertation entitled “Pharmacological rationale for the use of some medicinal plants as antiepileptic agents in Zaria, Nigeria” has been performed by me in the Department of Pharmacology and Clinical Pharmacy under the supervision of Dr. J. A. Anuka and Professor I. M. Hussaini. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

_____	_____	_____
Name of student	Signature	Date

CERTIFICATION

This dissertation entitled "PHARMACOLOGICAL RATIONALE FOR THE USE OF SOME MEDICINAL PLANTS AS ANTIEPILEPTIC AGENTS IN ZARIA, NIGERIA" by Ya'u Jamilu, meets the regulations governing the award of masters of science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presented.

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DEDICATION

This thesis is dedicated to Almighty Allah the Exalted and Most High and then to my beloved family.

ACKNOWLEDGMENT

In the name of Allah, the Most Gracious, the Most Merciful. All praise is due to Allah the Almighty, the Exalted and Most High. May Allah's peace and blessings be upon His final messenger, his pure family, his noble companions, and all those who follow them with righteous until the Day of Judgement.

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I am grateful to my family especially my mother, brothers and sisters for their unrelenting support, constant prayers, encouragement and understanding. I cannot end this without specially thank my wife “sweet heart” for her understanding, unrelenting support, and encouragement and for her “cooling effect”.

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ABSTRACT

The effects of the hydro-alcoholic root bark extracts of *C. edulis* and *B. aegyptiaca* were studied on pentylenetetrazole (PTZ) and Maximal electroshock (MES)-induced seizures in animal models. The effects of Flumazenil and Naloxone were studied on the anticonvulsant activity of the plant extracts to determine their possible mechanisms of anticonvulsant action. The intraperitoneal LD_{50} of the hydro-alcoholic root bark extracts of *C. edulis* and *B. aegyptiaca* were found to be 282.8 mg/Kg and 1131.3 mg/Kg respectively, this suggests that the extracts are mildly toxic by this route. Oral LD_{50} of the extracts were both greater than 5000 mg/Kg, suggesting non toxic profile. The *C. edulis* extract produced 40% while *B. aegyptiaca* offered 20% protection against seizures induced by PTZ when given intraperitoneally while the standard anticonvulsant drug used, diazepam offered 60% protection at 0.5 mg/kg and 100% protection at 1 mg/kg. Similarly, in the oral route no protection was obtained in both extracts. In MES-induced seizures, *C. edulis* extract protected the chicks by 90% while *B. aegyptiaca* showed no protection. Flumazenil and Naloxone reversed the anticonvulsant activities of both *C. edulis* and *B. aegyptiaca* extracts suggesting involvement of $GABA_A$-BDZ receptors and opioid receptors in the extracts anticonvulsant activities. *C. edulis* extract fractions aqueous portion (AP), diethyl ether filtrate (BP), diethyl ether precipitate (CS), ethyl acetate (EA) and n-butanol (NB) were found to have varied anticonvulsant activities; AP fraction showed 80%, BP 40%, CS 40%, EA 40% protection in a non dose dependent manner and NB offered no protection against PTZ-induced seizures in mice while fraction CS 60% seizure protection in an inverse dose dependent pattern, fractions AP, BP, EA and NB CS did not produce any significant seizure protection against MES-induced seizures in chicks, similarly phenytoin, standard

anticonvulsant drug used produced 100% protection. There was no significant difference between the fractions treated and the control groups in the duration of seizure. All the *C. edulis* fractions when tested against 4-aminopyridine-induced seizures showed no protection indicating lack of activity on potassium channels. Various phytochemical constituents such as anthraquinones, tannins, saponins, glycosides were found to be present and consistently alkaloids were absent in the extracts and the fractions. The results suggest the presence of bioactive component(s) that possess anticonvulsant activities. The data may provide pharmacological basis for the use of the plants in the management of epilepsy in Zaria, Nigeria.

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ABBREVIATIONS

4-AP	-	4-aminopyridine
A. B. U.	-	Ahmadu Bello University
ADD	-	Antiepileptic Drug Development
AEDs	-	Antiepileptic Drugs
AMPA	-	Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic Acid
ANOVA	-	Analysis of variance
AP	-	Aqueous Fraction
ASP	-	Anticonvulsant Screening Project
ATP	-	Adenosine Triphosphate
BA	-	*Balanites aegyptiaca*
BDZ	-	Benzodiazepines
BFNC	-	Benign Familial Neonatal Convulsions
BP	-	Supernatant/Filtrate of n-butanol fraction
Ca^{2+}	-	Calcium ion
cDNAs	-	Complementary Deoxyribonucleic Acids
CE	-	*Carissa edulis*
CNS	-	Central Nervous System
CS	-	Diethyl ether Precipitate Fraction
CT	-	Computerized Tomography
DZ	-	Diazepam
EA	-	Ethyl Acetate Fraction
EDM	-	Essential Drugs and Medicines

EEG	-	Electroencephalograph
Eipsp	-	Equilibrium Reversal Potential of an Inhibitory Postsynaptic Response
EK	-	Potassium Equilibrium Potential
FAO	-	Food and Agriculture Organization of United Nations
Flu	-	Flumazenil
GABA	-	Gamma Amino butyric Acid
GAT1	-	GABA Transporter
GIT	-	Gastrointestinal Tract
HCl	-	Hydrochloric Acid
HLTE	-	Hind Limb Tonic Extension
HSV	-	*Herpes simplex* Virus
HVA	-	High-voltage Activated
IAEA	-	International Atomic Energy Agency
IP	-	Intraperitoneal
IPSPs	-	Inhibitory Post Synaptic Potentials
K^+	-	Potassium ion
$KCNQ_2$	-	Gene Coded for Potassium Channels Expression
LD_{50}	-	Mean Lethal Dose
LVA	-	Low-voltage Activated
MES	-	Maximal Electroshock
MEST	-	Maximal Electroshock Test
MRI	-	Magnetic Resonance Imaging

mRNA	-	Messenger Ribonucleic Acid
MTLE	-	Mesial Temporal Lobe Epilepsy
Nal	-	Naloxone
NaOH	-	Sodium Hydroxide
NAPRI	-	National Animal Production Research Institute
NB	-	n-butanol Fraction
NINDS	-	National Institute of Neurological Disorder and Stroke
NMDA	-	N-methyl-D-aspartate
NPY	-	Neuropeptide Y
PH	-	Phenytoin
PHB	-	Phenobarbital
PRO	-	World Health Organization Regional Office for the Pacific
Sc	-	Subcutaneous
ScPTZ	-	Subcutaneous Pentylenetetrazole
SEM	-	Standard Error of Mean
TLC	-	Thin Layer Chromatography
TLE	-	Temporal Lobe Epilepsy
TRM	-	Traditional Medicine
VA	-	Sodium valproate
VGCCs	-	Voltage-gated Calcium Channels
VGKCs	-	Voltage-gated Potassium Channels
VGSCs	-	Voltage-gated sodium Channels
WHO	-	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

For a long time it was assumed that a single drug could be developed for the treatment of all forms of epilepsy, but the causes of epilepsy are extremely diverse, encompassing genetic and developmental defects and infective, traumatic, neoplastic and degenerative disease processes, and drug therapy to date shows little evidence of etiologic specificity (Porter and Meldrum, 2004). Epilepsies are common and frequently devastating disorders, affecting approximately 2.5million people in the United States alone (McNamara, 2001). The World Health Organization (WHO) estimated that 80% of the people of the world rely on medicinal plants for primary health care needs (Farnsworth, 1998). Also a current trend in the world is now moving towards the use of natural products in the treatment and or prevention of various disease conditions. Further, herbal medicine is currently enjoying a revival in popularity in the west and in fact it is the primary form of medicine in many parts of the world (Williamson *et al.*, 1996). Apart from being chronic, epilepsy adversely affects the patient's consciousness, behavior and socioeconomic status within the society. In most communities, there is stigmatization on the epileptic patients. Some traditional beliefs attribute the disease to evil spirits, and therefore some of the traditional treatments involve spiritual practice to drive away the evil spirits. Among the traditional practitioners only few are involved in the treatment of epilepsy with medicinal plants without any incantation or spiritual practice and also most of the traditional practice is done in secrecy (Chindo, 1999). There is a need to provide scientific basis for the ethnomedicinal use of plants with potentials in the treatment of epilepsy and research should:

i. Investigate the scientific basis for the use of a particular medicinal plant by the locals in the treatment and or prevention of epilepsy.

ii. Elucidate the possible mechanism of action of the plants.

iii Identify and characterize the active components in these plants.

This work will collect medicinal plants that are used in traditional medicine for the treatment of epilepsy; characterize the chemical constituents and determine anti-convulsant activity using different animal models.

1.2 Statement of Problems

Approximately, 1% of the world's population has epilepsy, the second most common neurologic disorder after stroke (Porter and Meldrum, 2004). World health organization (WHO) estimates that eight people per 1000 worldwide have epilepsy (WHO factsheet, 2001). The incidence of epilepsy varies significantly with age and ranges from as high as 560 cases/100,000/year for infants to as low as 20.3 cases/100,000/ year for ages between of 15 and 30 years. Above 30 years the incidence climbs at a slow rate but does not approach that of infancy. First degree relatives of patients with epilepsy have approximately 3 times more chances of having epilepsy than the non-relatives of epileptic patients (Darwin, 1989).

Life span of patients with epilepsy depends on the severity and underlying cause of the disorder. As many as half of patients have severe epilepsy that require medical attention

and would die directly from seizure. Early death occurs most commonly during the first 5 years after diagnosis. Survival decreases significantly when age of diagnosis is less than 1 year or greater than 60 years (Darwin, 1989).

Although, standard therapy permits control of seizures in 80% of these patients, millions have uncontrolled epilepsy (Porter and Meldrum, 2004). Various studies have attributed a teratogenic effect to antiepileptic drugs (AEDs), resulting in major malformations {central nervous system (CNS) malformations, congenital heart defects, orofacial clefts and skeletal malformations}, minor anomalies, and intrauterine or postnatal growth failure (Adab *et al.*, 2001). Also the management of women of childbearing age involves a number of important issues including contraception, fertility, effects of AEDs, effects of seizures on the fetus and management issues during and after the pregnancy. It is important to remember that a woman can become pregnant at any time and this should be taken into consideration when making decisions regarding medical treatments. Most of the currently AEDs used in the management of epilepsy are implicated to be harmful to fetus when used by women of childbearing age. Therefore, alternative, safe and efficacious molecule is required.

Generally, many drugs that are used as medicine were initially obtained from plants but later synthesized. After careful structural research, scientist have taken up the task of compiling and evaluating the medicinal and non-medicinal uses of plants so as to establish botanical and chemical profiles, thus, enhancing the pharmaceutical potential of plants (African Pharmacopoeia, 1985).

1.3 Justification

Clinical trials and experimental data suggest that the AED therapy does not alter the course or natural history of epilepsy. The therapy only suppresses seizures, but does not affect the underlying epileptogenic process itself (Shinner and Berg, 1996). Plant extracts can be an important source of natural and safer drugs for the treatment of epilepsy (Meldrum, 1997). Hence we aim to establish antiepileptic properties of plants used in the treatment of epilepsy with the hope of developing novel lead compound(s) in the future.

WHO encourages the inclusion of herbal medications in the healthcare because of their great potentials (Amos *et al*, 2001). The long historical use of medicinal plants has demonstrated the safety and efficacy of traditional medicine (WHO, 2000). Many people in the developing countries rely on traditional healing practices and medicinal plants (WHO/PRO, 1998). Also, traditional medicine is the most affordable and accessible systems of health care (Stanley, 2004). Traditional medicine has the potential of providing the healthcare need for Africa whose populations is mostly rural. With the great reliance on this type of medicine, it becomes pertinent to search for potent, effective and relatively safe plant medicines as well as scientific validation of the success claims about plants already in use by traditional medicine practitioners in order to enhance their safety and efficacy. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that are yet to be discovered. WHO reiterates that scientific research is needed to provide evidences of the safety and efficacy of beneficial medicinal plants (WHO, 2002).

The selection of potential anti-epileptic plants and evaluation of activity will provide scientific basis for the use of the plants. Isolation of active anti-convulsant compounds from medicinal plants may provide potent, cheap and readily available compounds. The provision of scientific basis for the therapeutic use of the plants is necessary in order to properly define their potentials as alternative drug therapy for epilepsy which is a disease of sociological and economic importance. Evidently, traditional medicine enjoys a very wide acceptability especially among the people of developing countries and it blends readily into their socio-cultural life.

1.4 Theoretical Framework

1.4.1 *Models for Epileptic Studies*

Many patients with epilepsy fail to experience adequate control of their seizure despite the optimal use of available antiepileptic drugs (AEDs) marketed in the US. Others patients do so only at the expense of significant toxic side effect. Since 1975, the epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH) through its Antiepileptic Drug Development (ADD) program, has collaborated with the pharmaceutical industry in developing new therapeutic agents for the treatment of seizure disorders (Stables and Kufferberg, 1997).

A single approach to identify potentially useful drugs cannot be used in a drug discovery program (such as the ADD). Therefore, combinations of different approaches have to be employed in order to identify potential compounds for the treatment of seizures. The approaches include;

1. *Mechanistic approach:* This assumes a specific mechanism(s) for seizure initiation, propagation and amelioration. Examples of this approach would be to identify compounds that work via inhibition of excitatory amino acid-mediated excitation or enhancement of GABA-mediated inhibitory transmission. To some extent, this approach limits the scope of discovery of novel substances. The advantage of this approach is that a large number of compounds can be evaluated in a short period of time, with limited amounts of materials and without the use of large numbers of animals.

2. *The non-mechanistic approach*: Non-mechanistic seizure models have clearly defined endpoints. Examples of such endpoints are the inhibition of the tonic hindlimb extension phase, following electrically-induced seizures or clonic seizures following administration of seizure producing doses of chemoconvulsants. These methods have advantage of requiring limited technical expertise and permit a direct comparison of the anticonvulsant profile of a new drug to that of 'clinically effective therapeutic agents'. Unfortunately, this approach provides little pertinent information regarding an active compound's mechanism of action, which is highly desirable for making drug development decisions.

3. *Seizure-type model approach*: There is a limited number of animal models that represent true 'epilepsies'. The models that are available require a high degree of technical expertise, are costly and labor intensive. With such models, drugs for symptomatic epilepsy are not readily identified, whereas those for the generalized epilepsies are much more easily identified.

In primary evaluation of anticonvulsant activity for a new compound or medicinal plant, the Anticonvulsant Screening Project (ASP) initiated at the University of Utah in the 1974 under NINDS contract, advocated the use of both electrical (maximal electroshock, MES) and chemical induced seizures (subcutaneous pentylenetetrazole, ScPTZ) for its initial screening procedures. In these animal models, the animals are 'normal' and not genetically predisposed to seizures. While the neuronal sensitivity of normal mice to anticonvulsants may be different than in epileptic animals, these models afford certain advantages over genetic epileptic prone animal models for the following reasons;

i. They are suited for routine screening of a large number of potential anticonvulsants.
ii. Normal animals are less expensive than are the inbred genetic animals.
iii. Genetically epileptic-prone rodents exhibit a greater number of false positive responses to non-anticonvulsant drugs.

The animal models chosen in these studies are experimental rodents that mimic human central nervous system disorders. They have been carefully chosen based on their simplicity, rapidity and reproducibility of results. The following is background information on the models used for the studies;

The Subcutaneous Pentylenetetrazole test (ScPTZ) induced seizure: This is a model that primarily identifies compounds that raise seizure threshold. Absence of clonic spasm in the observed time period indicates a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold (Swinyard *et al*., 1989). AEDs that inhibit this type of seizure (e. g. valproic acid, ethosuximide, phenobarbital and benzodiazepines) are effective in the treatment of myoclonic and absence seizures (Loscher *et al*., 1991).

Maximal Electroshock test (MEST): MEST is a standard AEDs test that evaluates the testing materials' ability to protect against hind limb tonic extension (HLTE) phase of the MEST (DeLorenzo *et al*., 2001). The MEST is a model for generalized tonic-clonic seizure which is highly reproducible with a consistent end-point (Stables and Kupferberg, 1997). The behavioral and electrographic seizures generated in this model are consistent with the human disorders (Swinyard *et al*., 1989). Abolition of the HLTE component indicates ability of test compound to inhibit MEST-induced seizure spread (White *et al*., 1998). AEDs that suppress the HLTE in MEST are effective in the therapy of generalized tonic–clonic and partial seizures. Example of such AEDs include; phenytoin, and carbamazepine (Loscher *et al*., 1991).

4-Aminopyridine-induced seizures: K^+ channel opening is one potential anti-epileptic mechanism that has not yet been exploited. K^+ channel plays a major role in the control of all aspect of neuronal excitability including resting membrane potential, responsiveness to synaptic inputs, spike frequency adaptation and neurotransmitter release (Wickenden, 2002). 4-aminopyridine is a K^+ channel antagonist (Yamaguchi and Rogawski, 1992), it is a powerful convulsant in man and animals. It readily penetrates the blood–brain barrier and induces seizure activity by enhancing spontaneous and evoked neurotransmitter release.

Although, both excitatory and inhibitory synaptic transmission is facilitated by 4-Aminopyridine (4-AP), the epileptiform activity induced by the drug is predominantly mediated by non *N-methyl-D-Aspartate* (NMDA) type excitatory amino acid receptors (Vogel and Vogel 1997). Drugs effective in this model can serve as alternative or adjunct therapy for the treatment of drug resistant or refractory epilepsy (Wickenden, 2002).

1.5 Aims and Objectives

1.5.1 *Aims of the Study*

The aim of the study is to investigate the anticonvulsant activity of Hydro-alcoholic root bark extracts of *Carissa edulis* and *Balanites aegyptiaca.*

1.5.2 *Objectives*

1. To select plants used in traditional treatment of epilepsy from folkloric claims of usefulness.
2. To evaluate the anti-convulsant activities of Hydro-alcoholic root bark extracts of *Carissa edulis* and *Balanites aegyptiaca* using animal models of epilepsy.
3. To characterize the phytochemical constituents of the plants.

1.6 Research Hypothesis

Carissa edulis and *Balanites aegyptiaca* used in traditional medicine for the treatment of epilepsy possess anti-seizure properties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Epilepsy

Epilepsy is an episodic neurological disorder that affects up to 1% of the population, and is perhaps best characterized as a family of disorders. Epilepsy is a progressive disorder comprising of many seizure types and syndromes. Although the specific mechanisms underlying this group of disorders are diverse, they all appear to have in common the feature of aberrant synchronized discharge of neurons leading to alterations in electroencephalograph (EEG) activity and behavior (Dichter, 1998). Such abnormal bursting activity in neurons has suggested to many, that a change in the intrinsic excitability of neurons is an underlying neurophysiological feature of the disorder, which has led to investigations into the mechanisms controlling neuronal excitability in the search for new treatments (Cosford *et al.*, 1998).

2.1.1 *Causes of Seizure*

The cause of convulsions must be clearly understood through some precise observations. The type of seizure depends on the site of the focus in the brain. Epileptic attack can be caused by biochemical insults to the brain, such as during hypoglycaemia, anoxia, hypocalcaemia, hyperventilation, water intoxication and sudden withdrawal of certain drugs such as barbiturates or alcohol (Bienvenu *et al.*, 2002). Epilepsy can also be caused by previous active pathology, such as birth trauma to the brain, during or following meningitis, trauma to the skull and brain later in life, cerebral abscesses, cerebral

infarction, cerebral haemorrhage or subarachnoid haemorrhage (Biller, 1997; Bienvenu *et al.,* 2002).

Further analysis shows that the blockade of post-synaptic gamma-amino butyric acid (GABA) receptors or an inhibition of GABA synthesis is the principal origin of brain discharge (Muhizi, 2002). Similarly, an epileptic attack can be triggered by a sensory stimulus, which is specific for individuals (Bienvenu *et al.,* 2002). So far, there is no single unifying explanation as to how these diverse factors cause seizures. Hence, it is difficult to determine the exact cause of epilepsy, even though it has been possible to investigate the pathophysiological events that are involved in the genesis of epilepsy (Sudarsky, 1990)

2.1.2 *Types of Epilepsy*

Optimum treatment of seizure disorders requires accurate classification (diagnosis) of seizure type as well as appropriate choice and use of medication (1983; Dreifuss, 1989; Pellock, 1989). In addition to clinical observation, seizure diagnosis and classification of the type of epilepsy also depend on neurological examination, medical history and techniques such as electroencephalography (EEG) and computerized tomography (CT). The EEG is often critical for identifying specific seizure types. TC scans are routinely utilized to access newly diagnosed patients, especially when partial seizure may be due to smaller focal lesions. Magnetic resonance imaging (MRI) primarily is used to locate brain lesion or anatomical defects that may be missed by conventional X-rays or TC scans.

The seizure classification used is based on the international classification of epileptic seizures (Dreifuss, 1989; Penry, 1986; Hussaini, 2006).

1. Partial seizures (Local or focal): There is local high frequency discharges (ictal) that lasts few minutes. Symptoms depend on the brain region involved. Partial seizures are those in which the discharge begins locally and often remains localized. These may produce relatively simple symptoms without loss of consciousnesses, such as involuntary muscle contractions, abnormal sensory experiences or autonomic discharge, or they may cause more complex effects on consciousness, mood and behavior, often termed psychomotor epilepsy (Rang *et al.,* 2003). Psychomotor epilepsy is often associated with a focus in the temporal lobe; the attack may consist of stereotyped purposeless movements such as rubbing or patting movements, or much more complex behavior such as dressing, walking or hair combing. The seizure usually lasts for a few minutes, after which the patient recovers with no recollection of the event (Webster and Jordan, 1989). It is subdivided into:

a. Simple; if consciousness is not affected. The symptoms can be motor, special sensory or somatosensory, autonomic or psychic symptoms

b. Complex: here there is loss of consciousness which could be with no other features, with features as in simple partial seizures, with automatisms.

c. Partial seizure secondarily generalized. This could be simple partial seizures evolving to generalized seizures, complex partial seizures evolving to generalized seizures or simple partial seizures evolving to complex partial seizures to generalized seizures.

2. Generalized (bilateral) Seizures (Convulsive or Non-convulsive): There are high frequency discharges that spread across the entire brain. The main categories are generalized tonic -clonic seizures (grand mal) and absences seizures (petit mal). A generalized tonic -clonic seizure consists of an initial strong contraction of the whole musculature, causing a rigid extensor spasm. Respiration stops and defecation, micturition

and salivation often occur. This tonic phase lasts for about 1 minute and is followed by a series of violent, synchronous jerks, which gradually dies out in 2-4 minutes (Sudarsky, 1990). The patient stays unconsciousness for a few more minutes, and then gradually recovers, feeling ill and confused. Injury may occur during the convulsive episode. With optional drug therapy, therefore, epilepsy is controlled completely in about 75% of patients, but about 10% continues to have seizures at intervals of one month or less, which severely disrupt their life and work (Rang *et al.*, 2003). While in absence seizures consciousness is impaired, with mild clonic components, atonic components, automatism or autonomic components alone or in combination. While in atypical absence seizure changes in tone is more pronounced than in typical absence or an onset and / or cessation that is not abrupt. The subclasses are as follows;

a. tonic-clonic (grand mal.) seizures
b. Absence seizures
c. Tonic seizures
d. Clonic seizures
e. Atonic seizure (astatic or akinetic)

3. Unclassified epileptic seizures: These include all seizures that cannot be classified because of inadequate data and some that defy classification in hitherto described categories. This includes some neonatal seizures (e.g. rhythmic eye movements, chewing and swimming movements).

Persistent seizures (Status Epilepticus): The term "status epilepticus" is used whenever a seizure persists for a sufficient length of time or is repeated frequently enough that recovery between attacks does not occur. Status epilepticus may be divided into.

a. Partial status epilepticus: when much localized motor status occurs it is called epilepsia partials continua.

b. Generalized status epilepticus e.g. absence status or tonic-clonic status (Darwin, 1989).

2.2 Mechanism of Epileptogenesis

2.2.1 *GABA Receptor Inhibition*

One classical theory of epileptogenesis rests on the hypopthesis that reduced inhibition within neuronal networks causes hyper-excitability thus leading to the occurrence of seizures (Olsen and Avoli, 1997; Avoli, 2000). GABA mediates its action in the mammalian brain via at least two classes of receptors, $GABA_A$ (ionotropic) and $GABA_B$ (metabotropic) receptors, which differ in terms of their pharmacological profile, mechanisms of transduction and regional distribution (De Sarro *et al.*, 2000). GABA released from pre -synaptic terminals, acts on both $GABA_A$ and $GABA_B$ receptors. $GABA_A$ receptors are post-synaptic while $GABA_B$ receptors are both pre- and post-synaptic. Previous studies have suggested that, the functional role of the pre-synaptic $GABA_B$ receptors is to regulate the release of both excitatory and inhibitory neurotransmitters (in particular, baclofen, a selective $GABA_B$ receptor agonist) is to elicit a down-regulation of $GABA_B$ function and to decrease transmitter release whilst $GABA_B$ receptor antagonists increase the release of both excitatory amino acids and GABA (De Sarro *et al.,* 2000). Both $GABA_A$ and $GABA_B$ receptors are involved in the control of

neuronal excitability and epiletogenesis but, whereas much is known about the involvement of $GABA_A$ receptors in the control of generalised convulsive seizures, the role of the $GABA_B$ receptors in epileptic process is not fully understood (De Sarro *et al.,* 2000).

2.2.2. *Glutamate Receptors Excitation*

Glutamate is the most important excitatory neurotransmitter in all rapidly conducting relay pathways of the motor and sensory systems of the outer tube of the central nervous system. It produces fast or prolonged synaptic excitation, and triggers various calcium dependent processes in the target cells, including production of nitric oxide (Bienvenu, *et al.,* 2002). Glutamate also plays a major role in synaptic plasticity and in the processes of learning and memory (Rang *et al.,* 2003).

Glutamate is a transmitter in the corticospinal, corticostriatal pathways, intrahemispheric and interhemispheric association pathways, hippocampal circuits, primary afferents, and somatosensory and special sensory pathways, cerebellar afferents and excitatory inter – neurons (Westmoreland *et al.,* 1994). Epilepsy may arise as a consequence of a dramatic release of glutamate from central nerve terminals. Sustained seizures of the limbic system in experimental animals result in brain damage that resembles that due to glutamate toxicity. Similar changes are seen at autopsy in patients with intractable epilepsy. In animals, such seizure related brain damage may be reduced by the administration of non-competitive NMDA receptor antagonists, but it would appear that not all seizure activity is suppressed by such drugs (Leonard, 2003). The precise mechanism whereby persistent

seizure activity results in neuronal degeneration is not completely understood. It seems possible that repetitive depolarization and repolarization of the nerve membrane eventually leads to an energy-deprived state within the cell, thereby preventing the restoration of the cell membrane potential. Each depolarization will also lead to an influx of calcium ions and efflux of potassium ions, which if prolonged, can result in cell death. The reduced efficiency of glial cells to remove potassium ions, and the ability of high extracellular concentration of potassium ions to depolarize neurons and cause neurodegenerative changes also play a critical role in causing the degenerative changes that are a feature of *status epilepticus* and intractable epilepsy (Leonard, 2003). Recent advances have indicated that gamma (g)-amino butyric acid (GABAA) receptors work synergistically with N-methyl-Daspartic acid (NMDA) receptors to increase the flux of Ca2+ ions into neurobasts and immature neurons. This is essential for the modulation of early CNS development (DeLorey and Olsen, 1999). Drugs have also been developed to modulate glutamic acid function. Reduction of excitatory glutamatergic neurotransmission is potentially important; AMPA receptor blockade probably contributes to the antiepileptic effect. Topiramate and NMDA receptor blockade contribute to the antiepileptic effect of drugs such as lamotrigine (Porter and Meldrum, 2004).

Apart from these two major aforementioned mechanisms of epileptogenesis, other mechanisms have been found to answer some of the questions rose which could be answered by these two major mechanisms. These include the following;

2.2.3 *GABA Transporter*

During et al (1995) reported that Human epileptogenic tissue from mesial temporal lobe epilepsy (MTLE) patients, who were analysed with implanted microdialysis probes, presents reduced levels of GABA transporters. It is, however, unclear whether these changes are the cause or the result of the epileptogenic conditions.

2.2.2 *Neuropeptide Y (NPY)*

Neuropeptides act as cotransmitters of classical neurotransmitters. Like classical transmitters they become released during epileptic seizures. Interesting acute seizures induce over-expression of some of these peptides (e.g. neurokinin B, somatostatin, cholecystokinin, dynorphin, neuropeptide Y) in brain areas involved in limbic seizures (Marksteiner and Sperk, 1988; Gruber *et al.,* 1993). Hippocampal granule cells with their axonal extensions, the mossy fibers, are especially "plastic" in temporal lobe epilepsy (TLE). Like other peptides (neurokinin B, dynorphin), neuropeptide Y (NPY) becomes even ectopically expressed in granule cells of epileptic rats (Marksteiner *et al.*, 1990; Sperk *et al.,* 1992; Marksteiner *et al.*,1992). At the same time, also NPY-Y2 receptors (located at the mossy fiber terminals) become up-regulated in these neurons, whereas Y1 receptors (targeted to the dendritic extensions of granule cells) are downregulated (Kofler *et al.,* 1997; Gobbi *et al.,* 1998). It is postulated that the over-expressed NPY may be released during epileptic seizures and may mediate an inhibition of glutamate release through Y2 receptors and thus exert an endogenous anticonvulsive mechanism (Marksteiner *et al.,* 1990; Greber *et al.,* 1994; Gruber *et al.,* 1994). Immunohistochemical and molecular studies have provided data indicating that neuromodulation in epileptic human hippocampus might be disturbed as well. Recently, attention is focused on neuropeptide Y,

a neuromodulator NPY, apart from playing a central importance in the regulation of neuronal excitability and, particularly, living interneuron discharge propensity (Baraban and Tallent, 2004). NYP decreases synaptic transmission by reducing presynaptic calcium influx via Y_2 receptor activation (Qian et al., 1997).

2.2.5 *Gap Junctions*

An additional mechanism that may contribute to epileptogenesis rests on gap junctions that are increasingly recognized to play a role in synchronizing neuronal networks under pathophysiological conditions such as epileptic seizures (Dermietzel and Sprays 1993; Bennette and Zukin, 2004; Connors and Long, 2004). Gap Junctions allow flow of electrical signals and small molecules, including dyes, between cells thus promoting neuronal synchrony. Procedures that is capable of blocking or enhancing the function of gap junction decreases or increases epileptiform synchronization, respectively in *in vitro* models of epilepsy (Carlen et al., 2000). The effects induced by gap junction decouplers in the synchronous activity generated by human neocortical slices superfused with 4-aminopyridine and glutamatergic transmission antagonist, suggests that interneuron activity in this preparation is synchronized via gap junctions.

2.2.5 *Gene Expression Patterns*

Recent investigation have shown that widespread changes of gene expression patterns occur in human epileptic hippocampus, some of which overlap with alterations found in the chronic MTLE condition induced by injection of pilocarpine in rats that include

proteins involved in cell-matrix interactions in cell growth and differentiation, in cellular signaling, and in transcriptional regulation (Becker et al., 2003).

There is now evidence that many epilepsy-associated genes participate in secondary cellular plasticity during brain development, which is likely to influence downstream events. These influences may account for the delayed temporal onset of seizures observed in some epileptic syndromes (DeLorey and Olsen, 1999).

2.3 Anti-Epileptic Drugs and their Mechanism of Action

The majority of currently available anti-epileptic drugs (AEDs) fall into one of two pharmacological classes, those that modulate neuronal voltage-gated channels such as sodium channels (e. g. carbamazepine, phenytoin, lamotrigine and topiramate) and voltage-operated calcium channels (e. g. ethosuximide, gabapentin and levetiracetam) and those that modulate inhibitory GABAergic neurotransmission (e. g. benzodiazepines, vigabatrin and tiagabine).

There is an increasing awareness that mutant ion channels play a role in episodic neurological disorders, especially those where excessive membrane excitability has been implicated (Dichter, 1998). This is intuitive since ionic balance controls membrane potential, and ion channels are major regulators of ion homeostasis.

2.3.1 *Voltage-Gated Sodium Channels*

While voltage-gated sodium channels (VGSCs) are required for excitatory synaptic transmission under normal physiological conditions, their overstimulation can result in profound neuronal damage (Clare *et al.,* 2000; Anger *et al.,* 2001). As a consequence antagonists of these channels may confer beneficial antiepileptic properties and/or neuronal protection following ischemic events (Taylor *et al.,* 1999; Mody, 1998; Urenjak and Obrenovich, 1996; Ragsdale and Avoli, 1998). Indeed, a large number of pharmaceutical agents that modulate these channels have found application as antiarrhythmic, neuroprotective and anticonvulsant agents in addition to exhibiting local anesthetic uses. However, most of the VGSC blockers which have been discovered and received widespread clinical usage exhibit relatively modest intrinsic potencies and frequently are relatively non-selective agents, possessing ancillary activities at other receptors which limit their utility. As a consequence, the identification of superior sodium channel antagonists with improved activity and enhanced safety profiles is urgently required. Success in this arena will necessitate an improved understanding of the molecular basis of voltage- and use-dependent blockade of these currents, rather than the empirical drug screening approaches used in the past. As many of the pharmaceutical agents in current clinical use clearly possess multiple mechanisms of action, considerable effort has been expended to rationally identify more selective derivatives that inhibit sodium channels exclusively, or are dual antagonists with only one additional cellular target.

Voltage-gated Na+ channel blockers: The drugs with this mechanism of action include carbamazepine, phenytoin and lamotrigine. They are useful for the treatment of partial seizures and clonic seizures of generalized seizures.

Topiramate reduces voltage-gated Na^+ currents in cerebella granule cells and may act on the inactivation state of the channel in a manner similar to that of phenytoin. It also activate a hyperpolarizing k^+ current, enhances postsynaptic $GABA_A^-$ receptor currents and also limits activation of AMPA-kainate subtype(s) of glutamate receptor(McNamara, 2006).

2.3.2 *Voltage-gated Calcium Channels*

Voltage-gated calcium channels (VGCCs) are key regulators of Ca^{2+} entry into neurons, and in this capacity they control a variety of Ca^{2+}-dependent functions, including neurotransmitter release, gene expression, mRNA stability, and neuronal excitability. The concept that VGCCs might be involved in epilepsy is not new, and is based partly on the physiological role of VGCCs, the activity of standard anticonvulsant compounds on these channels, and the ancillary anticonvulsant activity of known subtype-selective VGCC antagonists. What is new is that genetic studies have now revealed an unexpected correspondence between mutations in genes encoding VGCC subunits and absence epilepsy in mice, implicating anew that alterations in VGCC function might contribute to the pathogenesis of epilepsy. VGCCs are known to control a variety of cellular processes that regulate neuronal excitability; hence their role in epilepsy has been the subject of considerable research over the past 15 years (Stefani *et al.,* 1997). VGCCs can be divided into two groups, high-voltage activated (HVA) and low-voltage activated (LVA), and both groups can affect membrane excitability. Most obvious is the role of high-voltage activated (HVA) VGCCs in controlling the release of neurotransmitters, such as the excitatory neurotransmitter glutamate (Dunlop *et al.,* 1995). Also, by controlling the entry of Ca^{2+}

into neurons VGCCs indirectly influence the activity of Ca^{2+} -activated K+ channels and gene expression, which can result in both short and long-term changes in neuronal excitability. LVAs, or T-type VGCCs, appear to be more directly involved in controlling membrane potential. For example, T-type VGCCs cause depolarizing shifts in membrane potential that lead to low-threshold stimulation in thalamic neurons, which may underlie the synchronizing discharges characteristic of epilepsy (Huguenard *et al.,* 1996). These channels have been shown to be blocked by known AEDs, such as ethosuximide (Coulter *et al.,* 1989; Coulter *et al.,* 1990), further validating them as potentially useful therapeutic targets. However, not all T-type VGCCs appear to be equally sensitive to ethosuximide (Avery and Johnson, 1996; Leresche *et al.,* 1998). VGCCs are also implicated in epilepsy by the recent linkage of several mouse models of absence epilepsy to mutations in VGCC genes (Fletcher *et al.,* 1996; Letts *et al.,* 1998). Whether the epileptic phenotype in these animals results from ongoing functional changes in VGCC activity brought on by these mutations or from developmental changes is not yet known, but sufficient evidence exists to justify additional research devoted to examining the potential for VGCC modulators in the treatment of epilepsy. Currently lacking, however, is a complete set of potent, subtype-selective VGCC modulators that can be used to test this key hypothesis. The two most effective drugs for absence seizures, ethosuximide and valproic acid, probably act by inhibiting T- type Ca^{2+} channels in thalamic neurons (Lowestein, 2001).

Zonisamide inhibits the T-type Ca^{+} currents and also inhibits the sustained, repetitive firing spinal cord neuron presumably by prolonging inactivation of voltage-gated Na^{+} channels in manners similar actions of phenytoin and carbamazepine (McNamara, 2006).

2.3.3 *Voltage-Gated Potassium Channels*

One potential anti-epileptic mechanism that has not yet been exploited is K^+ channels opening. K^+ channels play a major role in the control of all aspects of neuronal excitability.

Potassium channel ligands offer a perhaps more subtle mechanism of seizure control than VGCC antagonists, because agonists or antagonists of voltage gated potassium channels (VGKC) indirectly modify presynaptic Ca^{2+} entry, neurotransmitter release and action. VGKCs generally regulate neuronal excitability by influencing the resting membrane potential and shaping action potentials and postsynaptic potentials (Meir *et al.,* 1999). VGKCs serve diverse functions in the CNS, many of which can be considered in the context of controlling or eliminating epileptic seizure activity. Presynaptic VGKCs regulate the release of both excitatory and inhibitory neurotransmitters throughout the brain. VGKCs in nerve terminals include (Meir *et al.,* 1999) delayed-rectifier potassium channels, fast transient A VGKCs, Ks slowly activating VGK Cs and K (Ca) calcium-activated potassium channels. In addition, terminals may express ligand-gated potassium channels gated by calcium, ATP or serotonin. Recent evidence obtained from mice with a disrupted gene for the α6 subunit of the $GABA_A$ receptor showed a compensatory increase in a voltage independent potassium-selective leak conductance in cerebellar granule neurons (Brickley *et al.,* 2001). As these potassium channels seemingly play an important role in maintaining normal levels of excitability of the granule cells in these mice, at least, it may be of interest to examine several of the non-voltage-gated potassium channels as possible

targets of AEDs. Postsynaptic VGKCs shape the response to transmitter by either influencing or directly setting the resting membrane potential. Generally, if VGKCs open, the membrane potential will move toward the EK (potassium equilibrium potential), whereas if they close, other ionic conductances active in the neuron will drive membrane potential changes, often, as in the case of VGSC and VGCCs, in a depolarizing direction. Thus, VGKC openers can be considered in a first approximation to be antiepileptic because they should 1) speed the re-polarization of the presynaptic terminal, which will shorten the duration of the action potential and decrease the amount of transmitter released and 2) dampen the postsynaptic response to transmitters by stabilizing the membrane potential toward EK. This obvious oversimplification neglects cases in which openers of presynaptic VGKCs would decrease the release of inhibitory transmitters, making matters worse in epileptic foci, or actually depolarizing postsynaptic cells where the Eipsp (equilibrium reversal potential of an inhibitory postsynaptic response) might be more negative than EK.

Positional mapping and linkage analyses of human VGKC genes have opened a molecular window for target based approaches to discover potential AEDs. The identification and subsequent genetic analysis of a family with BFNC (benign familial neonatal convulsions) in 1998 showed that a null allele for a marker localized on 20q13.3 occurred exclusively in individuals with BFNC and two non-penetrant individuals [166]. Using cDNAs spanning the deleted region, this study identified *KCNQ2*, a novel gene encoding a VGKC. Additional probands analyzed had missense, frameshift, and splice-site mutations, most of which cause loss of function. A second related VGKC gene, *KCNQ3*, was also mapped using positional cloning of an epilepsy locus on chromosome 8q24 (Charlier *et al.*, 1998).

However, not all polymorphic variations in these clearly lead to an epileptic phenotype (Steinlein *et al.,* 1999; Haug *et al.,* 2000). Because the cumulative lifetime risk of epilepsy in the general population is 2%, the approximate 16% incidence of seizures later in life suggests that the study of individuals with BFNC may provide hints for the pathogenesis of more general forms of epilepsy (Singh *et al.,* 1998; Charlier *et al.*, 1998).

2.3.4 *GABA Receptor-Mediated Inhibition*

The major amino acid neurotransmitters in the brain are GABA, an inhibitory transmitter and glutamic acid, an excitatory transmitter. GABA is widely distributed in the mammalian brain and has been shown to contribute to over 40% of the synapses in the cortex alone. While it is evident that a reduction in GABAergic activity is associated with seizures, and most anticonvulsant drugs either directly or indirectly facilitate GABAergic transmission, GABA also has a fundamental role in the brain by shaping, integrating and refining information transfer generated by the excitatory transmitters (Leonard, 2003). The GABAergic system has long been implicated in epilepsy with defects in GABA neurotransmission being linked to epilepsy in both experimental animal models and human syndromes. However, to date, no human epileptic syndromes may be the directly attributed to an altered GABAergic system (DeLorey and Olsen, 1999). The observed defects in GABA neurotransmission in human epileptic syndromes may be the indirect result of a brain besieged by seizures (DeLorey and Olsen, 1999). Effects on synaptic transmission have been sought for many anti-epileptic drugs. Enhancement of GABA–mediated inhibition can be produced in many different ways, involving either direct action on the $GABA_A$ - receptor-chloride channel ionophore complex, or action on reuptake or

metabolism of GABA (Porter and Meldrum, 2001). This mechanism provides protection against generalised tonic - clonic and focal seizures. Drugs have been developed to modulate GABA function; the inhibitors of GABA transaminases, which metabolize GABA, have been shown to be effective. These include;

GABA Analogs: Vigabatrin covalently binds and blocks GABA transaminase thus, increases the GABA transporter GAT-1. This increases the duration of GABA in the synaptic cleft thereby increasing duration of inhibitory post synaptic potentials (IPSPs). It is used as an adjunct for refractory partial Seizures (Hussaini, 2006).

Gabapentin binds with high affinity to the $\alpha_2\delta$ subunits of high voltage – activated Ca-channels. Little direct effect on Ca-currents, so real mechanisms of action is unknown (Hussaini, 2006).

Valproate: It mechanism of action is largely unknown but increases brain content of GABA. It is useful in most type of epilepsy (Hussaini, 2006).

Tiagabine inhibits the GABA transporter, GAT-1, and thereby reduces GABA uptake into neurons and glia, thus permitting more GABA to be available for receptor binding (McNamara, 2006)

2.4 Shortcomings of existing antiepileptic drugs

Since the introduction of Phenobarbital (luminal) by Hauptman in 1912 as an effective anticonvulsant agent a number of antiepileptic drugs (ADEs) have been introduced into the market. The first generation AEDs (phenytoin, carbamazepine, valproate, Phenobarbital, primidone, ethosuximide and benzodiazepines) is capable of preventing seizure generation irrespective of the underlying etiology. Although older generation antiepileptic drugs (AEDs) such as carbamazepine, phenytoin and valproic acid continue to be widely used in the treatment of epilepsy, these drugs have important shortcomings such as a highly variable and nonlinear pharmacokinetics, a narrow therapeutic index, suboptimal response rates, and a propensity to cause significant adverse effects and drug interactions. Further advances in the understanding of the biological causes of epilepsy over the last three decades have allowed the development of the second generation AEDs (oxacarbazepin, gabapentin, lamotrigine, felbamate, tiagabine, vigabatrin, topiramate, levetiracetam and zonisamide) that target the cellular mechanism that is responsible for epileptic discharges (Kwan and Brodie, 2000). The pharmacotherapy of epilepsy is confronted with four major problems. First, about 30 to 40% of epileptic patients do not respond at all to currently available AEDs (Pavia et al., 1987). Secondly, current AEDs are associated with toxicity and serious side effects, notably cognitive dysfunction, ataxia, behavioral disorders, gingival hyperplasia, anaemia, hepatotoxicity and teratogenesis (Theunissen et al., 1994; Battino et al., 2000). Thirdly, continous medication is necessary even after the seizures have been suppressed (Sasa, 2006). Lastly, currently available AEDs merely treat the symptoms and not the disorder (Sasa, 2006).

However, comparative risks associated with different AEDs have not been clearly defined and, although polytherapy seems to be more teratogenic than monotherapy, relative risks associated with specific AED combinations have not been adequately assessed. The teratogenic potential of newer generation AEDs is also unknown (Daniela *et al.,* 2002). Therefore, looking at problems enumerated for currently available AEDs, there is need for drugs that are devoid of these shortcomings. Hence, this study tends to search from the abundant compounds with potentials of being the ideal agent from the medicinal plants used in the traditional medicine.

2.5 Some Medicinal Plants used in the Management of Epilepsy

Passiflora incarnata, Scutellaria lateriflora and *Valeriana officinalis* are used as herbal treatment of epilepsy in Europe and America (Murray, 1998). A number of African medicinal plants have been shown to possess active principles which make them beneficial in the management of seizure disorders. These include *Dalbergia saxatilis* (Yemitan and Adeyemi, 2006) and *Schumanniophyton magnificum* (Ogbonnia *et al*., 2003). African traditional anti-epileptic plants include *Xylopia aethiopica, Heliotropium indicum, Cassia occidentalis, Eleusine indica* (Mann *et al*., 2003), *Disopyros physocalycina , Jatropha curcas, Securidaca longenpenduculata, Tetrapleura tetraptera* (Neuwinger 1996).

2.6 The Genus *Carissa*

The genus Carissa belongs to the order Gentianales (Apocynaceae), (Webb, 1969). Generally, the Apocynaceae family, including the genus Carissa contains numerous plants that have been used in traditional medicine and/or have yielded pharmacologically active

compounds (Reid, 1977). Their botanical, chemical, medicinal and ethnopharmacological properties is discussed.

2.6.1*Habitat*

Thirty to thirty five *Carissa* species are found in Africa, Madagascar, the Arabian Peninsula, the Indian subcontinent, New Guinea, New Caledonia and Australia (Foster, 1996).

2.6.2 *Morphology*

According to Foster (1996) members of the *Carissa* genus are evergreen perennial woody shrubs, scramblers or trees, producing white latex. The stem has opposite, simple or forked spines. Leaves are petiole and opposite, glabrous or with basifixed indumentums, collectors being absent. The inflorescence is terminal or extra-axillary, generally a pedunculate of umbelliform. Flowers are scented and pedicellate with the calyx lacking colleters. The corolla is of salverform with a cylindrical tube, usually somewhat bulging around the anthers. The flowers are normally glabrous or with internal and external indumentums, stamens are included, inserted near linear to linear-lanceolate. The style-head is subglobose and bifid at the apex. The fruit is aglobose to obloid-ovoid, syncarpous fleshy berry. It contains one to four seeds, which are flatted, globise, unwnged and ecomose.(Foster, 1996).

2.6.3 *Ethnopharmacology and Chemistry*

The medicinal uses of *C. edulis* will be discussed in detail later. Traditionally, *C. lancealata* has been used for the treatment of various medical conditions, including

toothache, respiratory infections and the cleansing of sores, the causes of which could be bacterial. Some indigenous communities in the Kimberly area of Western Australia burn leaves as a mosquito repellent and squeeze the wood of young plants to obtain a sap, which is rubbed on the skin for rheumatism. Also, decoction of leaves and twigs are used for the treatment of colds and flu-like symptoms (Rye *et al.*, 1992). Its chemical constituents include eudesmane-type sesquiterpenes, carrisone and the cardiac glycosides, odoroside H[25]. *C. carandas* is known for thousands of years in the Ayurvedic system of medicine. The root is credited with bitter, stomachic, antidiarrhoel and anthelmintic properties, while its leaves are prescribed in remittent fever, earache, soreness and syphilitic pain of the mouth (Kirithikar and Basu, 1918; Jayaweera, 1981). A tincture of fruits is used in skin infections (Ayurveda Pharmacopoeia, 1969) and a decoction of wood is employed as a tonic to strengthen the tendons of slim patients. The root of *C. carandas* has yielded a number of volatile principles, including 2-acetylphenol (Zaki *et al.,* 1983; Pino *et al.,* 2004) carinol (Pal *et al.,* 1975), carissone and carindone (Rastogi *et al.,* 1966; 1967). The leaves were reported to have triterpenoidal constituents (Siddiqui *et al.,* 2003) as well as tannins (Sarin and Kapoor, 1963). Carissol, oderoside H, digitoxigenin and the sugars D-glucose and D-digitalose were found from the fruit of the *C. carandas* (Rastogi *et al.,* 1967; Naim *et al.,* 1985). Medicinally of lesser importance, and therefore receiving considerably less attention in academic literature, are *C. bispinosa,C. Macrocarpa, C. congesta and C. spinarium.* The roots of *C. spinarium* are traditionally used for their purgative properties as well as to treat worm infested wounds in animals (Kirithikar and Basu, 1918). Limited chemical and pharmacological studies have not yet been undertaken with those particular Carissa species, but lignans, sesquiterpenes and several cardiac glycosides have been

isolated from them. The alcoholic extracts of aerial parts of *C.congesta*, were found to have CNS depressant activity in mic (Bhakuni *et al.,* 1968). The cardiotonic activity of *C. spinarium* was four to six times greater than that of *C. carandas* (Vohara and De, 1963).

2.7 *Carissa edulis*

2.7.1 *Taxonomy/Nomenclature of the Plant*

According to Burkill, (1985), this plant *C. edulis* falls under the following:

Kingdom: Plantae

Authority: Vahl

Order: Gentianales

Family: Apocynaceae

Genus: *Carissa*

Species: *edulis*

Common/Local Names:

Hausa - Cizaki (Gbile, 1980)

Malawi: Mpambala myoloko (Sofowora, 1986)

English: Carandas plum (H.M. Burkill), Arabic num-num and Natal plum

Fulani: Kanboro

Synonyms: *Carissa carandas*, *Carissa pubescens*, *Carissa pilosa schinz*, *Arduina edulis spreng*, *Azima pubescens suesseng*, *Carissa edulis var. tomentosa* (A. rich) stapf., *Jasminonerium edule* (Vahl) Kuntze and *Tasminonerium tomentosa* (A. rich) Kuntze.

2.7.2 *Botanical Description/ Morphology*

The plant, *C. edulis*, is a spiny evergreen shrub or a small tree that may reach a height of 5 feet and an equal breadth. The bark is grey and smooth with straight woody double pronged spine often in pairs and contains white latex of no recorded property (Dalziel, 1937). The leaves are opposite, leathery, shiny dark green, tip pointed, base rounded and the stalk is very short. The flowers are fragrant in pink-white terminal cluster. Each flower is five – lobed and tubular. Some plants bear flower that are functionally male which are larger than normal. The white or purple flowers which are covered for much of the year add attractiveness to the plant (Irvine, 1961). The rounded berries about half an inch long is green and rich in latex when unripe. As it ripens, the smooth skin turns to purple-black. The flesh is tender, very juicy although bits sour like unripe cherries. Unripe fruits taste tart. Berries are consumed both ripe and unripe. The root when crushed emits a strong smell of methylsalicylate, and if rubbed onto the fingers produces a prickly sensation (Burkill, 1985).

2.7.3 *Geographical Description/Habitat*

The plant is a native of South Africa. It grows in tropical African region and Arabia (Irvine, 1961), the Transvaal, Botswana, north and northeast Namibia. It is a shrub of dry deciduous forest and coastal thickets, extending across the regions from Senegal to West Cameroon and throughout the drier parts of tropical Africa and across Asia to Indochina. It has been reported as a parasite on henna plants in Northern Nigeria (McElderry, 1945). The abundant branching habit and the presence of stiff spines 5cm long make the plant suitable for planting as a protective hedge (Dalziel, 1937). The plant is browsed by goats and camels in the dry parts of Sudan (Irvine, 1961). The plant is also found in majority of

Northern Nigeria in Kaduna, Zaria, Kogin Kano, Maiduguri, Southern Nigeria, Madagascar, India, Ghana and Togo (Hutchingson, 1963).

The Natal plum is a subtropical to near tropical enduring temperature as low as 25^0F although young plants need protection when the temperature drops below 29^0F. Best growth is obtained in full sun. The shrub thrives on dry, rocky hillsides in clay soil. The plant has moderate drought tolerance and high resistance to soil salinity. It cannot stand water logging. The peak period for blooming and fruiting of the Natal plum is May through September (Bekele – Tesemma *et al.*, 1993).

2.7.4 *Ethno Medicinal Uses of the Plant*

The plant parts are used in folk medicine for wide variety of remedies such as fever, sickle cell anaemia and hernia (Ibrahim, 1997). They were also reported to be used for the treatment of oedema, toothache, cough, ulcer, worm infestation and a source of dye (Burkill, 1985; Omino and Kokwaro, 1993; Sofowora, 1986). In Guinea, the boiled leaves are applied as a poultice to relieve toothache (Pobeguin, 1912). The root bark of the plant and other plants are use for management of epilepsy (Suleiman and Saude, personal communication) and also the root bark is mixed with spices and used as an enema for lumbago and other pains in Ghana (Dalziel, 1937). The root scrapings are used in the Soudano-guinean region for glandular inflammation (Aubreville, 1950). The root decoction is taken in Senegal to which pimento is added as an anthelminthic especially against taenia (Kerharo and Adam, 1974). Also, it is known in Tanganyika for anthelminthic action in both man and animals (Irvine, 1961). The ground up root is a remedy for venereal disease

(Watt and Breyer Brandwijk, 1962). In East Africa, the root is used for chest complaints (Bally, 1937); cough remedy, tonic and abortifacient. The Masai takes it with goat meat as a treatment for gastric ulcer (Watt and Breyer – Brandwijk, 1962). In Ghana, they are often added to the food of a sick person as an appetizer (Irvine, 1961). In Kenya, a piece of root is fixed into a hut roof as a snake repellant (Watt and Breyer-Brandwijk, 1962). Vinegar can be made from them by fermentation (Dalziel, 1973; Irvine, 1961) and are made into jam in the Soudan area of the region (Aubreville, 1950) and in Kenya (Irvine, 1961).

2.7.5 *Pharmacology of Carissa edulis*

In pharmacological studies, root wood extracts of *C. edulis* were found to induce a diuresis in rats (Nedi *et al.* 2004). Also, analgesic activity of the water extracts of the root bark, stem bark, leaves, fruits and seeds of *C. edulis* were evaluated in mice and was found to have analgesic activity, with the fruits having the highest activity, followed by the leaves, seeds root bark (Ibrahim *et al.*, 2007). Furthermore, the leave aqueous and methanolic extracts of *C. edulis* were investigated for antimicrobial activities and were found to show inhibitory activity on the organisms tested (Ibrahim *et al,* 2005). Also, an extract of the leaves of *C. edulis* significantly reduced the blood glucose level in steptozotocin diabetic rats, indicating the presence of compounds with hypoglycemic ativity (El-Fiky *et al,* 1996). Aqueous extracts of *C. edulis* have exhibited significant activity against the *Herpes simplex* virus (HSV) *in vitro* and *in vivo* for both wild type and resistant strains of HSV (Tolo *et al,* 2006).

2.7.6 *Chemical Constituents of the Plant*

The chemical compositions of *C. edulis* have extensively been reported. The extract yields benzenoids, phenylpropanoid, lignans, three sesquiterpenes and coumarins (Achenbach, 1983; Bentley, 1984), steroids, terpenes, tannins, flavonoids and cardiac glycosides (Ibrahim, 1997).

2.8 *Balanites aegyptiaca*

2.8.1 *Taxonomy/ Nomenclature of the Plant*

According to Burkill, (1985), this plant *Balanites aegyptiaca* falls under the following:

Kingdom: Plantae

Subkingdom: Tracheobionta (vascular plant)

Division: Magnoliophyta (angiosperm, flowering plants)

Class: Magnoliopsida (dicot)

Subclass: Rosidae

Order: Sapindales

Family: Zygophyllacea/Balanitaceae (caltrop)

Genus: Balanites

Species: aegyptiaca

Authority: Del

Synonyms: *Balanites ferox* G. Don, *Balanites fischeri* Mildbr and Schltr, *Balanites zizyphoides* Mildbr and Schltr, *Balanites suckertii* Chiov, *Balanites latifolia* (Tiegh) Chiov, *Balanites racemosa* Chiov, *Balanites roxburgii* (Roxb) Planchon, *Agialida Senegalensis* Tiegh, *Agialida nigra* Tiegh, *Agialida abyssinica* Tiegh, *Agialida aegyptiaca* (L) Adans,

Agialida cuneifolia Tiegh, *Agialida latifolia* Tiegh, *Ximenia aegyptiaca* (L) and *Ximenia ferox* (Pois).

Common/Local names:

English: Soapberry tree (Daziel); thorn tree; desert date

Hausa: aduwa

Fula/Fulfulde: murotauki

Kanuri: bettor

Mauritania (Arabic): teichat

Senegal (Arabic) Manne and Niger: Hadjlidj

Mali: Dogon mənə

Hindu: betu, hingot, hingotia, lalo, zachun

Spanish: mirobalano de Egipto

German: zachunbaum

2.8.2 *Botanical Description*

A tree reach up to 12m high, usually less, with a crown of tangled thorny twigs, drooping at the distal ends, tending to form thickets; on impoverished soils of the drier soudano-sahelian savanna but often on sites liable to inundation occurring from Mauritania to northern Nigeria, and widespread in the dry tropical areas of Africa and into Palestine. The plant hitherto known under this name in India is *B. roxburgii* (Roxb) Planchon. Spines are about 2 to 5 cm long, naked except for a few scale-leaves or rarely a single short lateral spine. Scale leaves are rare on young spines, falling quickly, and triangular, acute, 0.75 mm long. Foliage-leaves are only found on the stems, closely and minutely puberulous, often

glabrescent, or sometimes densely tomentellous, subsessile or with a petiole, leathery; apex bluntly acute to obtuse or rounded. Flowers are variously arranged in loose or tight cymose fascicles at spinous nodes, on spineless stems, or closely arranged on shoots of short internodes, sometimes more or less spiciforme; pedicels finely tomentellous. Sepals sometimes fall early, tomentellous on the outside. Petals are narrowly obovate-elliptic, yellowish or blue green. The ovary is pubescent to tomentose, style, 1-2 mm long. The fruit elongating markedly in early development, becoming ovoid to ellipsoidal, usually rounded or truncate at both ends, usually ripening yellow.

2.8.3 *Geographical Description/ Habitat*

The tree grows throughout Africa in a belt from Tanzania to the Ivory Coast and Arabia. The tree is drought and fire resistant and withstands up to 2 months flooding in areas near the river.

2.8.4 *Ethnopharmacology*

In Senegal, the root bark powdered is drunk for snake bite and some people use it to wash the wound (Abdoul, 1962; Kerharo and Adam, 1974). It also has application for treating insanity, jaundice, yellow fever and syphilitic conditions. The Fula of Senegal use a root macerate as a purgative and to soothe colic (Kerharo and Adam, 1964; 1974). In Tanganyika, the pounded root is eaten with porridge as a galactogogue and sliced and boiled with flour (mtama) the liquid is drunk for syphilis. Trunk bark is also anthelmintic and is used as such by the Fula of Senegal (Kerharo and Adam, 1964) and elsewhere (Watt and Breyer-Brandwijk, 1962). The Fula (Fulfulde) of northern Nigeria prepare the bark as a

cough medicine (Jackson, 1973), the root bark of the plant and other plants are use for management of epilepsy (Suleiman and Saude, personal communication) and in Adamawa the Margi tribes take the liquid in which bark has been boiled, together with a strong laxative as an abortifacient (Meek, 1931). Yedina at Lake Chad burns the bark as a healing fumigation for circumcision wounds (Dalziel, 1937). In Borno the fruit is an ingredient, along with tamarind and others, as an antidote to arrow-poison to be taken internally (Dalziel, 1937). The fruit and seed-oil are used in Senegal as an embrocating for rheumatism (Kerharo and Adam, 1964; 1974) and in spite of edibility the fruit is considered anthelmintic, emetic as well as purgative and a remedy for liver and spleen conditions (Watt and Breyer-Brandwijk, 1962). The kernels are commonly sold in northern Nigerian markets as a medicine for feverish chills and aches. Along with other oil seeds, it is used to yield an oily extract (kufi, Hausa) which is applied to sores on camels and for parasite skin conditions e. t. c. (Dalziel, 1937). Fruit-pulp has been found to be effective molluscicide acting on fresh water snails which are the intermediate hosts of the *schistosoma* trematode and on the free living larval stages of this parasite. Also, B. aegyptiaca is lethal to Cyclops, the water-flea, which carries guinea worm disease (Dale and Greenway, 1961; Eggling and Dale, 1952; Irvine, 1961; Oliver, 1960).

2.8.5 *Non-Medicinal Uses of* B. aegyptiaca

The leaves are eaten as vegetable by the Bagirmi in Chad (Barth, 1857), by the Fula of Northern Nigeria (Jackson, 1973) and in time of shortage in Jebel marra of Sudan (Hunting Tech Survey, 1968). The plant's wood is suitable for turnery and cabinetry and is used for tool handles (Dalziel 1937; Hunting tech survey, 1968). Also, it is considered as one of the

best firewood. The ashes are used in Mali and the Chad region in indigo dyeing (Irvine, 1961). The bark is widely used in drier north of Africa as fish poison (Aubreville, 1950; chevalier, 1937).

2.8.6 *Pharmacology of the Plant*

Mohamed *et al*., (1999) investigated the effect of the plant's aqueous extract on jaundice, where it showed a significant decrease in serum bilirubin level in experimental obstructive jaundice in rats. Similarly, aqueous and organic leaves extracts of *B. aegyptiaca* and *Moringa oleifera* were tested for their activity against *Salmonella typhii* isolated from blood clot culture using the disc diffusion method. The extracts of *B. aegyptiaca* demonstrated higher activity than those of *M. oleifera* and their activities were comparable to those of ciprofloxacin, cotrimoxazole and chloramphenicol commonly used for treating typhoid fever (Doughari *et al*., 2007). Furthermore, Koko *et al*., (2000) investigated the fasciolicidal efficacy of *Albizia anthelmentica* and *Balanites aegyptiaca* and were found to be effective compared to albendazole. Finally, the level of antinutritional factors in some wild edible fruits of Northern Nigeria among which *B. aegyptiaca* fruit is one were investigated and was found that the values were not up to the toxic levels of the antinutrients (Umaru *et al*., 2007).

2.8.7 *Chemical Constituents of the Plants*

The kernel (10%) contains 40 %- 58 % of liquid glyceride oil. The mesocarp contains 38 % sugars, 15% organic acids and 46 % other organic substances. Stem bark contains a saponin. The plant contains balanitin -1, -2 and -3; balanitin -1 for example possesses a

yamogenin aglycone with a branched glucose and rhamnose side chain. The plant is reported to contain 5.6 % diosgenin. Seeds contain furocoumarins.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Materials

The root samples of *Carissa edulis* and *Balanites aegyptiaca* were collected from Basawa village of Sabon Gari Local Government of Kaduna State, Nigeria, in July 2006. The plants were identified and authenticated by Malam Musa and Umar Gallah of the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University (A. B. U), Zaria, Kaduna State, Nigeria. Voucher specimens were deposited at the Herbarium for future reference and voucher numbers of the plants are 601 and 2064 for *C. edulis and B. aegyptiaca* respectively.

3.1.1 *Plant Extraction*

Method of maceration extraction method described by Sim, (1965) was adopted in this work. The roots of *C. edulis* and *B. aegyptiaca* were cleaned and the bark was scrapped. The root barks were air dried for 14 days until constant weight was obtained. It was then powdered with pestle and mortar and sieved with mesh 20. A portion (100g) of the powdered root bark of each plant was extracted with 700ml of ethanol (60%$^{v}/_{v}$) for 14 days using cold maceration method and the marc was re-extracted for 7 days with occasional shaking. The filtrates were merged together and the solvent was evaporated using thermostated water bath at 60º C to obtain an average yield of 25.77%$^{w}/_{w}$ and 14.9%$^{w}/_{w}$ for *C. edulis and B. aegyptiaca* respectively. The extracts were stored in desiccators until when needed for the study. The solutions of the extracts were always freshly prepared for each study.

3.1.2 *Animals*

Swiss Albino mice of either sex weighing between 15 to 30g were obtained from Animal House of Department of Pharmacology and Clinical Pharmacy, A. B. U., Zaria. One-day old ranger cockerels of body weight range from 26 to 45g both males and females were purchased from National Animal Production Research Institute (NAPRI), Shika, Zaria, Kaduna State, Nigeria.

The animals were maintained in a well ventilated room in the laboratory animal house under ambient laboratory conditions of temperature and light. They were fed on standard laboratory animal feeds and water *ad libitum.*

3.1.3 *Drugs and Chemicals*

4-Aminopyridine (Merck-Schuchardt), Diazepam 10mg in 2ml (Roche Product Ltd Welwyn Garden City) , Dragendorff reagent (BDH Ltd Poole, England), Ethanol (Sigma, St. Louis U.S.A), Ethyl acetate, Diethyl ether, Ferric chloride (BDH Ltd Poole, England), Glacial acetic acid (Searle Essex, England), N-Butanol, Meyer reagent (BDH Ltd Poole, England), Pentylenetetrazole (Sigma Chemical Co.USA), Phenobarbital (Lab. Renaudin, France), Phenytoin sodium capsules (Parke-Davis and Co. Ltd), Vanillin, Sulphuric acid (BDH Ltd Poole, England), Sodium sodium valproate (Sanofi Synthelabo, One Onslow St.Surrey, Canada), Hydro-alcoholic root bark extract *of Carissa edulis* and *Balanites aegyptiaca*, flumazenil and naloxones were purchased from BIOMOL Research Lab (Plymouth Meeting, PA, USA). The doses 5, 10 and 20 mg/kg used for the intraperitoneal and 50, 100, 200 and 400 mg/ Kg for oral studies were selected after preliminary

pharmacological screening. These doses gave optimum and consistent results during the screening.

3.1.4 *Equipment*

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

3.2 Fractionation of *Carissa edulis* Extract

About 30g of Hydro-alcoholic root bark extract of *C. edulis* was dissolved in 100 ml of distill water. Then 150 ml of ethyl acetate was used to partition the dissolved extract. The ethyl acetate portion was separated using separating funnel, concentrated to dryness and was denoted EA. The aqueous portion was further partitioned with 150 ml of n-Butanol and separated. The n-butanol portion was divided into two parts; one part and the remaining aqueous portion were concentrated to dryness and tagged as NB and AP respectively. To other half of n-butanol portion, diethyl ether was added drop wise; the precipitate was collected with filter paper and dried in the oven. The supernatant/filtrate was concentrated to dryness. Both the precipitate and filtrate powder were denoted letters as CS and BP respectively.

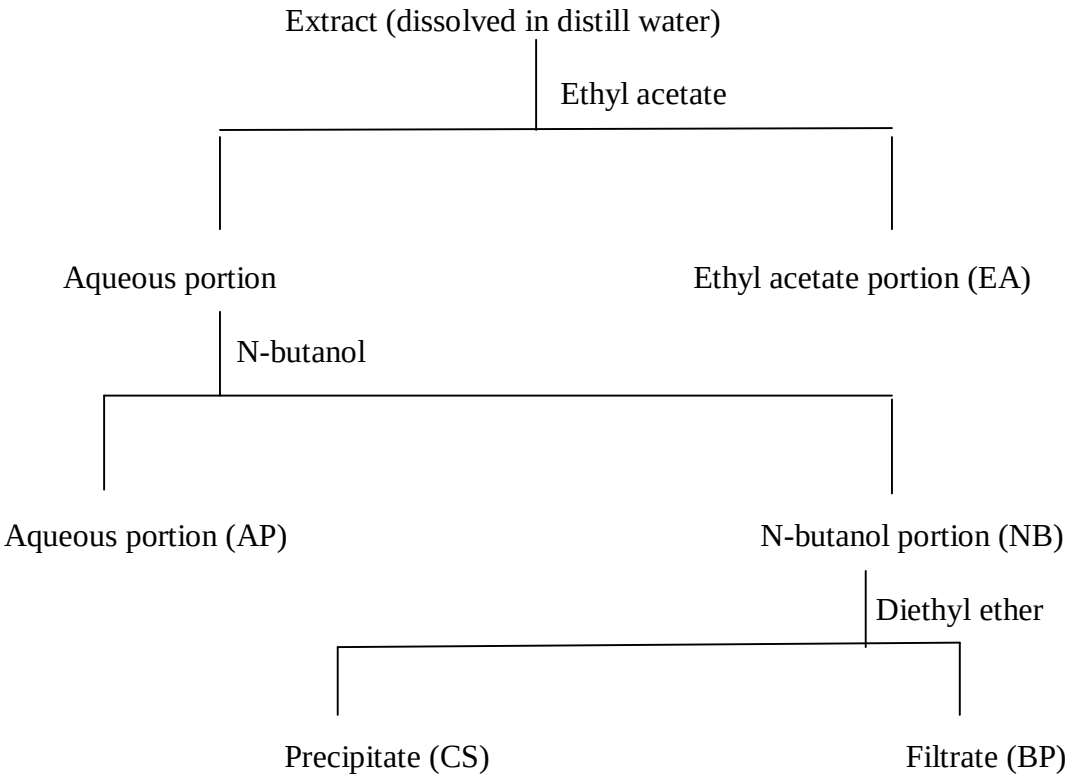


Fig 3.1: Scheme depicting different fractions of Hydro-alcoholic root bark extract of *C. edulis;* AP, BP, CS, EA and NB

3.3 Thin Layer Chromatography (TLC) Analysis

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

Alkaloids: Dragendoff's reagent was used as visualizing reagent. Appearance of orange red spots would indicate alkaloids.

Anthraquinones: Bontrager's reagent (5% ethanolic potassium hydroxide) was used as visualizing reagent. Red color spots indicate Anthraquinones while yellow spots represent anthraones and anthranols.

Cardiac Glycosides: Kedde's reagent (3,5-dinitrobenzoic acid + 2M NaOH) was used test the presence of cardenolides. Pink – violet spots indicate cardenolides. Butadienolides were visualized using 0.5 ml of Anisaldehyde + 10 ml glacial acetic acid + 85 ml methanol followed by 5 ml of concentrated sulphuric acid. Prominent blue spots denote butadienolides.

Tannins: Vanillin/HCl reagent was used as visualizing reagent. Appearance of red spots indicates condensed tannins.

Triterpenes: 1% ethanolic Vanillin spray followed by 10% ethanolic sulphuric acid was used as visualizing reagent. Appearance of red spots would indicate triterpenes.

Flavonoids: Ferric chloride solution was used as visualizing reagent. Appearance of blue spots indicates flavonoids.

3.4 Pharmacological Studies

3.4.1 *Acute toxicity Studies in mice (LD_{50})*

LD_{50} determination was conducted using the method of Lorke (1983) for oral and intraperitoneal routes in mice. Briefly; the method was divided into two phases. In the

initial phase, three groups each containing three mice was treated with the Hydro-alcoholic root bark extract of the plants at doses of 10,100 and 1000mg/kg body weight *i.p*. and oral then observed for signs of toxicity and death for 24 hours. In the second phase, three or four groups each containing one mouse was administered (*i.p.* and oral) with three or four more specific doses of the extract depending on the outcome of the first phase. The second phase doses were as follows:

Animal	**Routes of administration**	**Doses (mg/kg)**			
		CARISSA EDULIS			
Mice	Intraperitoneal	50	100	200 ($^0/_1$)	400 ($^1/_1$)
	Oral	1600	2900	5000 ($^0/_1$)	
		BALANITES AEGYPTIACA			
Mice	Intraperitoneal	200	400	800 ($^0/_1$)	1600 ($^1/_1$)
	Oral	1600	2900	5000 ($^0/_1$)	

The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1and 1/1).

3.4.2 *Anticonvulsant Studies*

Pentylenetetrazole (PTZ)-induced convulsion in mice: The method of Swinyard et al., *(*1989*)* was employed to induce convulsion in mice using PTZ. Thirty mice were divided into 6 groups each containing 5 mice. The first group received normal saline 10 ml/kg body weight *i.p*, the second, third and fourth groups received 5, 10 and 20 mg/kg body weight *i*.p

of ethanol extract of *C. edulis*, while the fifth and sixth group were injected with diazepam 1 and 0.5 mg/kg body weight *i.p.* Thirty minutes after treatment, the animal in all the groups received PTZ 90 mg/kg subcutaneous (Sc). Mice were observed over a period of 30 min and absence of an episode of clonic spasm of at least 5 seconds duration indicated the extract or a compound's ability to abolish the effect of PTZ on seizure threshold.

Maximal electroshock- induced convulsion in chicks: The method described by Swinyard and Kufferberg, (1985) as modified by Sayyah *et al.,* (2002) was used in the study. 50 one-day old cockerels were randomly divided into 5 groups each containing 10 chicks. The first group received normal saline 10 ml/kg body weight *i.p,* second group, third and fourth groups were treated with 5, 10 and 20 mg/kg of the extract *i.p,* and the fifth group was injected with 20 mg/kg of phenytoin *i.p*. Thirty minutes later, maximal electroshock was administered to induce seizure in the chicks using Ugo Basile electroconvulsive machine (model 7801) connected to Clande Lyons stabilizer with corneal electrodes placed on the upper eyelid of the chicks. The shock duration, frequency, current and pulse width were set and maintained at 1.6s, 150 pulse/s, 80 mA and 0.8 ms, respectively. A current of about 80 mA which produced tonic seizures in 90% of the negative control chicks was used throughout the study. Seizures were manifested as hind limb tonic extension (HLTE) (Swinyard, 1969). The ability to prevent this feature or prolong the latency and/ or onset of the HLTE was considered as an indication of anticonvulsant activity (Swinyard, 1969; Sayyah *et. al., 2002*).

4-Aminopyridine-induced Seizure in Mice: The method used was described by Yamaguchi and Rogawski, (1992). A group of 30 Swiss albino mice were randomly divided into 6 groups each containing 5 mice. The first group served as control and received normal saline (10ml/kg *i.p*). The second, third, fourth and fifth groups were pre-treated with 1.25, 2.5, 5 and 10 mg/kg per kg body weight of *C. edulis* extract fraction AP, *i.p*. The sixth group was treated with Phenobarbital (30mg/kg *i.p*). 30 minutes post treatment; 4-Aminopyridine was administered at a dose of 15mg/kg body weight to each mouse, subcutaneously (*sc)*. The same procedure was conducted for the other fractions. Ability of the fraction to protect the mice from lethality within 30 minutes observation period was considered as an indication for anti-convulsant activity (Yamaguchi and Rogawski, 1992).

3.4.3 *Mechanistic study*

Effects of flumazenil on anticonvulsant activity of Hydro-alcoholic Root Bark Extracts of C. edulis and B. aegyptiaca: The effects of selective $GABA_A$-BZD receptor antagonist; flumazenil were also studied (File *et al*. 1982; File and Pellow, 1986), on the anticonvulsant activity of the extracts in order to investigate the probable involvement of $GABA_A$-BZD receptors. We selected 2 groups of 5 mice each. In the first group, mice were given flumazenil (10 mg/kg) 15 min before the administration of *C. edulis* (5 mg/kg) extracts (45 min before the injection of PTZ). In the second group, the animals received flumazenil 15 min before the administration of diazepam (0.5 mg/kg) (45 min before the injection of PTZ). The anticonvulsant activity of the extract and diazepam in mice pretreated with flumazenil was assessed and compared with controls and extract treated animals. The same was done with *B. aegyptiaca* (100 mg/kg).

Effects of naloxone on anticonvulsant activity of Hydro-alcoholic Root Bark Extracts of C. edulis and B. aegyptiaca: Further investigation was carried out on the probable modulatory properties of opioid receptors on the anticonvulsant activity of the extracts in PTZ-induced seizure. Here, naloxone was used as an opioid receptor antagonist (Mannino and Wolf, 1974; Cowan *et al*. 1979; Lauretti et *al*. 1994) at the doses of 0.3 mg/kg, 15 min before the administration of extracts (5 mg/kg for *C. edulis* and 100 mg/kg for *B. aegyptiaca*) and 45 min before the injection of PTZ. The anticonvulsant activity of the extracts in groups pretreated with naloxone was assessed and compared with animals pretreated only with extracts and control groups, which received naloxone and vehicle.

3.5 Statistical Analysis

Results were expressed as mean ± standard error of mean (SEM) and Percentages where appropriate. Statistical analysis was performed by student t-test and analysis of variance (ANOVA); when a statistically significant result was obtained with ANOVA, a post hoc Dunnets test was performed for multiple comparisons. Values of $P<0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 *Carissa edulis* Fractions

From the Hydro-alcoholic root bark extract of *Carissa edulis* 5 fraction were obtained from partitioning with three different solvents; ethyl acetate (EA), n-butanol (NB) and diethyl ether (DE). While BP and CS were sub fractions of NB where CS was precipitated with diethyl ether and BP was the filtrate. All the fractions were solid, no oil was obtained. The physical appearances of the fraction were as follows; Fraction AP was found to be dark brown and sticky, fraction BP and NB were brown powder, fraction CS cream powder, fraction EA was brown sticky semi solid. All the fractions have pleasant characteristic odor.

4.2 Thin Layer Chromatographic (TLC) Analysis

Thin layer chromatographic analysis revealed the presence of tannins, cardiac glycosides (cardinolides), anthraquinones and saponins (steroids and terpenoids) and flavonoids while alkaloids were absent in *Carissa edulis* and *Balanites aegyptiaca* crude extracts. Similarly, the AP fraction contained tannins, and saponins (steroids and triterpenes). BP and NB fractions contained tannins, saponins and cardiac glycosides. The CS fraction revealed the presence of only saponins. Also, in the EA fraction tannins, anthraquinones, saponins and flavonoids were found. These constituents (Table 4.1) may be responsible for the plants biological activities observed and reported in Figures 4.1-3 and Tables 4.1-26. +, ++ and +++ were assigned based on the intensity of the coloration of the spots in the presence of various detecting reagents.

Table 4.1: Phytochemical Constituents of *C. edulis* and *B. aegyptiaca* and Fractions of *C. edulis* Extract (AP, BP, CS, EA and NB) from TLC Analysis

Constituents	Crude Extracts		Fractions of *C. edulis* Extract				
	C. edulis	B. aegyptiaca	AP	BP	CS	EA	NB
Alkaloids	-	-	-	-	-	-	-
Anthraquinones	+	+	-	-	-	++	-
Tannins	+++	+++	++	+	-	++	+
Steroid/Triterpenes	++	+	+	+	++	+	+
Cardiac glycosides	++	+	-	+++	-	-	+
Saponins	++	+	+	+	++	+	++
Flavonoids	+	+	-	-	-	+	-

Key= + = Few Present, ++ = Moderately Present, +++ = Abundantly Present and - = Absent

4.3 Results of Acute Toxicity studies

The median lethal dose (LD_{50}) of the *C edulis* extract in mice was found to be 282.8 mg/kg body weight administered intraperitoneally. At 400 mg/kg dose there was 100 % mortality and the maximum dose that the rodent survived was 200 mg/kg in the second stage. The oral LD_{50} value for *C. edulis* was above 5000 mg/kg since there was no mortality observed in both stages. The median lethal dose (LD_{50}) of the *B. aegyptiaca* extract in mice was found to be 1131.3 mg/kg body weight intraperitoneally. At 1600 mg/kg dose, no mice survived. No mortality observed at 800 mg/kg; a maximum survival dose. Also, the oral LD_{50} value for *B. aegyptiaca* was above 5000 mg/kg, in all the stages no death was recorded (Table 4.2).

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Table 4.2: LD_{50} Values of *Carissa edulis* and *Balanites aegyptiaca*

Plants	Routes of administration	LD_{50} Values (mg/kg)
Carissa edulis	Intraperitoneal	282.2
Carissa edulis	Oral	>5000
Balanites aegyptiaca	Intraperitoneal	1131.3
Balanites aegyptiaca	Oral	>5000

4.4 Anticonvulsant Screenings

4.4.1: Effect of Intraperitoneal Administration of Hydro-alcoholic root bark Extract of *C. edulis* 30 min and 60 min before Pentylenetetrazole (PTZ) Administration

The extract of *C. edulis* administered 30 min prior to PTZ produced 40% protection against threshold seizure However, when the extract was given 60 min prior to PTZ injection, no protection against seizure was observed. There was no statistically significant difference between the mean onset of seizure in control mice compared with rodents administered with the extract. However, there was protection against PTZ-induced mortality but was not dose-dependent. The standard anticonvulsant drug used, diazepam (0.5 mg/kg) increased the mean onset of seizure by 51% (from 8.61 ± 1.21 min to 13.0 ± 2.00 min) and offered 60% protection against seizure. When the dose of diazepam was increased to1 mg/kg none of the mice convulsed (Table 4.3).

Table 4.3: Effect of Hydro-alcoholic root bark extract of *Carissa edulis* (CE) and diazepam (DZ) on PTZ –induced seizure in mice

Drugs (mg/kg)	Mean Onset of seizure (min)	Protection against seizure (%)	Protection against mortality (%)
Control	8.61±1.21	0.0	80.0
CE (5)	6.67 ± 1.76	40.0	100.0
CE (10)	9.00 ± 2.89	0.0	40.0
CE (20)	12.0 ± 2.08	20.0	100.0
CE[a] (5)	4.20 ± 1.24	0.0	0.0
CE[a] (10)	4.60 ± 0.93	0.0	0.0
CE[a] (20)	8.00 ± 1.70	0.0	0.0
DZ (0.5)	13.0 ± 2.00	60.0	100.0
DZ (1)	-	100.0	100.0
DZ (3)	-	100.0	100.0

Control: Normal saline, CE: *Carissa edulis* and DZ: diazepam was administered 30 min and CE[a] was *C. edulis* administered 60 min intraperitoneally before the injection of PTZ (90 mg/kg) subcutaneously; Values are the mean ± SEM, n = 5.

4.4.2: Effect of Intraperitoneal Administration of Hydro-alcoholic root bark Extract of *C. edulis on* Maximal Electroshock Test (MEST)-induced Seizure in Chicks

The reduced efficacy of *C. edulis* in mice may be due to the inability of the constituent(s) in the extract to penetrate the blood-brain-barrier (BBB). To circumvent this problem, I used one-day old chicks which lack BBB (Waelsh, 1995, Key and Marley, 1962) to test the effect of *C. edulis* on MEST-induced seizure. The extract offered protection against seizure induced by maximal electroshock in chicks with the highest percentage protection (90%) observed at doses of 10 and 20 mg/kg. The 20 mg/kg dose (6.84 ± 0.00) showed significant difference ($p<0.05$) in the mean onset of seizure compared to control group. The standard anticonvulsant drug, phenytoin (20 mg/kg) produced 100% inhibition of hind limb tonic extension (HLTE) induced by maximal electroshock (Table 4.4).

Table 4.4: Effect of Hydro-alcoholic root bark extract of *C. edulis* (CE) and Phenytoin (PH) on MEST-induced convulsions in chicks

Dose (mg/kg)	(%) Protection against seizure	Mean Onset of seizure (s)	Mean Duration of seizure (min)	Mortality Rate
Control	10.0	4.26±0.31	7.00±1.27	0.0
CE (5)	60.0	3.54±0.54	6.00±1.00	0.0
CE (10)	90.0	3.00±0.00	3.00±0.00	0.0
CE (20)	90.0	6.84±0.00*	8.00±0.00	0.0
PH (20)	100.0	0.0	0.0	0.0

Values were presented as Mean ± SEM, * = P< 0.05, and n = 10, CE= Carissa edulis and PH= Phenytoin

4.4.3: The effect of Oral Administration of Hydro-alcoholic root bark Extract of *C. Edulis* on Pentylenetetrazole (PTZ)-induced Seizure in Mice

The extract of *C. edulis* at the doses tested (50, 100, 200 and 400 mg/kg) offered no protection against PTZ-induced seizure. However, 200 and 400 mg/kg doses provided significant difference ($p<0.05$) in the mean onset of seizure compared to control group. The extract protected the mice from mortality but was not dose-dependent. *C. edulis* (400 mg/kg) provided 40% protection compared with control group 20%. The standard anticonvulsant drug used, phenobarbital (30 mg/kg) produced 100% protection against both threshold seizure and mortality (Table 4.5).

Table 4.5: Effect of Hydro-alcoholic root bark extract of *Carissa edulis* (CE) and Phenobarbital (PHB) on Pentylenetetrazole (PTZ)-induced seizures in mice

Drugs (mg/kg)	Mean Onset of seizure (min)	Protection against seizure (%)	Protection against Mortality (%)
Control	5.40 ± 0.75	0.0	20.0
CE (50)	4.60 ± 0.40	0.0	0.0
CE (100)	5.00 ± 0.71	0.0	20.0
CE (200)	10.44 ± 1.81*	0.0	20.0
CE (400)	8.80 ± 0.97*	0.0	40.0
PHB (20)	0.0	100.0	100.0

Control: Normal saline, CE: *Carissa edulis* and PHB: phenobarbital were administered orally 30 min before the injection of PTZ (90 mg/kg); Values are the mean ± SEM, n = 5. *P< 0.05

4.4.4: Effect of Oral Administration of Hydro-alcoholic root bark Extract of *C. edulis* on Maximal Electroshock Test (MEST)-induced seizure in Chicks

The extract of *C. edulis* did not produce any inhibition of seizure induced by maximal electroshock in chicks. However, the mean recovery times from the seizure were reduced, though only 400 mg/kg dose showed significant difference ($p<0.05$). No mortality was observed in all the groups. Phenytoin (20 mg/kg), the anticonvulsant drug used produced 80% protection against hind limb tonic extension (HLTE) (Table 4.6).

Table 4.6: Effect of Hydro-alcoholic root bark extract of *Carissa edulis* (CE) and Phenytoin (PH) on Maximal electroshock test in chicks

Drugs (mg/kg)	Mean recovery time (min)	Protection against seizure (%)	Protection against Mortality
Control	12.0 ± 4.36	0.0	100.0
CE (50)	5.44 ± 0.88	0.0	100.0
CE (100)	7.10 ± 0.66	0.0	100.0
CE (200)	7.00 ± 1.65	0.0	100.0
CE (400)	4.70 ± 0.67*	0.0	100.0
PH (20)	19.0 ± 11.0	80.0	100.0

Control: Normal saline, CE: *Carissa edulis* and PH: phenytoin were administered orally 30 min before the injection of PTZ (90 mg/kg); Values are the mean ± SEM, n = 10,* P< 0.05.

4.4.5: Effect of Intraperitoneal administration of Hydro-alcoholic root bark Extract of *B. aegyptiaca* 30 min and 60 min before Pentylenetetrazole (PTZ) Administration

Administration of the extract of *B. aegyptiaca* (100 mg/kg) 30 min before PTZ injection offered 20% protection against threshold seizure and the same dose of the extract showed no protection 60 min prior to PTZ injection. Diazepam, the standard anticonvulsant drug produced 60% protection at 0.5 mg/kg while 1 mg/kg offered 100% protection with no mortality observed in all the diazepam doses. The highest protection against mortality was observed in 50 mg/kg dose 30 min and 100 mg/kg dose 60 min before PTZ injection. However, there was no significant difference observed in the mean onset of seizure (Table 4.7).

Table 4.7: Effect of Hydro-alcoholic root bark extract of *Balanite aegyptiaca* (BA) and Diazepam (DZ) on Pentylenetetrazole (PTZ)-induced seizure in mice

Drugs (mg/kg)	Mean Onset of seizure (min)	Protection against seizure (%)	Protection against mortality
Control	5.40 ± 0.87	0.0	40.0
BA (25)	7.00 ± 1.64	0.0	20.0
BA (50)	6.60 ± 1.66	0.0	80.0
BA (100)	7.75 ± 1.65	20.0	40.0
BA[a] (25)	3.80 ± 0.37	0.0	0.0
BA[a] (50)	6.80 ± 1.59	0.0	0.0
BA[a] (100)	4.80 ± 1.11	0.0	80.0
DZ (0.5)	13.00 ± 2.00	60.0	100
DZ (1)	0.0	100	100
DZ (3)	0.0	100	100

Control: Normal saline, BA: *Balanite aegyptiaca* and DZ: diazepam were administered intraperitoneally 30 min before the injection of PTZ (90 mg/kg); [a] *Balanite aegyptiaca* was administered 60 min before the injection of PTZ; Values are the mean ± SEM; n = 5.

4.4.6: Effect of Intraperitoneal Administration of Hydro-alcoholic root bark Extract of *B. aegyptiaca* on maximal electroshock Test (MEST)-induced Seizure in Chicks

The extract of *B. aegyptiaca* did not produce any significant inhibition of seizure induced by MEST in chicks. The standard anticonvulsant drug used, phenytoin (20 mg/kg) produced 100% seizure protection. However, there was no significant difference between the mean duration of seizure in the control and the extract treated groups (Table 4.8).

Table 4.8: Effect of Hydro-alcoholic root bark extract of *B. aegyptiaca* (BA) and Phenytoin (PH) on maximal electroshock test in chicks

Treatment (mg/kg)	Protection against seizure (%)	Mean Onset of seizure (s)	Mean Duration (min)
Control	10.0	6.24±1.35	5.11±0.59
BA (100)	10.0	4.95±1.19	4.56±0.50
BA (50)	10.0	4.79±1.25	5.00±0.80
BA (25)	20.0	3.58±0.34	4.38±0.50
PH (20)	100	0.0	0.0

Values were presented as mean ± SEM, n=10, BA represent B. aegyptiaca and PH is Phenytoin

4.4.7 Effect of Oral Administration of Hydro-alcoholic root bark Extract of *B. aegyptiaca* on Pentylenetetrazole (PTZ)-induced seizure in mice

The extract of B. aegyptiaca exhibited 20% protection against PTZ-induced seizure at 50 and 200 mg/kg doses while phenobarbital (30 mg/kg), the standard anticonvulsant drug offered 100% protection against threshold seizure and mortality. Similarly, the extract produced highest (80%) protection against mortality at 200 mg/kg dose. However, there was no significant difference between the mean onsets of seizure in unprotected mice compared to the control group (Table 4.9).

Table 4.9: Effect of Hydro-alcoholic root bark extract of *Balanite aegyptiaca* (BA) and Phenobarbital (PHB) on Pentylenetetrazole (PTZ)-induced seizure in mice

Drugs (mg/kg)	Mean Onset of seizure (min)	Protection against seizure (%)	Protection against mortality (%)
Control	4.80 ± 1.11	0.0	0.0
BA (50)	7.50 ± 2.33	20.0	20.0
BA (100)	8.60 ± 2.20	0.0	60.0
BA (200)	5.75 ± 0.48	20.0	80.0
BA (400)	4.00 ± 0.55	0.0	20.0
PHB (30)	0.0	100	100

Control: Normal saline, BA: *Balanite aegyptiaca* and PHB: phenobarbital were administered orally 30 min before the injection of PTZ (90 mg/kg); Values are the mean ± SEM; n = 5.

4.4.8: Effect of Oral Administration of Hydro-alcoholic root bark Extract of *B. aegyptiaca* on Maximal Electroshock Test (MEST)-induced seizure in chicks

The extract of *B. aegyptiaca* at the doses tested (50, 100, 200 and 400 mg/kg) offered no protection against maximal electroshock-induced seizure. The 200 mg/kg dose of the extract produced significant reduction ($p<0.05$) in the mean recovery time from the seizure by 60%. Phenytoin (20 mg/kg), the standard anticonvulsant drug used exhibited 80% inhibition against hind limb tonic extension (HLTE). No mortality was observed in all the groups including the control except with the dose of 100 mg/kg of the extract which produced 10% mortality (Table: 4.10).

Table 4.10: Effect of Hydro-alcoholic root bark extract of *Balanite aegyptiaca* (BA) and Phenytoin (PH) on Maximal electroshock test (MEST)-induced seizure in chicks

Drugs (mg/kg)	Mean recovery time of seizure (min)	Protection against seizure (%)	Protection against mortality (%)
Control	12.00 ± 4.36	0.0	100.0
BA (50)	5.70 ± 0.78	0.0	100.0
BA (100)	5.78 ± 0.72	0.0	90.0
BA (200)	4.80 ± 0.98*	0.0	100.0
BA (400)	7.30 ± 1.02	0.0	100.0
PH (20)	19.00 ± 11.0	80.0	100.0

Control: Normal saline, BA: *Balanite aegyptiaca* and PH: phenytoin were administered orally 30 min before the injection of PTZ (90 mg/kg); Values are the mean ± SEM; n = 10; *P<0.05.

4.4.9: Effect of Flumazenil on the Anticonvulsant Activity of *C. edulis* and Diazepam on Pentylenetetrazole (PTZ)-induced Seizure in Mice

Administration of Flumazenil (10 mg/kg), a selective antagonist of benzodiazepine (BDZ) site in the $GABA_A$-BDZ receptor complex 15 min before the extract, reduced the mean onset of seizure in 5 mg/kg dose of the extract treated group by 40% and completely blocked the protection (40%) provided by the same dose against seizure. Similarly, flumazenil (10 mg/kg) reduced the mean onset of seizure in diazepam (0.5 mg/kg) treated mice by 25.6% and reduced the percentage protection against seizure by 33.3% (Table: 4.11).

Table 4.11: Effect of flumazenil (Flu) on the anticonvulsant activity of Hydro-alcoholic root bark extract of *Carissa edulis* (CE) and diazepam (DZ) on Pentylenetetrazole (PTZ)-induced seizure model in mice

Drugs (mg/kg)	Mean Onset of seizure (min)	Protection against seizure (%)	Protection against mortality (%)
Control	8.61 ± 1.21	0.0	80.0
DZ (0.5)	13.0 ± 2.00	60.0	100.0
DZ (0.5) + Flu(10)	9.67 ± 2.19	40.0	80.0
CE (5)	6.67 ± 1.76	40.0	100.0
CE (5) + Flu (10)	4.00 ± 0.84	0.0	80.0

Control= Normal saline, DZ = diazepam and CE = *Carissa edulis* were administered 30 min intraperitoneally before injection of PTZ (90 mg/kg); Flu = flumazenil (10 mg/kg) was administered 15 min before CE or DZ; values are the mean ± SEM; n = 5.

4.4.10: Effect of Naloxone on the Anticonvulsant Activity of *C. edulis* on Pentylenetetrazole (PTZ)-induced Seizure in Mice

Naloxone (0.3 mg/kg) an opioid receptor antagonist administered 15 min prior to the extract (5 mg/kg) increased the mean onset of seizure by 33%. However, the seizure protection in the extract-treated group was abolished in the presence of naloxone. Similarly, protection against mortality was increased by 50% (Table: 4.12).

Table 4.12: Effect of naloxone (Nal) on the anticonvulsant activity of Hydro-alcoholic root bark extract of *Carissa edulis* (CE) on Pentylenetetrazole (PTZ)-induced seizure model in mice

Drugs (mg/kg)	Mean Onset (min)	Protection against seizure (%)	Protection against mortality (%)
Control	5.40 ± 0.87	0.0	40.0
CE (5)	5.75 ± 0.63	20.0	40.0
CE (5) + Nal (0.3)	7.40 ± 1.21	0.0	60.0
Nal (0.3)	8.00 ± 1.52	0.0	0.0

Naloxone (0.3 mg/kg) was administered 15 min before CE: *Carissa edulis*. PTZ (90 mg/kg) was injected 30 min after the administration of CE; Control: Normal saline; Values are the mean ± SEM; n = 5.

4.4.11: Effect of Flumazenil on the Anticonvulsant Activity of *B. aegyptiaca* and Diazepam on Pentylenetetrazole (PTZ)-induced Seizure in Mice

Administration of flumazenil (10 mg/kg) 15 min before the injection of the extract (100 mg/kg) antagonized the anticonvulsant activity of the extract by abolishing the 20% protection obtained with 100 mg/kg dose alone. There was no significant difference between the latency of seizure in mice that received extract (100 mg/kg) pretreated with flumazenil and the control group. On the other hand, flumazenil (10 mg/kg) reduced the anticonvulsant activity of diazepam (0.5 mg/kg) by 33.3%. However, there was no significant difference between the flumazenil pretreated and control groups (Table: 4.13).

Table 4.13: Effect of flumazenil (Flu) on the anticonvulsant activity of *Balanite aegyptiaca* (BA) and diazepam (DZ) on PTZ-induced seizure in mice

Drugs (mg/kg)	Mean Onset (min)	Protection against seizure (%)	Protection against mortality (%)
Control	5.40 ± 0.87	0.0	40.0
DZ (0.5)	13.00 ± 2.00	60.0	100
DZ (0.5) + Flu	9.67 ± 2.19	40.0	80.0
BA (100)	7.75 ± 1.65	20.0	40.0
BA (100) + Flu	8.20 ± 1.28	0.0	60.0

Control: Normal saline, DZ: diazepam and BA: *Balanite aegyptiaca* were administered intraperitoneally 30 min before injection of PTZ (90 mg/kg); Flu: flumazenil (10 mg/kg) was administered 15 min before the injection of BA or DZ; Values are the mean ± SEM; n = 5.

4.4.12: Effect of Naloxone on the Anticonvulsant activity of *B. aegyptiaca* on Pentylenetetrazole (PTZ)-induced Seizure in Mice

Pretreatment with naloxone (0.3 mg/kg) 15 min before the injection of the extract (100 mg/kg) abolished seizure protection offered by the extract (20%) with reduction in the protection against mortality by 33.3%. However, there was no significant difference observed in the mean onset of seizure between the naloxone pretreated (4.40 ± 0.6 min) and the control group (5.4 ±0.87) (Table: 4.14).

Table 4.14: Effect of naloxone (Nal) on the anticonvulsant activity of *Balanite aegyptiaca* (BA) on PTZ-induced seizure in mice

Drugs (mg/kg)	Mean Onset (min)	Protection against seizure (%)	Protection against mortality (%)
Control	5.40 ± 0.87	0.0	40.0
BA (100)	7.75 ± 1.65	20.0	40.0
BA (100) + Nal (0.3)	4.40 ± 0.60	0.0	60.0
Nal (0.3)	8.00 ± 1.52	0.0	0.0

Naloxone (0.3 mg/kg) was administered 15 min before BA: *Balanite aegyptiaca*. PTZ (90 mg/kg) was injected 30 min after the administration of BA and control (Normal saline); Values are the mean ± SEM; n = 5.

4.4.13: Effect of fraction AP on Pentylenetetrazole (PTZ)-induced Seizure in Mice

The fraction AP of *C. edulis* exhibited seizure protection against PTZ-induced seizure at doses tested (1.25, 2.5, 5 and 10 mg/kg) where 1.25 and 10 mg/kg produced 20% and 80% protection respectively. Furthermore, 5 and 10 mg/kg doses produced highest mortality protection 80%. However, there was no significant difference in the latency of seizure between the fraction treated and control groups. The standard anticonvulsant drug used, sodium valproate (200 mg/kg) produced 100% protection against both seizure and mortality (Fig: 4.1).

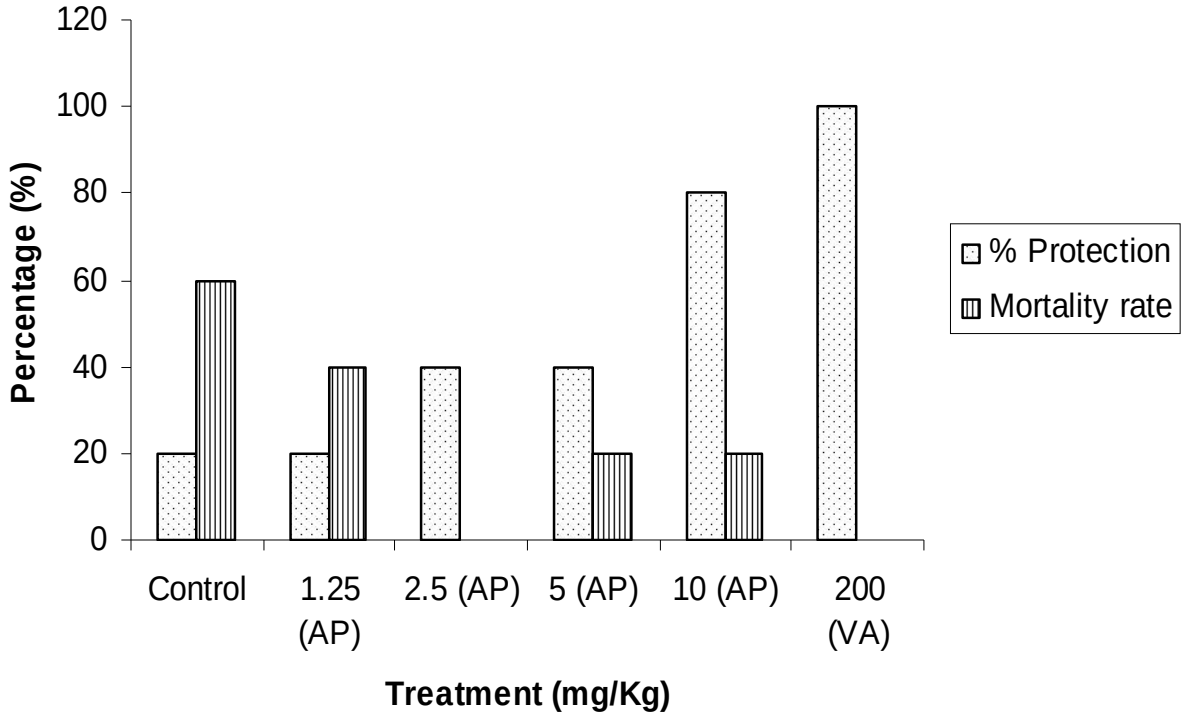


Fig 4.1: Effect of aqueous portion (AP) fraction of Hydro-alcoholic root bark extract of *Carissa edulis* and sodium valproate on PTZ-induced seizures in mice. The fraction was given at doses of 1.25, 2.5, 5 and 10 mg/kg and sodium valproate (VA) at dose of 200 mg/kg.

4.4.14: Effect of Fraction BP *C. edulis* on Pentylenetetrazole (PTZ)-induced Seizure in Mice

The fraction BP of *C. edulis* produced highest protection (40%) at the highest dose (10 mg/kg) against PTZ-induced seizure, although, the effect was no dose dependent. There was complete protection against mortality at the doses tested except the lowest dose (1.25 mg/kg) with 80%. Sodium valproate (200 mg/kg), the standard anticonvulsant drug used offered 100% protection to the mice against PTZ-induced seizure. However, there was no statistically significant difference between the fraction BP treated and control groups in the mean onset of seizure (Table: 4.15).

Table 4.15: Effects of BP fraction of Hydro-alcoholic root bark extract of *C. edulis* and sodium valproate (VA) on PTZ-induced convulsions on mice

Dose (mg/kg)	Protection (%) against seizure	Mean Onset of Seizure (Min)	Mortality Rate
Control	0.0	11.80 ± 3.34	40.0
BP (1.25)	20.0	10.25 ± 2.78	20.0
BP (2.5)	20.0	13.75 ± 3.01	0.0
BP (5)	20.0	13.75 ± 3.28	0.0
BP (10)	40.0	6.33 ± 0.67	0.0
VA (200)	100.0	0.0	0.0

Values were presented as Mean ± SEM, n=5, BP= n- Butanol fraction filtrate and VA= sodium valproate.

4.4.15: Effect of Fraction CS of *C. edulis* on Pentylenetetrazole (PTZ)-induced seizure in Mice

The highest dose (10 mg/kg) of CS fraction of *C. edulis* tested produced 40% protection against seizure induced by administration of PTZ, while other doses offered no protection. However, the highest (10 mg/kg) dose offered only 40% protection against mortality while the remaining doses had 80% protection compared to the control group 60%. The standard anticonvulsant drug used, sodium valproate (200 mg/kg) exhibited 100% protection against both the seizure and mortality. There was no significant difference found in the latency of seizure (Table: 4.16).

Table 4.16: Effects of CS fraction of Hydro-alcoholic root bark extract of *C. edulis* and sodium valproate (VA) on PTZ-induced convulsions on mice

Dose (mg/kg)	Protection against seizure (%)	Mean Onset of Spasms (Min)	Mortality Rate
Control	0.0	11.80 ± 3.34	40.0
CS (1.25)	0.0	5.80 ± 1.16	20.0
CS (2.5)	0.0	8.80 ± 1.88	20.0
CS (5)	0.0	9.00 ± 2.02	20.0
CS (10.0)	40.0	7.67 ± 1.20	60.0
VA (200	100.0	0.0	0.0

Values were presented as Mean as ± SEM, n=5, CS= n-butanol fraction precipitate and VA= sodium valproate.

4.4.16: Effect of Fraction EA of *C. edulis* on Pentylenetetrazole (PTZ)-induced Seizure in Mice

EA fraction of *C. edulis* offered protection to the mice against seizure induced by PTZ from 20% in the control group to 40% at 10 mg/kg dose, the EA fraction highest dose tested. Similarly, the mortality rate was reduced by 66.7% at 10 mg/kg dose. However, there was no significant difference in the mean onset of seizure between the fraction treated and the control groups. The standard anticonvulsant drug used, sodium valproate (200 mg/kg) produced 100% protection against both the seizure and mortality (Fig: 4.2).

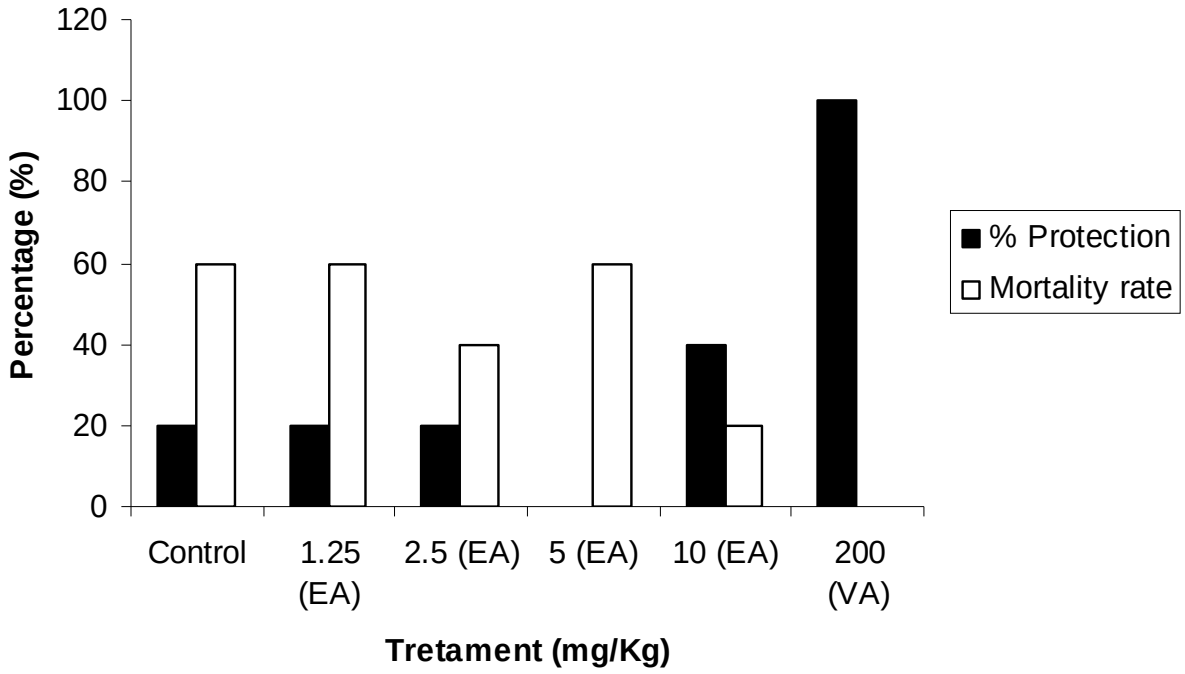


Fig 4.2: Effect of ethyl acetate (EA) fraction of Hydro-alcoholic root bark extract of *Carissa edulis* and sodium valproate on PTZ-induced seizures in mice. The EA fraction was administered intraperitoneally at doses of 1.25, 2.5, 5 and 10 mg/kg and sodium valproate (VA) at dose of 200 mg/kg**.**

4.4.17: Effect of Fraction NB of *C. edulis* on Pentylenetetrazole (PTZ)-induced Seizure in Mice

The fraction NB of *C. edulis* produced no significant difference in both the seizure and mortality protection compared to the control group. Similarly, in the mean onset of seizure, there was no significant difference but, sodium valproate (200 mg/kg), the standard anticonvulsant drug used offered 100% protection against seizure and mortality (Fig 4.3).

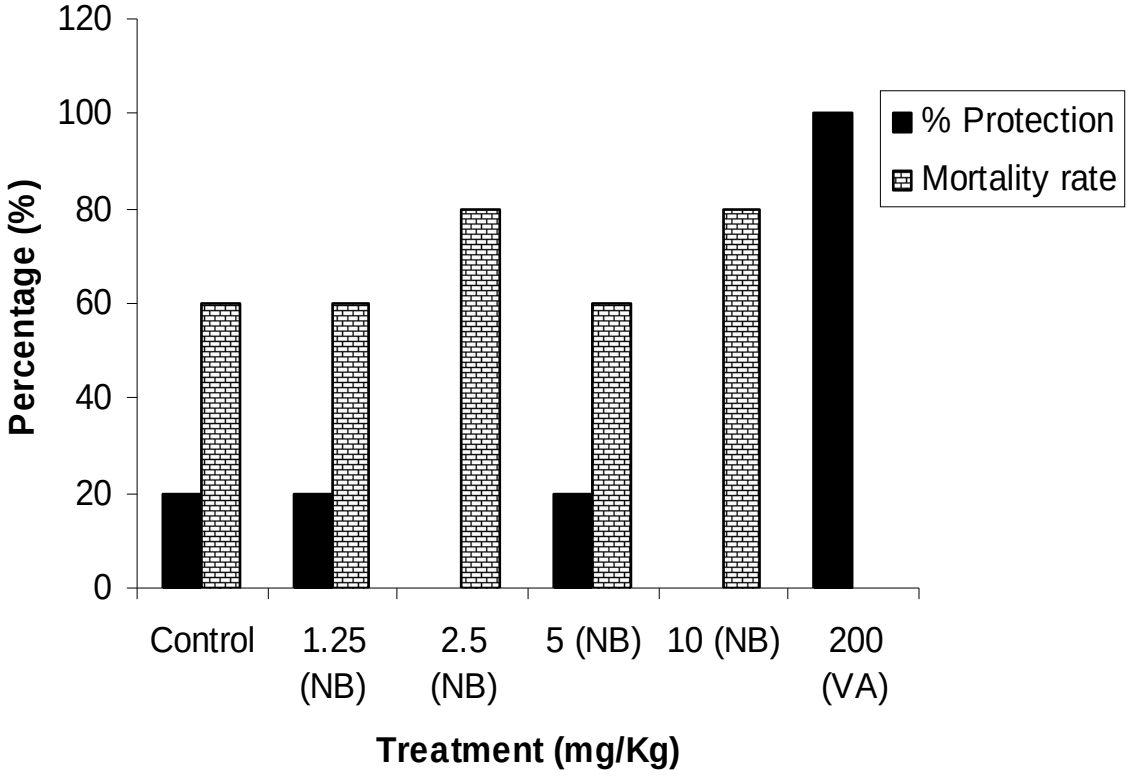


Fig 4.3: Effect of n-butanol (NB) fraction of Hydro-alcoholic root bark extract of *Carissa edulis* on PTZ-induced seizures in mice. The fraction NB was given intraperitoneally at doses of 1.25, 2.5, 5 and 10 mg/kg and sodium valproate (VA) at dose of 200 mg/kg.

4.4.18: Effect of Fraction AP of *C. edulis* on Maximal Electroshock Test (MEST)-induced Seizure in Chicks

2.5 mg/kg dose of fraction AP of *C. edulis* produced 20% seizure protection similar to the control group. Furthermore, there was no significant difference observed between the fraction AP treated and control group in the mean onset of seizure. However, phenytoin (20 mg/kg), the standard anticonvulsant drug used exhibited 100% protection against seizure. No mortality was observed in all the groups (Table: 4.17).

Table 4.17: Effects of AP fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenytoin (PH) on MEST-induced convulsions in chicks

Dose (mg/kg)	Protection (%) against seizure	Mean Duration of seizure (min)
Control	20.0	8.25 ± 0.96
AP (1.25)	10.0	9.11 ± 0.68
AP (2.5)	20.0	6.63 ± 0.80
AP (5)	0.0	8.00 ± 0.54
AP (10)	0.0	9.50 ± 2.54
PH (20)	100.0	0.0

Values were presented as Mean ± SEM, n=10, AP= Aqueous fraction and PH= Phenytoin

4.4.19: Effect of Fraction BP of *C. edulis* on Maximal Electroshock Test (MEST)-induced Seizure in Chicks

The fraction BP of *C. edulis* offered no significant seizure protection compared to the control group. However, the standard anticonvulsant drug used, phenytoin (20 mg/kg) offered 100% seizure protection to the chicks. There was no significant difference observed between the mean duration of seizure in the fraction treated and the control groups (Table 4.18).

Table 4.18: Effects of BP fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenytoin (PH) on MEST-induced convulsions in chicks

Dose (mg/kg)	Protection against seizure (%)	Mean Duration of seizure (min)
Control	20.0	8.25 ± 0.96
BP (1.25)	10.0	7.78 ± 1.12
BP (2.5)	0.0	7.20 ± 0.55
BP (5)	10.0	8.44 ± 0.58
BP (10)	0.0	9.40 ± 0.93
PH (20)	100.0	0.0

Values were presented as Mean ± SEM, n=10, BP= n-Butanol fraction filtrate and PH= Phenytoin.

4.4.20: Effect of Fraction CS of *C. edulis* on Maximal Electroshock Test (MEST)-induced Seizure in Chicks

The fraction CS of *C. edulis* showed inverse dose-dependent decrease in the percentage protection against seizure from 60% to 10% at the doses tested (1.25, 2.5, 5 and 10 mg/kg). On the other hand, no significant difference was observed in the mean duration of seizure between the fraction CS treated groups and the control group. Phenytoin (20 mg/kg) offered 100% seizure protection to the chicks (Table: 4.19).

Table 4.19: Effects of CS fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenytoin (PH) on MEST-induced convulsions in chicks

Dose (mg/kg)	Protection against seizure (%)	Mean Duration of seizure (Min)	Mortality Rate
Control	10.0	6.13 ± 1.09	0.0
CS (1.25)	60.0	4.75 ± 0.63	0.0
CS (2.5)	50.0	5.20 ±0.80	0.0
CS (5)	50.0	5.40 ± 0.93	0.0
CS (10)	20.0	6.38 ± 0.75	0.0
PH (20)	100.0	0.0	0.0

Values were presented as Mean ± SEM, n=10, CS= n-butanol fraction precipitate and PH= Phenytoin.

4.4.21: Effect of Fraction EA of *C. edulis* on Maximal Electroshock Test (MEST)-induced Seizure in Chicks

The fraction EA of *C. edulis* offered no significant seizure protection compared to the control group. However, the standard anticonvulsant drug used, phenytoin (20 mg/kg) offered 100% seizure protection to the chicks. There was no significant difference observed between the mean duration of seizure in the fraction treated and the control groups (Table: 4.20).

Table 4.20: Effects of EA fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenytoin (PH) on MEST-induced convulsions in chicks

Dose (mg/kg)	Protection against seizure (%)	Mean Duration of seizure (min)
Control	20.0	8.25 ± 0.96
EA (1.25)	0.0	7.10 ± 1.16
EA (2.5)	30.0	6.86 ± 1.08
EA (5)	10.0	6.899 ± 1.30
EA (10)	0.0	7.90 ± 0.81
PH (20)	100.0	0.0

Values were presented as Mean ± SEM, n=10, EA= Ethyl acetate and PH= Phenytoin

4.4.22: Effect of Fraction NB of *C. edulis* on Maximal Electroshock Test (MEST)-induced Seizure in Chicks

The fraction NB of *C. edulis* offered no significant seizure protection compared to the control group. However, the standard anticonvulsant drug used, phenytoin (20 mg/kg) offered 100% seizure protection to the chicks. There was no significant difference observed between the mean duration of seizure in the fraction treated and the control groups (Table: 4.21).

Table 4.21: Effects of NB fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenytoin (PH) on MEST-induced convulsions in chicks

Dose (mg/kg)	Protection against seizure (%)	Mean Duration (min)
Control	10.0	6.13 ± 1.09
NB (1.25)	20.0	6.75 ± 0.53
NB (2.5)	10.0	5.78 ± 0.32
NB (5)	0.0	7.00 ± 1.02
NB (10)	20.0	5.88 ± 0.58
PH (20)	100.0	0.0

Values were presented as Mean ± SEM, n=10, NB= n-Butanol fraction and PH= Phenytoin.

4.4.23: Anticonvulsant Screening of *C. edulis* Fractions (AP, BP, CS, EA and NB) on 4-Aminopyridine-induced Seizure in Mice

All the doses of the fractions AP, BP, CS, EA and NB of *C. edulis* when tested against 4-Aminopyridine-induced seizure offered no protection to the mice and with 100% mortality. Furthermore, no significant difference was observed between the mean onset of mortality in the fraction treated and the control groups. However, phenobarbital, the standard anticonvulsant drug used produced 100% protection against seizure and mortality (Tables 4.22 – 4.26).

Table 4.22: Effects of AP fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenobarbital (PHB) on 4-aminopyridine-induced convulsions on mice

Dose (mg/kg)	Protection against seizure (%)	Mean Onset of Mortality (min)
Control	0.0	13.2 ± 0.80
AP (1.25)	0.0	10.0 ± 2.07
AP (2.5)	0.0	12.4 ± 1.44
AP (5)	0.0	11.6 ± 1.53
AP (10)	0.0	13.4 ± 1.72
PHB (30)	100.0	0.0

Values were presented as Mean ± SEM, n=5, AP= Aqueous fraction and PHB= Phenobarbital.

Table 4.23: Effects of BP fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenobarbital (PHB) on 4-aminopyridine-induced convulsions on mice

Dose (mg/kg)	Protection against seizure (%)	Mean Onset of Mortality (min)
Control	0.0	4.80 ± 0.37
BP (1.25)	0.0	5.80 ± 0.58
BP (2.5)	0.0	4.80 ± 0.20
BP (5)	0.0	3.80 ± 0.37
BP (10)	0.0	5.40 ± 0.68
PHB (30)	100.0	0.0

Values were presented as Mean ± SEM, n=5, BP= n- Butanol fraction filtrate and PHB= Phenobarbital.

Table 4.24: Effects of CS fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenobarbital (PHB) on 4-aminopyridine-induced convulsions on mice

Dose (mg/kg)	Protection against seizure (%)	Mean Onset of Mortality (min)
Control	0.0	5.40 ± 0.24
CS (1.25)	0.0	6.40 ± 0.74
CS (2.5)	0.0	6.00 ± 0.77
CS (5)	0.0	4.80 ± 0.66
CS (10)	0.0	7.80 ± 0.73
PHB (30)	100.0	0.0

Values were presented as Mean ± SEM, n= 5, CS= n-butanol fraction precipitate and PHB= Phenobarbital.

Table 4.25: Effects of EA fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenobarbital (PHB) on 4-aminopyridine-induced convulsions on mice

Dose (mg/kg)	Protection against seizure (%)	Mean Onset of Mortality (min)
Control	0.0	13.20 ± 0.80
EA (1.25)	0.0	15.40 ± 0.40
EA (2.5)	0.0	10.20 ± 0.58
EA (5)	0.0	11.20 ± 0.86
EA (10)	0.0	11.60 ± 0.51
PHB (30)	100.0	0.0

Values were presented as Mean ± SEM, n=5, EA= Ethyl acetate fraction and PHB= Phenobarbital.

Table 4.26: Effects of NB fraction of Hydro-alcoholic root bark extract of *C. edulis* and phenobarbital (PHB) on 4-aminopyridine-induced convulsions on mice

Dose (mg/kg)	Protection against seizure (%)	Mean Onset of Mortality (min)
Control	0.0	4.50 ± 0.29
NB (1.25)	0.0	4.60 ± 0.40
NB (2.5)	0.0	4.20 ± 0.58
NB (5)	0.0	4.60 ± 0.60
NB (10)	0.0	5.60 ± 0.60
PHB (30)	100.0	0.0

Values were presented as Means ± SEM, n=5, NB= n-Butanol fraction and PHB= Phenobarbital.

CHAPTER FIVE

5.0 DISCUSSION

The thin layer chromatographic (TLC) analysis of Hydro-alcoholic root bark extract of *Carissa edulis* revealed the abundance of saponins (triterpenes and steroids), less anthraquinones, moderate amount of cardiac glycosides (cardinolides), abundant tannins and flavonoids while alkaloids were absent. These chemical constituents might be responsible for the observed pharmacological activities of the extract. The phytochemical constituents seemed to vary in different fractions (AP, BP, CS, EA and NB) of the extracts. For example, the AP fraction contained a high amount of tannins, less saponins (triterpenes and steroids) and flavonoids, while alkaloids, anthraquinones and cardiac glycosides (cardinolides) were absent. The BP fractioned only contained fewer amounts of tannins and saponins (triterpenes and steroids), abundant cardiac glycosides (cardinolides) and flavonoids. The CS fraction yielded only moderate amount of saponins (triterpenes and steroids). Furthermore, EA fraction harbored moderate amount of anthraquinones and tannins with less saponins (triterpenes and steroids). However, alkaloids and cardiac glycosides were absent in the EA fraction, NB fraction contained fewer amounts of tannins, saponins (triterpenes and steroids) and cardiac glycosides (cardinolides) while alkaloids and anthraquinones were absent.

On the other hand, Hydro-alcoholic root bark extract of *Balanites aegyptiaca* was found to contain abundant tannins and fewer amounts of anthraquinones, saponins (triterpenes and steroids) and cardiac glycosides (cardinolides) while alkaloids are absent. The presence of various phytochemical constituents might be responsible for the different pharmacological

activities observed when the *C. edulis and B. aegyptiaca* extracts were screened for anticonvulsant activities.

The LD_{50} values for the hydro-alcoholic root bark extract of *C. edulis* were found to be 282.8 mg/Kg body weight following intraperitoneal injection and 5000 mg/Kg after oral administration in mice. This suggests that the extract might be moderately toxic intraperitoneally while non toxic when administered orally. The oral route of administration is used in traditional medicine which seems to be relatively non toxic.

The LD_{50} of value of Hydro-alcoholic root bark extract of ***B. aegyptiaca*** is 1131.3 mg/Kg body weight intraperitoneally which suggests the extract is mildly toxic. However, LD_{50} value of greater than 5000 mg/Kg body weight orally was found to be relatively non toxic and the oral route is the widely used route of administration of the plant extract in the traditional medicine.

For long time it was assumed that a single drug could be developed for the treatment of all forms of epilepsy, but the causes of epilepsy are extremely diverse, encompassing genetic and developmental defects and infective, traumatic, neoplastic and degenerative disease processes (Roger and Brain, 2004); thus the single drug is yet to be found. The Hydro-alcoholic root bark extract of *C. edulis* and *B. aegyptiaca* protected the animals against ScPTZ-induced seizures, though not in dose dependent manner using 30 min post treatment and also it decreased the rate of mortality. However, at 60 min, the extract offered no

protection against seizure but protected the animals against mortality. This indicates that the best time of pretreatment with the extract was 30 min.

PTZ and MES tests are of relevance regarding the clinical spectrum of experimental compounds (Rogawski and Porter, 1990; Kupferberg and Schmutz, 1998). These two different animal models characteristically described three types of seizure activities. These were demonstrated by the activity against PTZ-induced seizures which correlates with anti-absence activity and activity against electrically-induced seizure which represents activity against generalized tonic-clonic and partial seizures (Delgado and Remers, 1998). Gamma amino butyric acid (GABA) is a major inhibitory neurotransmitter in the brain, and the inhibition of its neurotransmission has been thought to be the underlying factor in epilepsy (Amabeoku *et al.,* 1998). The enhancement of the GABAergic neurotransmission is reported to antagonize seizures, while the inhibition of the neurotransmission promotes seizures (Gale, 1992; Leonard, 2003; Erasmus, 2004). PTZ has been shown to diminish the GABAergic tone (Macdonald and Baker, 1997) by the inhibition of benzodiazepine (BDZ) site of the GABA receptors (Rehavi *et al.*,1982; Locher and Schmudt, 1988; De Deyn *et al.,* 1992). PTZ is a known convulsant and anticonvulsant effect against PTZ test identifies compounds that can raise seizures threshold in the brain (White *et al.,* 1998; Stables and Kupferberg, 1997). The pharmacological profile of the ScPTZ seizures model is consistent with the human condition (Swinyard *et al.,* 1989). The ScPTZ-induced seizures are similar to the symptoms observed in the absence seizures and drugs such as valproate, diazepam and ethosuximide which are useful in treatment of absence seizures suppress PTZ-induced seizures (McNamara, 2006). Therefore, inhibition of PTZ-induced seizures suggests effects

on GABAergic neurotransmission. Also, it has been found empirically that drugs which inhibit PTZ-induced seizures and raise the threshold for production of electrically-induced seizures, are generally effective against absence seizures (Rang *et al.*, 2003).

The protection of mice against PTZ-induced seizures by the standard anticonvulsant drug (diazepam) was expected, since various authors have shown that diazepam exerts its anticonvulsant activity by enhancing GABA-mediated inhibition (Olsen, 1981; Amabeoku *et al.*, 1998; Leonard, 2003; Rang *et al*., 2003; Shaun, 2004). Ability of the Hydro-alcoholic root bark extract of *C. edulis* and *B. aegyptiaca* to protect the animals against PTZ-induced seizures may suggest anticonvulsant activity for the management of absence seizures (petit mal), though that of *B. aegyptiaca* protection was mild. This may suggests that the extract may be useful in petit mal (absence) epilepsy, especially as an adjuvant to other medicinal plants, since most of the times the traditional practitioners use combinations of more than one plant. Generally, compounds with the anticonvulsant activity in the petit mal epilepsy are effective in PTZ-induced seizures model (Vida, 1995).

The experimental animal models of epilepsy induced by the systemic administration of chemicals have been used to study the mechanism of drugs action. Some studies have indicated that PTZ diminishes the GABAergic tone (Macdonald and Barker, 1977) by the inhibition of BDZ site of the GABA receptors (Rehavi *et al.*, 1982). In order to establish the role of BDZ receptors participation in the *C. edulis* and *B. aegyptiaca* extract-induced anticonvulsant activity, flumazenil, a specific antagonist of the benzodiazepine site in the $GABA_A$-BDZ receptor complex (File *et al.*, 1982; File and Pellow, 1986; Brogden and Goa, 1988) was used. Antagonism of anticonvulsant activity of the extracts by flumazenil indicates that the extracts mediate their anticonvulsant activity against PTZ-induced

seizures in part through the facilitation of the inhibitory activity of the GABAergic system probably through a competitive agonistic action in the BDZ site of the GABA receptors.

Naloxone, a non specific opioid receptors antagonist (Mannino and Wolf, 1974; Cowan *et al.,* 1979; Lauretti *et al.,* 1994) has been shown to antagonize the anticonvulsant activity of the Hydro-alcoholic root bark extracts of *C. edulis* and *B. aegyptiaca.* Selective κ-opioid receptor activation produces a dose-dependent suppression of the bicuculine-induced seizures (Yajima *et al.,* 2000). Bicuculine produces convulsions through its antagonism of $GABA_A$ receptor-mediated inhibition (Macdonald and Baker, 1978) and was completely blocked by pretreatment with nor-binaltorphimine. It is well documented that κ-opioid receptor agonists affect mostly Ca^{2+} channels, resulting in the blockade of Ca^{2+} entry (Werz and Macdonald, 1985). Although, the mechanism of the anticonvulsant effect with κ-opioid receptor agonists is presently unclear, one possibility is that postsynaptically localized κ-opioid receptor may contribute to the inhibition of excitability induced by postsynaptic blockade of $GABA_A$ receptor by PTZ through the reduction of Ca^{2+} entry. Since it is also known that at cellular level, one of the basic mechanisms of actions of AEDs such as ethosuximide and Valproic acid is the suppression of T-type calcium currents in thalamic neurons (Rho and Sankar, 1999; Meldrum, 1996; Macdonald and Kelly, 1994). The suppression of the T-type calcium channel may be involved in the extract's activity. On the basis of this, it would be suggested that κ-opioid receptor may participate in the *C. edulis* and *B. aegyptiaca* extracts mediated PTZ anticonvulsant effect since naloxone reduced the effect of the extracts on seizures. The biphasic activity observed in PTZ-induced seizures

studies may probably be due to possible interactions between constituents of the crude extracts.

The hydro-alcoholic root bark extract of *C. edulis* showed significant protection (90%) against MES-induced seizures which is comparable to the standard drug phenytoin (100%). Similarly, the extract of *B. aegyptiaca* protected the animals against MES mildly in a biphasic manner. MES test is a non-mechanistic seizures model that has clearly defined end points such as inhibition of hind limb tonic extension (HLTE) (Stables and Kupferberg, 1997). There is no false negative in MES test and the currently available AEDs that are clinically effective in the management of generalized tonic-clonic and partial seizures such as carbamazepine, phenytoin, primidone, phenobarbital, valproate and lamotrigine all suppress HLTE in MEST (Browning, 1992; Rho and Sankar, 1999). Protection against HLTE by the extracts as compared with standard antiepileptic drug (phenytoin) used indicates that the extracts may have one or more compounds that can be useful in the management of generalized tonic-clonic and partial seizures probably as adjunct agent. Anticonvulsant activities have been reported for saponins and flavonoids (Shibata, 2001; Yemitan *et al.,* 2001; Kavvadias *et al*., 2004) which were found in C. edulis and B. aegyptiaca extracts. These constituents may be responsible, in part or in combination for the observed anticonvulsant effect of the extracts.

Oral administration of *C. edulis* and *B. aegyptiaca* extracts did not protect the mice from PTZ and MES-induced seizures. However, *i.p* injection of the extracts showed significant increase in the latency of seizures. This could be due to pharmacokinetic factors which

result in the poor absorption of the extracts' active principle(s) from the GIT following oral garvage or enzymatic breakdown of the extract in the acidic condition of the stomach or extensive first pass effects or combined together, thus, limit the bioavailability of the active component(s) of the extracts in the plasma and consequently reduced the amount of the drug that will penetrate through blood brain barrier to reach the site of action. These may probably be the reason behind the observed differences on seizure with the extracts when given orally compared to parenteral (intraperitoneal) route. Therefore, I would suggest that the best route of administering the dosage form for these extracts is parenteral.

The fractions AP, BP, CS, EA and NB of the Hydro-alcoholic root bark extract of *C. edulis* were found to have anticonvulsant activities in different capacities in PTZ and MES-induced seizures. The AP fraction's protection against seizures induced by PTZ and MES-induced seizure is not dose-dependent and there is no significant difference in latency and duration of seizures. This suggests that AP fraction may contain at least one or more components that antagonizes chemically (PTZ) and electrically (MES)-induced seizures in mice and chicks, respectively. AEDs' effectiveness in the therapy of generalized seizures of (absence or myoclonic) petit mal type such as phenobarbital, Valproic acid, ethosuximide and benzodiazepines exhibit dose-dependent suppression of various seizures pattern induced by PTZ (Loscher *et al.*, 1991).

Moderate anticonvulsant activity of AP fraction in ScPTZ animal model suggests the presence of bioactive compound(s) effective in the therapy of absence or myoclonic seizures. Also, mild anticonvulsant action of BP and CS fractions against PTZ-induced

seizures may suggest the presence of a compound that is devoid of or with little anti-absence or myoclonic seizures. This findings is in conformity with Swinyard *et al.,* (1952) and Swinyard, (1969) that not all antiepileptic drugs have protective value against ScPTZ-induced convulsions. However, exhibition of biphasic activities by EA and NB fractions against ScPTZ test may be due to the presence of more than one compounds in these fractions that are antagonistic to one another thus, they interfere with the anticonvulsant activities of other constituent(s). Increased in mortality shown by the NB fraction might be due to presence of chemical substance(s) that could augment the lethality of PTZ in the animals tested.

The fractions AP, BP, EA and NB of C. edulis and B. aegyptiaca showed weak anticonvulsant activity while CS fraction exhibited moderate anticonvulsant activity against MES and the effects were not dose dependent. Inhibition of hindlimb tonic extension (HLTE) is the common feature of MES in rodents, cat, monkey and humans and the response of rodent brain to the anticonvulsant is similar to that of humans (Swinyard, 1972). Protection against HLTE also indicates the ability of a testing material to inhibit or prevent seizure discharge within the brainstem seizure substrate (Browning, 1992). The weak inhibitory effect of AP, BP, EA and NB fractions and moderate action of CS fraction suggest that these fractions may possess compounds that might not be very effective in the management of generalized tonic-clonic and partial seizures alone but may be useful in combinations with other drugs. Further purification and characterization of the fractions would allow us to delineate the anticonvulsant activities of individual chemical constituents of these plants.

The absence of activity against 4-aminopyridine-induced seizures produced by the entire fractions may suggest that the component(s) in these fractions have no activity against potassium channels, since 4-aminopyridine is a known potassium channel blocker (Yamaguchi and Rogawski, 1992). Thus, the compounds present in these individual fractions may likely exhibit their actions via the GABA receptor and partly through κ-opioid receptors mediated neural inhibition as was shown by the crude extract.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

Based on the results presented herein, it may be concluded that the Hydro-alcoholic root bark extracts of *Carissa edulis* and *Balanites aegyptiaca* contain bioactive component(s) that possess anticonvulsant activities which may be responsible for the use of the plants in the management of epilepsy (Grand mal and Petit mal epilepsies) in traditional medicine. Furthermore, both *C. edulis* and *B. aegyptiaca* extracts may be acting through GABAergic mediated neural inhibition and partly via κ-opioid receptors system.

More work will be carried out to elucidate the active component(s) of the extracts.

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