SOME NEUROPHARMACOLOGICAL STUDIES OF EXTRACT AND FRACTIONS OF *CRINUM ZEYLANICUM*(L.) (AMARYLLIDACEAE)BULB IN LABORATORY ANIMALS

BY

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DECLARATION

This is to certify that the work reported in this dissertation was carried out by me in the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria and the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Idu industrial lay out, Abuja, under the supervision of Professor J.A. Anuka and Professor (Mrs.) O.A.Salawu. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this Dissertation has been submitted elsewhere for a degree.

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CERTIFICATION

This thesis entitled **“**SOME NEUROPHARMACOLOGICAL STUDIES OF EXTRACT AND FRACTIONS OF*CRINUM ZEYLANICUM*(L.) (AMARYLLIDACEAE**)**BULB IN LABORATORY ANIMALS” by TijaniAdeniyiYahaya meets the regulations governing the award of the degree of DOCTOR OF PHILOSOPHY (PHARMACOLOGY) of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To the everlasting memory of my late parents Sheikh Ahmad-Tijani Adesokanand Mrs. AsanatArabaAttahTijani

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ABSTRACT

Crinum zeylanicum is a medicinal plant used in folk medicine for management of general debility, infantile seizures and pain. Some neuropharmacological effects of methanolic bulb extract of *Crinum zeylanicum* were studied in laboratory animals.The Phytochemical screening of the methanolic bulb extract and its solid residue fraction (SRF) were carried out using standard procedures. Quantitave determination of the elemental content of *Crinum zeylanicum* bulb and its methanolic bulb extract was carried out using atomic absorption spectrophotometer. The oral and intraperitoneal acute toxicities of themethanolic bulb extract and its solid residue fraction were carried out in mice and rats using modified Lorke's method. The effect of the methanolic bulb extract was studied on gross behaviour in mice using Irwin method, on pentobarbitone-induced sleep time, spontaneous motor activity, exploratory behaviour also in mice usinghole board, motor coordination (rota-rod) andapomorphine –induced stereotypy. The anticonvulsant activity of the methanolic bulb extract and SRF was evaluated using chemical convulsants in mice (Pentylenetetrazole, picrotoxin, strychnine, pilocarpine, and 4-aminopyridine) and electrically using maximal electric shock in a day old chicks. The analgesic activity of the extract was evaluated using acetic acid induced writhing test in mice, formalin induced pain in rats and hot plate test in mice.Chronic oral toxicity study of the methanolicbulb extract was carried out in male and female Wistar rats for 90 days. The assessment of toxic effect of the extract was evaluated on feed and water intake, change in body weight, haematological and biochemical parameters. Histopathological evaluation of the internal organs was also carried out.The Phytochemical test revealedpresence of balsam, sterols, terpenes, resins carbohydrate, tannins, phylobatannins, saponins, flavonoids, alkaloids and volatile oils while

anthraquinones, phenols and glycoside were absent. Sterols, alkaloids and terpenes were present in SRF. The elemental analysis showed presence of sodium, potassium, calcium, magnesium, manganese, lead, iron and nickel in the bulb material and the methanolic bulb extract while chromium was present only in the bulb. The oral median lethal dose of the methanolic bulb extract was greater than 5000 mg/kg body weight while the intraperitoneal median lethal dose was 3807.88 mg/kg body weight in mice and rats respectively.The oral and intraperitonealmedian lethal doses (LD_{50}) of the solid residue fraction were 470 and 77.49 mg/kg body weight respectively. The Irwin test revealed that the extract possesses central depressant effect.The methanolic bulb extract and SRF significantly ($p<0.01$) shortened the onset of sleep and prolonged the duration of sleep. The methanolic bulb extract significantly ($p<0.001$) decreased spontaneous motor activity but had no effect on motor co-ordination.The extract significantly ($p<0.001$) decreased the number of head dips in the hole board. The methanolic bulb extractsignificantly ($p<0.0001$) decreasedapomorphine-induced stereotypy in mice.The methanolic bulb extract and SRF protected mice against pentylenetetrazole- and pilocarpine-induced seizures in mice.Themethanolic bulb extract did not protect while SRF protected mice against picrotoxin-induced seizures in mice dose-dependently.The extract did not protect mice against strychnine and 4-aminopyridine- seizures butsignificantly ($p<0.05$) delayed onset of tonic hind limb extension in mice. The extract did not protect while SRF at 100 mg/kg and 20 mg/kg phenytoin protected 90 and 100% of the chicks respectively against maximal electric shock-induced seizures. The methanolic bulb extract showed significant analgesic effect against acetic acid-induced pain in mice, formalin-induced pain in rats and thermal-induced pain in mice on hot plate. The methanolic bulb extract did not produce adverse clinical signs and death at doses of 250, 500 and 1000 mg/kg administered for 90 days in rats. The methanolicbulb extract at 500 and 1000 mg/kg suppressed feed intake and increased water intake significantly ($p<0.001$) in the 12th and 13th weeks of the study in female rats. The methanolic bulb extract had no effect on feed intake, water intake and body weight of male rats.The changes in the haematological parameters in male and female rats were within normal range values. Significant ($p<0.05$-0.001) increase in liver function enzymes and lipid profiles were observed in female and male rats at 42 and 90-days while other biochemical parameters remained normal.The relative organ weight of all organs studied in female and male rats remained normal at 42 and 90-days. Histopathology revealedlivercongestion and cellular infiltrationat 1000 mg/kg body weight after 90 days of administration in female and male rats.The results obtained provided evidence based scientific support for its potential therapeutic benefits and safety of themethanolic bulb extract in the management of epilepsy, psychosis and painful disorders.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

LIST OF PLATES

ABBREVIATIONS

AD:	Alzheimers disease
AChEI:	Acetylcholinesterase inhibitors
NMDA:	N-methyl-D- Aspartate
HD:	Huntington’s disease
PD:	Parkinson’s disease
GABA:	Gamma amino butyric acid
CNS:	Central nervous system
PNS:	Peripheral nervous system
MAO B:	Monoamine oxidase B
DOPAC:	Dihydroxyphenylacetic acid
COMT:	Catechol-O-methyl transferase
nAChRs:	nicotinic acetylcholine receptors
EEG:	Electroencephalograph
ILAE:	International League Against Epilepsy
GTC:	Generalized tonic-clonic
GCSE:	Generalized convulsive status epilepticus
NCSE:	Non-convulsive status epilepticus
VPA:	Valproate
CBZ:	Carbamazepine
OXC:	Oxcarbazepine
PHT:	Phenytoin
PRM:	Primidone
PB:	Phenobarbital

PGB:	Pregabalin
LTG:	Lamotrigine
TPM:	Topiramate
ZNS:	Zonisamide
AEDs:	Anti-epileptic drugs
SLE:	Seizure-like events
WBC:	White blood cells,
RBC:	Red blood cells,
HB:	Haemoglobin,
HCT:	Haematocrit,
PLT:	Platelets,
LYM:	Lymphocyte,
MXD:	Monocyte
NEUT:	Neutrophil
ALP:	Alkaline phosphatase
AST:	Aspartate transaminase
ALT:	Alanine transaminase
T/Bil:	Total bilirubin,
HDL:	High density lipoprotein,
LDL:	Low density lipoprotein,
Trig:	Triglyceride,
T/Chol:	Total cholesterol

CHAPTER ONE

1.0 INTRODUCTION

1.1 Statement of the Research Problem

The nervous system is a complex, sophisticated system that regulates and co-ordinates the body's basic functions and activities. It is made up of the Central Nervous System (CNS), consisting of the brain and spinal cord and the Peripheral Nervous System (PNS) which consists of all other neural elements.

When the brain is healthy, it functions quickly and automatically. However, when problems occur, the results can be devastating. Major types of disorders of the brain include (a) Neurogenetic diseases e.g. Huntington's disease and muscular dystrophy. (b) Developmental disorders e.g. Central palsy, (c) Degenerative diseases of adult life e.g. Parkinson disease and Alzheimer's disease, (d)Metabolic disease e.g. stroke and vascular dementia, e) Trauma e.g. spinal cord injury and head injury, (f) Infectious diseases e.g. Acquired immunodeficiency syndrome (AIDS), dementia, brain tumors and (g) Convulsive disorders e.g. epilepsy (Dichter and Ayala,1987).

Huntington's disease (HD) is a dominantly inherited disorder characterized by gradual onset of motor incoordination, cognitive decline in midlife and prominent neuronal loss in the striatum (caudate/putamen) of the brain (Lipton and Rosenberg, 1994)). Symptoms of the disorder include movement disorder typified by brief jerk like movements of the extremities, trunk, face and neck (chorea) or as personality change. The outcome of HD is invariably fatal, because as the disease progresses, the affected person becomes totally disabled, unable to communicate and eventually die from the complications of immobility.

Parkinson's disease (PD) is characterized by progressive degeneration of the substantial nigra, with subsequent depletion of striatal dopamine and disequilibrium of the extrapyramidal motor circuits. In normal adults, the rate of dopaminergic cell loss occurs linearly, with a 5% decline per decade. However, in PD, cell loss accelerates exponentially, with 45% decline during the first decade after diagnosis. It is observed that Parkinson's symptoms do not appear until a critical threshold of 80% loss of striatal dopamine concentration has occurred (Churchyard *et al.,* 1997). Imbalances of other neurotransmitters (e.g. acetycholine, gammaaminobutyric acid {GABA}, glutamate, norepinephrine, serotonine) (Lang and Lozano, 1998) are also characteristic of Parkinson disease (Duvoisin, 1967). The cardinal clinical features of PD are tremor at rest, rigidity, akinesia or bradykinesia, and postural instability. The cause of PD remains obscure, but factors such as age related neurodegeneration, genetic constitution, and toxin exposure may play a role in its etiology.

Epilepsy is one of the most common neurological problems worldwide. Approximately 4 million people in the European Union and the United States suffer from epilepsy. Research findings showed that approximately 3% of the general population will have epilepsy at some point in life (Duncan *et al.,* 2006). Epilepsy knows no geographical, social, or racial boundaries, occurs in men and women and affects people of all ages. Its occurence is more frequent in the young people (in the first two decades of life) and people over the age of 60 (Sander, 2004). The term epilepsy encompasses a number of different syndromes with cardinal feature of a predisposition to a recurrent unprovoked seizure (French and Pedley, 2008). Seizures, in turn, are sudden, brief attacks of altered consciousness, motor, sensory, cognitive, psychic, or autonomic disturbances, or inappropriate behaviour caused by abnormal excessive or synchronous neuronal activity in the brain (Kwan and Sander, 2004). The nature of each seizure is determined by the point of origin of the hyper excitability and its degree of spread in the brain. If given a sufficient stimulus (e.g. hypoxia, convulsant agents, hypoglycemia), a normal brain can discharge excessively, producing a seizure. Conventionally, the diagnosis of epilepsy requires that the patient must have had two unprovoked seizures. However, a person with an isolated non recurrent, externally provoked seizure that are also caused by excessive discharge of cerebral neurons is not thought to have epilepsy as long as the seizures are not recurrent and each seizure is preceded by a provocation (e.g., substance abuse, fever, exposure to alcohol combined with lack of sleep). Clinically, there are two major seizure types' i.e partial (local, focal) and generalized seizures. Partial seizures are those in which the discharge begins locally and often remains localized, while generalized seizures involve the whole brain, including the reticular system, resulting in production of abnormal electrical activity at both hemispheres (Marson *et al.*, 2007).

The causes of epilepsy include brain damage resulting from malformations of brain development, neurosurgical operations, head trauma, other penetrating wounds of the brain, brain tumor, high fever, bacterial or viral encephalitis, intoxication, stroke, or acute or inborn error of metabolism (Berg, 2008). Signs and symptoms depend on the area of the brain in which seizure activity occurs and on the type of seizure. In simple partial seizures, symptoms may be motor, sensory, psychic (states of consciousness), and/or autonomic (involuntary activity controlled by the autonomic nervous system). Motor signs include alternating contraction and relaxation of muscle groups (tonic-clonic convulsion), rapid eye movements and turning of the head to the same side, asymmetrical posturing of the limbs, speech arrest and vocalization. Sensory symptoms include seeing flashes of lights or colors, illusions and hallucinations, hearing humming, buzzing or hissing noises, experiencing unpleasant odors and tastes, dizziness and lightheadedness. Psychic symptoms include detachment, depersonalization, dreamy state and memory distortion manifested as flashback, déjà vu (feeling that one has seen something before), time distortion, unprovoked emotion: fear, pleasure, displeasure, depression, anger, elation and eroticism. Autonomic signs and symptoms include rumbling noises produced by gas in the intestines, flushing, incontinence , nausea, vomiting, piloerection (goose bumps), pupillary dilation, sweating and tachycardia (rapid heart rate) (Danesi, 1985)

Complex partial seizures are easily triggered by emotional stress. The limbic structures (i.e., hypothalamus, hippocampus, and amygdala) of the brain may be damaged by seizure activity. However, in grand- mal epilepsy, generalized tonic-clonic seizures may begin as simple or complex partial seizures. The tonic phase consists of fall, Loss of consciousness, extension of arms, legs, and/or face, clenched fingers and jaw and autonomic symptoms characterized by increased blood pressure and heart rate, increased bladder pressure, flushing, sweating, increased salivation, increased bronchial secretion, apnoea (cessation of breathing) . During the clonic phase, muscles relax completely, and then muscle tone returns resulting in rhythmic jerks of the body and head while signs such as drooling, biting of the tongue, cheek, or lip and urinary incontinence are common in the post ictal phase (Meldrum,1990).

It has been reported that more than 80% of people with epilepsy live in developing countries, where the condition remains largely untreated due to unavailability of treatment, inadequate health delivery systems, lack of trained personnel, lack of essential drugs, and traditional beliefs and practices that often do not consider epilepsy as treatable condition (Salih and Mustafa, 2008).

1.2 Justification for the study

In spite of substantial progress made in the treatment of neurological disorders, epilepsy remains a significant therapeutic challenge. Cumulative evidence has shown that the older anti-epileptic drugs (AEDs) such as phenytoin (PHT), carbamazepine (CBZ), phenobarbital (PB), primidone (PRM), and valproate (VPA) are effective against a broad range of seizure types and do not differ in terms of their efficacy when given as monotherapy in the treatment of newly diagnosed patients with partial seizures or primary and secondary generalized tonic-clonic seizures (Kwan and Sander, 2004). The use of these agents, is however limited by complex pharmacokinetics and serious adverse effects, including effects on liver cytochrome P450 enzymes. In general, PRM and PB appear to be associated with the lowest tolerability due to serious adverse effects. Four of the five older AEDs are hepatic enzyme inducers, enhancing the metabolism of many commonly prescribed medications, such as warfarin, beta-blockers, antibiotics, chemotherapeutic agents, antidepressants, antipsychotics, and immunosuppressants, and may also increase the clearance of certain hormones and vitamins. The induction of hepatic enzymes may limit the effectiveness of oral contraceptives thus contributing to sexual dysfunction. Similarly, increased metabolism of vitamin D can lead to increased bone turnover, osteopenia, and eventually osteoporosis. The deleterious effect of rapid metabolism of vitamin D on bone turnover is of particular concern in patients with epilepsy because of high risk of falls and fractures associated with seizures (Marson *et al.,* 2007). Nine newer, second-generation AEDs have been approved by the Food and Drug Administration (FDA) since 1993. They include felbamate (FBM), gabapentin (GBP), lamotrigine (LTG), levetiracetam (LEV) oxcarbazepine (OXC), pregabalin (PGB), tiagabine (TGB), topiramate (TPM), and zonisamide (ZNS). Three of these, TPM, LTG, and LEV, have a broad spectrum of activity with proven efficacy in the treatment of both partial and generalized seizures. ZNS also appear to be efficacious in generalized epilepsy based on large open label series, but there are no randomized controlled trials demonstrating this. Broad spectrum activity simplifies the treatment of patients where the diagnosis of partial versus generalized onset is unclear or the rare case where the patient has both partial and generalized onset seizures (Smith *et al.*, 1998).

The newer AEDs offer fewer side effects but have done little to bring effective seizure control to the significant population living with active and uncontrolled epilepsy. More than a third of all epilepsy patients in the United States have persistent seizures using existing therapies. Many others tolerate serious debilitating adverse effects, including sedation, blood dyscrasias and spermatogenesis (Loscher, 2002., Patsalos, 1999) and diminished cognition, as a price for seizure control. The development of new pharmacological agents that can overcome these side effects and affordable treatment cost have made traditional herbs and herbalists very useful and indispensable in the struggle for therapeutic management of epilepsy in developing countries and future AED development. The plant kingdom is a major target in the search for new drugs and lead compounds to treat epilepsy and other neurological disorder (Adeyemi and Yemitan, 2005; Adeyemi *et al*., 2007). There is therefore need for research into medicinal plants with possible anti-convulsant effects based on vast ethno-botanical knowledge and practices of the indigenous people in the treatment of seizure.

1.3 Theoretical Framework

The development of phytotheraputic products from medicinal plants begins with acute toxicity studies. The results of these studies will reveal the possible clinical signs elicited by the substances under investigation, provide information on the median lethal dose (LD_{50}) of the product and identify the range of doses that could be used subsequently for efficacy and long term toxicity studies. The method described by Lorke (1983) with slight modification was used for the acute toxicity studies. The results obtained from the acute toxicity studies guided the choice of doses used for the neuropharmacological studies of the extract and fractions of *Crinum zeylanicum* bulb.

(i) Gross behavioural study

A systematic, quantitative procedure assessing the behavioral state of mice for the evaluation of drugs described by Irwin (1964, 1968) was used for gross behavioural study. This method was applied in the beginning of *Crinum zeylanicum* pharmacological screening to detect its possible psychotropic effects.

(ii) Potentiation of pentobarbitone sleeping time

The test is used to evaluate CNS-active properties of drugs. Hypnotics, sedatives, tranquilizers as well as antidepressants at high doses are known to prolong pentobarbitone- induced sleep after a single dose of pentobarbitone. The loss of righting reflex is measured as criterion for the duration of pentobarbitone-induced sleeping time (Miya et al., 1973). Mice are used in this test, since metabolic elimination of pentobarbitone is rapid in this species. The method described by Roland et al., (1969) was used for evaluating CNS activities of *Crinum zeylanicum* in mice.

(iii) Studies on spontaneous motor activity

The Spontaneous Motor Activity (SMA) is used to evaluate gross behavioural effects of drugs in laboratory animals (Carpendo et al., 1994). It measures the level of excitability of the central nervous system (Mansur et al., 1971) which correlates well with drug effect in humans. Any agent that suppresses SMA may possess central sedative properties (Ozturk et al., 1996).

(iv) Test for motor co-ordination (Rotarod Test)

The test is used to evaluate the activity of drugs interfering with motor coordination. Ozturk et al., (1996) suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. The apparatus consists of a horizontal wooden rod or metal rod coated with rubber with 3 cm diameter attached to a motor with the speed adjusted to 2 rotations per minute. Any compound that interferes with motor co -ordination reduces the time spent on the revolving rod by an animal before falling off.

(v) Hole-Board Test

The apparatus known cosists of Letica board (LE 3333) of 60 cm ×30 cm with 16 evenly distributed holes. This method is employed in the evaluation of certain components of behaviour, such as curiosity or exploration(File and Pellow, 1985) . Poking the nose into a hole is a typical behaviour of the mice indicating a certain degree of curiosity. An agent that decreases this parameter reveals a sedative behaviour (File and Pellow, 1985) Anxiolytics have been shown to increase the number of head dips (Takeda *et al.,* 1998).

(vi) Apomorphine –induced stereotype behavioural studies in mice

The model is capable of identifying antagonist of dopamine receptors in the nigrostriatal system (Tarsy and Baldessarini, 1986). Dopamine agonist such as apomorphine induces stereotype behaviour which includes sniffing, jumping/climbing, limb licking and circling. The anti-dopaminergic property of any compound can be determined by its ability to antagonize apomorphine-induced stereotypy which may be correlated with its neuroleptic potentials.

(vii) Pentylenetetrazole-Induced Convulsion

This is a model that primarily identifies compounds that raise seizure threshold. The pharmacological profile of the subcutaneous pentylenetetrazole (Sc.PTZ) seizure model is consistent with human condition (Swinyard *et al.,* 1969). The Sc. PTZ utilizes a dose of pentylenetetrazole that produces clonic seizures lasting for a period of at least five seconds in 97% (CD97) of animal tested. The test compound is administered intraperitoneally (i.p.) in mice at the anticipated time the convulsant is administered subcutaneously. Absence of clonic spasms in the 30 minutes observation period indicates compound ability to abolish the effect of pentylenetetrazole on seizure threshold (Swinyard *et al.,* 1969).

(viii) Maximal Electroshock Seizure Test (MEST)

The electroshock assay in mice or a day old chicks is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics but also by other centrally active drugs. The MEST is highly reproducible with consistent end points. The behavioural and electrographic seizures generated in this model are consistent with the human disorder (Swinyard *et al.,* 1989).

(ix) Picrotoxin-induced convulsions

Picrotoxin- induced convulsions are used to further evaluate CNS-active compounds. Picrotoxin is regarded as a GABAA-antagonist modifying the function of the chloride ion channel of the GABAA receptor complex (Vogel and Vogel, 1997). .

(x) Strychnine-Induced Convulsion in mice

The convulsant action of strychnine is due to interference with postsynaptic inhibition mediated by glycine. Glycine is an inhibitory transmitter to motor neurons and inters neurons in the spinal cord. Strychnine acts as selective, competitive antagonist to block the inhibitory effects of glycine receptors (Rajendra *et al.,* 1997). Subcutaneous administration of strychnine produces hind limb tonic extensor jerk s in mice (White *et al.,* 1998). Latency to hind limb extensor jerk or mortality is defined as the interval between the end of the strychnnine (STN) injection and the occurrence of first episode of hind limb tonic extensor jerk or death (Abdulwahab *et al .,* 2009). Compounds which reverse the action of strychnine have been shown to have antiepileptic effects (Larson, 1996).

(xi) Pilocarpine-induced convulsion

Pilocarpine (a muscarinic agonist) treatment was introduced as a possible model for human temporal lobe epilepsy (TLE) by Turski (1983) in rats and mice. In general, a single high dose of pilocarpine (320 to 400 mg/kg) generates status epilepticus (SE) characterized by spontaneous recurrent seizures, tonic- hind limb extension and death in mice. When treating with pilocarpine, scopolamine methylnitrate should be administered prior to pilocarpine to reduce peripheral cholinergic effects (Cavalheiro, 1995), and the pilocarpine-induced SE is almost always interrupted with an antiepileptic drug (e.g., diazepam; Mello et al., 1993).

(xii) 4-aminopyridine-induced convulsion in mice

The potassium channel antagonist 4-aminopyridine is a powerful convulsant in animals and man. In mice, parenterally administered 4-aminopyridine induces clonic-tonic convulsion and lethality (Vogel and Vogel, 1997). 4-aminopyridinine is believed to induce seizure activity by enhancing spontaneous and evoked neurotransmitter release. The ability of the compound(s) to protect mice from lethality within 30mins is considered an indication of anticonvulsant activity which is believed to act by opening potassium channels (Rogawski and Porter, 1990).

(xiii) Writhing tests

Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing. The test is suitable to detect peripheral analgesic activity although some psychoactive agents also show activity. An irritating agent such as acetic acid is injected intraperitoneally to mice and the stretching reaction is evaluated (Koster et al., 1959; Nakamura et al., 1986)

(xiv) Formalin test in rats

The formalin test identifies mainly centrally active drugs, whereas peripherally acting analgesics are almost ineffective. Therefore, the formalin test may allow dissociation between inflammatory and non-inflammatory pain, a rough classification of analgesics according to their site and their mechanism of action (Chau 1989). Cowan (1990) underlined the aspect that the formalin-test is a model of chronic pain whereas most other methods measure only the effect on acute pain.

(xv) Hot plate

The hot plate test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics (Tjølsen et al., 1992). The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses.

(xvi) Chronic toxicity study

Oral chronic toxicity study was carried out according to WHO (1992) guideline. The guideline prescribed between three months to six months toxicity study for a compound intended for one to three months clinically through the intended route of administration. The chronic toxicity study provides information on the possible health hazards likely to arise from repeated exposure over a considerable part of the entire lifespan (in rodents). The study will provide information on the toxic effects of the substance, indicate target organs and the possibility of accumulation. It will also provide an estimate of the no-observed-adverse effect level which can be used for establishing safety criteria for human exposure.

1.4 objectives of the study

The main objective of this research is to evaluate the central nervous system effects of methanolic extract of *Crinum zeylanicum* so as to provide scientific rational for its continued use in traditional medical practice as an anti-convulsant.

Other specific objectives include:

a.) To collect, identify and prepare extracts of *Crinum zeylanicum* bulb using solvents of varying polarities.

b.) To carry out acute toxicological study of the extract, to detect behavioural signs of toxicity, calculate or determine the median lethal dose (LD_{50}) orally and intaperitoneally in mice and rats and to determine doses to be used in efficacy and long term toxicological studies.

c.) To carry out some neuropharmacological studies of the extract using various animal models: pentobarbitone- induced hypnosis in mice, spontaneous motor activity (SMA), exploratory activity, rotarod performance (motor co-ordination), apomorphine-induced stereotype behavior in mice and analgesic effect of the extract.

d.) To evaluate the anti-convulsant activity and possible mechanism (s) of action of the extract using various models of convulsion /seizure: pentylenetetrazol(PTZ)- induced convulsion, strychnine- induced convulsion, picrotoxin- induced convulsion, pilocarpine -induced convulsion, 4 –amino pyridine induced convulsion in mice and maximal electric shock-induced seizure in chicks.

e.) To carry out anti-convulsant guided fractionation of the crude methanolic bulb extract of *Crinum zeylanicum*.

f.) To carry out phytochemical analysis of the crude methanolic bulb extract of *Crinum zeylanicum* and its fractions.

g.) To carry out elemental analysis of the crude methanolic extract.

h.) To carry out 90- day chronic toxicological studies of the methanolic bulb extract of *Crinum zeylanicum* to evaluate its long term toxic effects in rat.

1.5 Statement of Research Hypothesis

Crinum zeylanicum bulb does not contain biologically active phytochemical constituents that are useful in management of convulsion and other neuropsychological disorders.

CHAPTER TWO

2.0 LITERATURE REVIEW

A proper understanding of the physiological and anatomical organization of the nervous system is essential to correctly identifying the sites and mechanisms of central nervous system (CNS) disorders, and how drugs can modify these disorders. The nervous system gathers information from the external environment, integrates the stimuli in the form of electrical impulses and initiates appropriate biological responses. The nervous system is divided into two functional units; the central nervous system (CNS) consisting of the brain and spinal cord, and the peripheral nervous system (PNS), which consists of all the other nerve cells in the body.

2.1. **Central nervous system**

The central nervous system is made up of networks of neurons, which coordinates and modifies motor function based on the intensity and frequency of sensory inputs, hormonal and other blood borne signals.

2.1.1 *The organisational structure of the brain and spinal cord*

Embryologically, the central nervous system may be divided into three sections namely; the prosencephalon (forebrain), the mesencephalon (mid-brain) and the rhombencephalon (hind-brain).

Prosencephalon or Forebrain; this is further divided into telencephalon, (cerebrum) and the diencephalon (thalamic nuclei). The cerebrum consists of two hemispheres that constitute the bulk of the nervous system. The cerebral hemispheres are composed of the cerebral cortex, certain limbic structures, the corpus striatum, and a multitude of nervous system pathways. The cerebral cortex is divided into four lobes; the frontal, temporal, parietal, and occipital lobes. The pathways formed by axons from neurons in the cerebral cortex transmit information throughout the CNS. Some of these pathways form the corpus callosum and the corona radiata, through which motor and sensory information pass, as well as other pathways inter connecting the four cerebral lobes (Brodal, 1992). The occipital lobe is almost exclusively devoted to the senses of vision. It is divided into a multitude of different visual areas, of which the largest is the primary visual cortex, the major destination for visual information. The parietal lobe is partially devoted to the sense of touch.

It is also very important for perceiving the spatial lay out of the environment and effectively moving through it. The temporal lobes contain the primary auditory cortex, an area for hearing similar to the primary visual and somatosensory cortices, as well as secondary auditory area that further process what we hear, including in the left hemisphere, the decoding of words and sentences. The temporal lobes also contain more specialized visual areas for recognizing detailed objects such as faces. The temporal lobes are critical for memory as they contain hippocampal formation and amygdala. The frontal lobes are essential for planning and movement. The rearmost portion of the frontal lobes is the primary motor cortex, responsible for more complex movements. The rest of the frontal lobes, not directly responsible for movement, are collectively termed the prefrontal cortex. The prefrontal cortex occupies about 30 percent of the brain in humans and is indispensible for rational activity.

Diencephalon/thalamic nuclei; this is made up of epithalamus which is composed of several small nuclei and the pineal gland, the dorsal thalamus, (the major sensory relay area to and from the cerebral cortex), the hypothalamus (maintains homeostasis) and the ventral thalamus that functions primarily with the basal ganglia. The hypothalamus is responsible for regulating the vital functions (body temperature, circadian rythms, blood pressure and glucose level of humans) and feelings of thirst, hunger, anger, aggression and lust. The hypothalamus governs sexual, reproductive development and behavior as well as differentiates men from women, caused by early hormonal influences during nervous system development. The thalamus is the gateway to the forebrain and all incoming sensory information except smell sense (has a direcrct route to cortex) go through it before reaching the cortex.

Brain stem; it is a collective name for the mid brain, pons, and medulla oblongata. It is the caudal continuation of the CNS below the forebrain. It is about 1.5cm long and is significantly narrower than the forebrain.

The mid brain consists of the tectum, the tegmentum, the cerebral peduncles, and the associated substantia nigra. The brain stem houses the most basic programs of survival such as breathing, swallowing, vomiting, and irritation. Since the brain stem is also simply the spinal cord continuing up into the head, it performs functions for the head similar to those that the spinal cord performs for the rest of the body.

The **rhombencephalon** or **hind brain** consists of reticular formation, basal ganglia, and cerebellum. Reticular formation is located in the brain stem and contain network of neurons that project up into the cerebral cortex and basal ganglia and affect general arousal. The reticular formation is also involved in inducing and terminating different stages of sleep.

Cerebellum; is a large protuberance connected to the back of the brain stem. Its size and convoluted surface makes it look like a supplementary brain. The cerebellum is extremely important for proper motor function. Damage to the little lobes at the very bottom causes head tilt, balance problems, and a loss of smooth compensation of eye position for movement of the head. Damage to the ridge that runs up its back affect limb coordination . The most obvious role of the cerebellum is in motor learning (Bloom, 1996)

The basal ganglia: the basal ganglia are a system of sub cortical structures crucial for planning and producing movement. They receive input from the entire cerebral cortex and projects to the motor centers of the brain cell and, via the thalamus, back to the cortex's motor planning area. Damage to the basal ganglia can produce symptoms ranging from the tremors and rigidity of Parkinson's disease to the uncontrollable jerky movements of Huntington's disease. The basal ganglia may also have a role in producing non verbal actions and understanding the non verbal behaviours of other people (Lieberman, 2000).

Hippocampus and amygdale: These are the two structures within the forebrain that are essential for memory and emotions respectively.

The hippocampus plays an important role in the storage of new memories, while the amygdala, which is located immediately in front of the hippocampus, serves a vital role in learning to associate things in the world with emotional responses.

Spinal cord: The spinal cord is the connection center for the reflexes as well as the afferent (sensory) and efferent (motor) pathways for most of the body below the head and neck. The spinal cord begins at the brainstem and ends at about the second lumbar vertebra. The sensory, motor, and interneurons are found in specific parts of the spinal cord and nearby structures. Sensory neurons have their cell bodies in the **spinal (dorsal root) ganglion**. Their axons travel through the **dorsal root** into the gray matter of the cord. Within the **gray matter** are interneurons with which the sensory neurons may connect. Also located in the gray matter are the motor neurons whose axons travel out of the cord through the **ventral root**. The **white matter** surrounds the gray matter and contains the spinal tracts which ascend and descend the spinal cord. Surrounding both the spinal cord and the brain are the **meninges**, a three layered covering of connective tissue. The **dura mater** is the tough outer layer beneath which is the **arachnoid**. The arachnoid has abundant space within and beneath it (the **subarachnoid space**) which contains cerebrospinal fluid, as does the space beneath the dura mater (**subdural space**). The cerebrospinal fluid supplies buoyancy and provide cushioning effect for the spinal cord and brain. The **pia mater** is a very thin layer which adheres tightly to the surface of the brain and spinal cord. It follows all contours and fissures (sulci) of the brain and cord.The spinal cord is a rope of neural tissue that runs inside the hollows of the vertebrae, from just above the pelvis up into the base of the skull. In the normal adult the spinal cord ends between lumbar vertebrae L1 and L2. A damage to the spinal cord at the fourth cervical vertebra (C4) result in quadriplegia, a total loss of motor and sensory functions from the level of the superior surface of the shoulders and below (Brodal, 1992).

The spinal cord is organized in segments. There are 8 cervical, 12 thoracic, 5 lumbar, 5 sacral segments and 1 coccygeal segment. This arrangement is reflected in the dermatomes of the body (Richard *et al.*, 1997) such that sensory nerves from the fourth and twelfth thoracic vertebrae (T4 and T12) subserve a narrow band of skin at the level of the nipples and umbilicus, respectively.

2.1.2 Peripheral Nervous System

The peripheral nervous system is composed of 31 pairs of spinal nerves and 12 pairs of cranial nerves. The peripheral nervous system can be divided into a motor nervous system and an autonomic nervous system. The motor axons originate from neurons in the ventral horn, pass through the ventral root into the spinal nerve and terminate in skeletal (somatic) muscle, cardiac muscle, smooth (visceral) muscle or glands (Brodal, 1992; Richard *et al.*, 1997). The spinal cord receives (consciously perceived) sensory information from exteroceptors, proprioceptors, and interoceptors which travel from sensory organs through the spinal nerve and dorsal root before synapsing in the dorsal horn of the cord. The exteroceptors transmit sensations of pain, touch, and temperature while the proprioceptors are responsible for muscle, joint, and tendon perceptions. These receptors are transducers that change sensory modalities into action potentials, which are generated by the receptive neurons.The parasympathetic nervous system composed of cranio-sacral outflow, that is , the fibres originate in the cranial nerves and from the sacral regions of the spinal cord (Brodal, 1992).The cranial outflow of the parasympathetic sytem supplies the visceral structures in the head via the oculomotor, facial, and glossopharyngeal nerves, and those in the thorax and upper abdomen are supplied via the vagus nerves.

The sacral outflow supplies the pelvic viscera via the pelvic branches of the second to fourth sacral spinal nerves. It slows the heart, lowers the blood pressure, constricts the pupils, and constricts the air passage. In addition it speeds up the digestion of food and plays an important part in defeacation and micturition. It is the 'emptying mechanism' of the body (Brodal, 1992). The sympathetic nervous system is associated with the spinal nerves in a continuous column from the first thoracic nerve to the third lumbar nerve. Although sympathetic neurons originate within the thoracic portion and part of lumbar potion of the spinal cord, they innervate effector organs throughout the body. The somatic motor neuron projects its axon out of the spinal cord and innervates a skeletal muscle. The sympathetic nervous system is involved in organizing energy mobilization resulting in selective energy expenditure, catabolic function and cardio pulmonary adjustments for intense activity and simultaneous prepare the body for emergency in a “Plateht- or- flight” reaction (Jansen *et al.,* 1995).

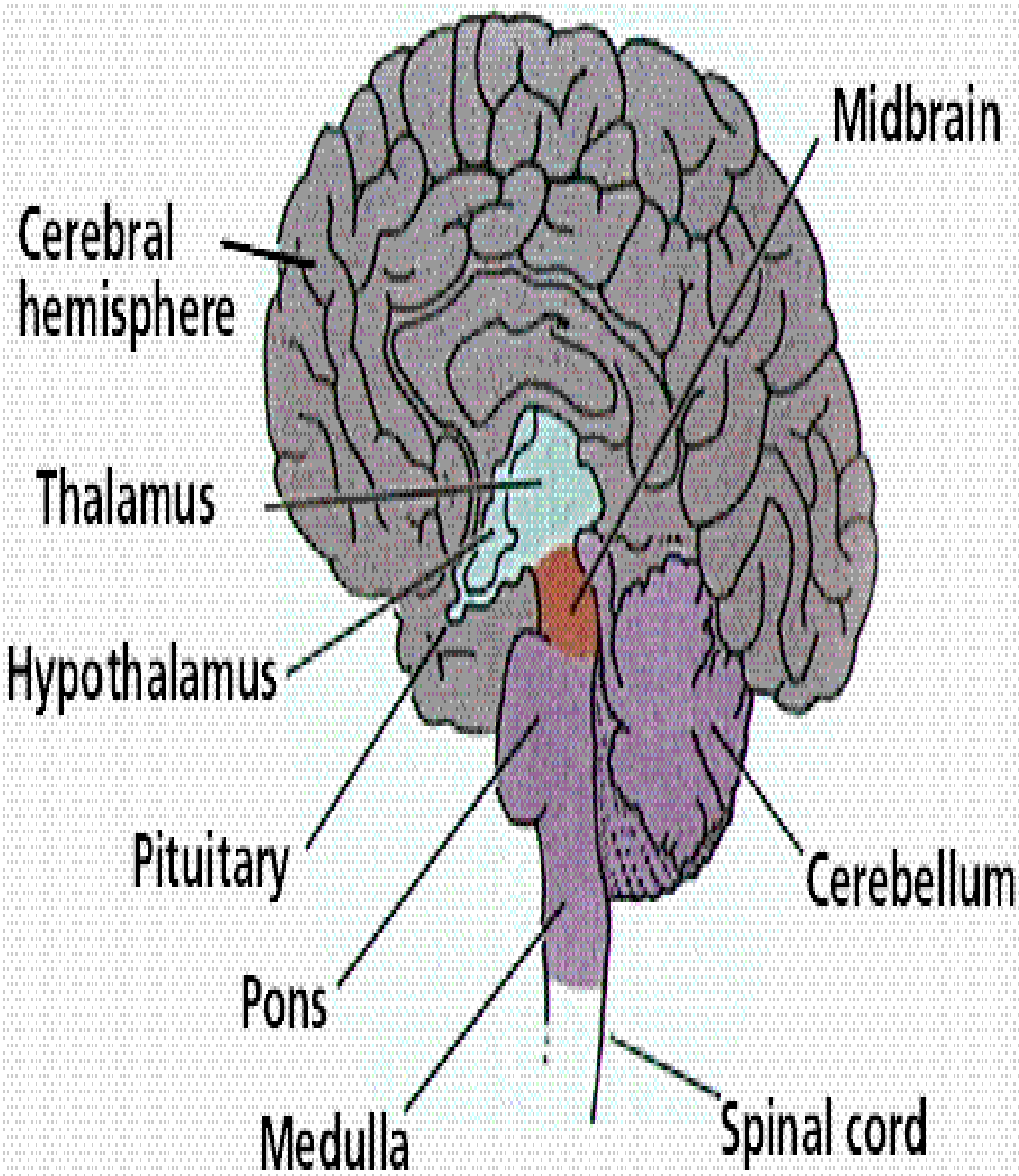

Figure 2.1: Diagram of the human brain

Adapted from Geschwind 1979

2.2 Disorders of the nervous system

The normal functioning of the central nervous system depends on the fine balance between the inhibitory and excitatory neurotransmitters which mediate transmission of impulses to and from the central nervous systemDopamine (DA) is a neurotransmitter in the central and peripheral nervous systems where it regulates numerous physiological processes. Within the central nervous system (CNS), dopamine is known to regulate emotion, reward, cognition, memory, endocrine functions, and motor control. Alterations in dopaminergic transmission are known to be involved in the etiology and/or therapy of a number of neurological and psychiatric disorders, including Parkinson’s disease, Tourette’s syndrome attention-deficit hyperactivity disorder (ADHD), schizophrenia, and substance use. One of the hallmarks of these disorders is that they are all treated with drugs that either enhance or impede dopaminergic transmission.

2.2.1 Parkinson’s disease

Parkinson’s disease is a neurodegenerative disorder of unknown cause. Age is the most consistent risk factor and incidence in the general population above 75 years of age is 254:100 000 (Rajput *et al.,* 1984). With an aging population, the management of Parkinson’s disease is likely to prove an increasingly important and challenging aspect of medical practice. Classically, Parkinson’s disease presents with resting tremor, rigidity, and akinesia often in an asymmetric fashion, but later usually bilateral. However, initial symptoms may be subtle and vague, for example discomfort or mild stiffness in the limbs, and may be misinterpreted. Moreover clinical features are variable with some patients presenting with akinesia and rigidity only and others with a tremor dominant type. The most common differential diagnoses are essential tremor, arteriosclerotic pseudo-parkinsonism, drug induced parkinsonism (Marti-Masso *et al.,* 1996) and the so-called Parkinson plus syndromes namely multiple system atrophy, (Shy and Drager, 1960; Wenning et al., 1994) progressive supranuclear palsy (Lees, 1987; steel et al., 1964) and corticobasal degeneration (Litvan,1997).

Pathophysiology of Parkinson's disease

Dopaminergic projections from the SNc to the striatum (putamen and caudate) synapse on two populations of dopamine receptor mediated efferent neurons (referred to as the direct and indirect pathways), which, in turn, mediate motor activity via a complex neuronal circuit involving the extrapyramidal system. In IPD, the degeneration of the SNc neurons results in reduced activity within these two efferent pathways. The direct pathway involves activation of striatal D1 dopamine receptors (which are coupled to adenylate cyclase) and stimulates the inhibitory γ-aminobutyric acid (GABA)/substance P efferents to the globus pallidus interna (GPi) and substantia nigra pars reticulata. The GPi and substantia nigra pars reticulata efferents are inhibitory to the thalamus (Smith et al., 1998). In IPD, the reduced activation of D1 receptors results in greater inhibition of the thalamus. The indirect pathway involves activation of striatal D2 dopamine receptors (which are coupled to a guanosine triphosphate binding protein that opens potassium channels to hyperpolarize neurons, thereby reducing the excitability of the neuron) (Smith et al.,1998). Activation of striatal D2 dopamine receptors inhibits GABA/enkephalin efferents (medium spiny neurons) to the globus pallidus externa. The globus pallidus externa projects GABA neurons to the subthalamic GPi output are inhibitory on the glutamatergic thalamic projections. In IPD, the reduced activation of D2 receptors translates into greater inhibition of the thalamus. In IPD, restoring activity at the D2 receptor appears to be of more importance than the D1 receptor for mediating clinical improvements. Overall, loss of the presynaptic nigrostriatal dopamine neurons in IPD results in inhibition of thalamic activity and reduced activation of the motor cortex (Schapira et al., 2006). Dopaminergic therapies help to restore motor activity. In addition to dopamine, the synaptic organization of the basal ganglia also involves a variety of other neurotransmitters and neuromodulators, including acetylcholine, adenosine, enkephalins, GABA, glutamate, serotonin, and substance P. The role for drug modulation of other neurotransmitters and receptor types (e.g., adenosine A2A receptors) is currently being investigated

The drugs used in the management of Parkinson's disease include:

L-dopa

Degeneration in the basal ganglia in the brains of Parkinson's disease patients primarily affects dopaminergic neurons in the substantia nigra resulting in dopamine deficiency. Exogenous L-dopa replaces endogenous deficient neurotransmitter. L-dopa is taken up by remaining dopaminergic neurons where it undergoes decarboxylation in the presynaptic terminal to form dopamine. Usually L-dopa is combined with benserazide or carbidopa which do not cross the blood-brain barrier but inhibit the conversion of L-dopa to dopamine peripherally by blocking the enzyme aromatic acid decarboxylase that catalyses this reaction. Dopaminergic adverse effects are thus reduced, central delivery is amplified, and dosage of L-dopa can be reduced (Silva *et al.*, 1997). By giving long acting controlled release preparations, fluctuations in plasma concentration of L-dopa can be reduced (Koller and Pahwa, 1994). L-dopa remains the single most effective drug for the treatment of Parkinson's disease (Lang and Lozano, 1998). For the first five to ten years of L-dopa treatment, most symptoms usually improve, although higher doses may be needed to treat tremor. The drug is tolerated well and side effects, particularly psychiatric symptoms, orthostatic hypotension, and nausea are limited. After several years of a favourable response to L-dopa (the "L-dopa honeymoon") patients often develop disabling motor complications including fluctuations between the "on" and "off" state and dyskinesias (Lesser *et al.,* 1979). This is mainly related to an increasing loss of dopaminergic nerve terminals. L-dopa can no longer be stored in these terminals and patients' symptoms begin to fluctuate according to the plasma concentration of circulating L-dopa. In addition to the development of treatment related complications there is theoretical concern that L-dopa is neurotoxic as it has the potential to form free radicals and other toxic substances as breakdown products when metabolized (Basma *et al.*,1995). These free radicals might injure surviving dopaminergic neurons and thus speed the progression of Parkinson's disease (Fahn, 1996). However, in vivo toxicity of L-dopa to neurons in the substantia nigra has not been demonstrated (Quinn *et al.*, 1986) and the emphasis on the toxic potential of L-dopa has been criticized. Nevertheless, it appears prudent to delay treatment with L-dopa, provided adequate improvement of parkinsonian symptoms is achieved with other drugs. Alternatives to L-dopa are dopamine agonists, amantadine, anticholinergic drugs, and selegeline.

Dopamine Agonists

Drugs belonging to this class act directly on dopamine receptors, mimicking the endogenous neurotransmitter.They can be classified into ergot derivates (bromocriptine, pergolide, lisuride, and cabergoline) and the non-ergolines (apomorphine, pramipexole, and ropinirole). There are several theoretical advantages of dopamine agonists over L-dopa. Firstly, they usually have a long duration of action that more closely mimics the physiological release of dopamine from normal nigral neurons and may help to prevent or reduce motor fluctuations (Chase *et al.*, 1996). However, half life varies between agonists as well as between individual patients (Goetz *et al.*, 1985). Secondly, they can have a L-dopa sparing effect. Thirdly, due to sparing of L-dopa and stimulation of presynaptic auto receptors resulting in a decrease of dopamine turnover including potentially toxic metabolisms, they may also be neuroprotective. In addition, dopamine agonists are not metabolized by oxidative pathways and do not produce free radical metabolites (Mena *et al.,* 1992). They may also have direct anti-oxidative effects (Yoshikawa *et al.,* 1994). They are commonly used as adjunctive therapy to L-dopa after motor complications have developed (Nohria and Partiot, 1997) but may also be considered as monotherapy before starting L-dopa, particularly in younger patients (Oertel et al., 1996). Patients on dopamine agonist monotherapy patients often do not show motor fluctuations until L-dopa is added to the regimen (Hely *et al.,* 1994). They usually take longer than L-dopa to reach effective doses and require supplementary L-dopa for relief of symptoms after a varying period of time (Lang and Lozano, 1998). A common side effect of dopamine agonists is nausea due to stimulation of the area postrema in the medulla, a region that is outside the blood-brain barrier. The peripherally acting dopamine antagonist domperidone can alleviate this symptom without worsening parkinsonian symptoms. Psychiatric side effects such as hallucinations are similar to those caused by L-dopa (Hely *et al.,* 1994).

Most long term studies assessing dopamine agonist monotherapy in previously untreated Parkinson patients have investigated bromocriptine. About one third of these patients showed good response and some may not require L-dopa for 2–5 years (Lang and Lozano, 1998). A study by Ogawa *et al.,* (1997) supported the usefulness of bromocriptine monotherapy at an early stage of Parkinson’s disease with adjunctive L-dopa when necessary. Pergolide, which stimulates both D_1 and D_2 receptors unlike bromocriptine which only stimulates D_2- receptor has been demonstrated to be beneficial in Parkinson’s disease. Newer dopamine agonists pramipexole (Guttman,1997), ropinirole, (Adler *et al.,*1997) and cabergoline (Rinne *et al.,* 1997) were demonstrated to have some benefit in previously untreated patients but it is not yet known whether they have better long term efficacy with fewer complications than bromocriptine or pergolide. An electrophysiological study has shown that pramipexole binds selectively and with high affinity to dopamine D2 and D3 receptors and has a greater efficacy for stimulating dopamine receptors than cabergoline dopamine agonist (Piercey *et al.*, 1996). It appears to be safe, well tolerated, and effective as an add-on therapy in advanced Parkinson's disease with treatment complications such as motor fluctuations (Guttman, 1997).

Apomorphine

The use of apomorphine in Parkinson's disease was first reported by Schwab *et al.*, (1951) who noticed improvement in tremor and rigidity. It was later shown that oral apomorphine reduced frequency of attacks but treatment was limited by nausea, vomiting, postural hypotension, and sedation (Cotzias *et al.*, 1970). However, given subcutaneously by repeated injections it is a well tolerated treatment (Ostergaard *et al.*,1995). Rapid subcutaneous absorption response to a bolus occurs after 10–15minutes and the effect lasts for 20–60 minutes. Infusion pumps are generally well tolerated but widespread application is limited by the complexity of the technique. Alternatively, rectal, intranasal, or sublingual preparations have proved effective (Kapoor *et al.*,1990). Side effects are postural hypotension, cognitive impairment, and disabling dyskinesias during "on" phases. When infusion pumps are used, particular attention to the subcutaneous infusion site is needed, as allergic reactions, aseptic necrosis, or infection may occur.

Monoamine Oxidase B Inhibitors

Selegeline is an example of this class of drug. It selectively and irreversibly inhibits intracellular and extracellular monoamine oxidase B (MAO B) and therefore reduces or delays the breakdown of dopamine to dihydroxyphenylacetic acid (DOPAC) and hydrogen peroxide. The latter has been implicated in oxidative damage in dopaminergic neurons in the substantia nigra. It also inhibits reuptake of dopamine from the synaptic cleft. Adding selegeline to L-dopa may allow a reduction of the L-dopa dose of 10%–15%, occasionally up to 30% (Oertel and, Quinn, 1996). Mild L-dopa response fluctuations can often be reduced by adding selegeline. Monotherapy in de novo patients' delays the need for additional treatment by approximately a year. Side effects of L-dopa, especially dyskinesias and psychiatric problems, are enhanced by selegeline. Orthostatic hypotension may also occur (Churchyard *et al.,* 1997)

Amantadine

This antiviral agent has been used in Parkinson's disease for almost 30 years and several possible mechanisms of action have been advocated. It may increase dopamine synthesis, it may be a dopamine and noradrenaline presynaptic re-uptake blocker, and it also has a mild anticholinergic action (Bayley and stone, 1975). Amantadine influences predominantly akinesia and rigor but has also a mild effect on rest tremor. Two thirds of parkinsonian patients show an improvement on amantadine monotherapy. The beneficial effects of amantadine may be short lived. Kornhuber *et al.,* (1991) proposed that therapeutic benefits of amantadine in parkinson's didease is mediated via antagonism of excitatory neurotransmitter glutamate at the NMDA –receptors.

This might have implications for the treatment of dyskinesias as functional overactivity of glutaminergic projections to the basal ganglia has been shown to be of importance in the development of dyskinesias. Cautious use of amantadine is recommended in renal failure as it is excreted in the urine. Side effects include hallucinations, insomnia, nightmares, and ankle oedema.

Anticholinergics

Anticholinergic drugs improve tremor and stiffness to a greater degree than akinesia and are overall mildly effective (Duvoisin, 1967). Due to a peripheral parasympathomimetic action, side effects such as glaucoma, dryness of the mouth, blurred vision, urinary retention, and constipation can occur. Anticholinergics have a relatively high potential for causing or worsening confusional states and impairing concentration (Pondal *et al.*, 1996). They should therefore be used very cautiously in the elderly. Currently available drugs in the UK are biperiden, procyclidine, orphenadrine, benzhexol, and benztropine.

Catechol O-methyl transferase (COMT) inhibitors

L-dopa is converted to dopamine by a reaction catalysed by the enzyme aromatic acid decarboxylase which is inhibited by carbidopa and benserazide. Significant peripheral metabolism of L-dopa is also mediated by catechol-omethyltransferase (COMT) which catalyses the O-methylation of L-dopa to 3-O methyldopa. As aromatic acid decarboxylase is inhibited by conventional L-dopa preparations the peripheral metabolism is shunted towards the reactions catalysed by COMT. The addition of a COMT inhibitor as adjunctive therapy to L-dopa plus either carbidopa or benserazide reduces peripheral metabolism of L-dopa, prolongs the plasma half life of L-dopa and increases the amount available in the brain (Kaakkola et al., 1994). Peak concentrations of L-dopa are not altered by adding COMT inhibitors. In the brain, COMT activity catalyses the metabolism of L-dopa to 3-O methyl dopa and DOPAC to homovanillic acid. Therefore COMT inhibition translates into less fluctuation of L-dopa plasma concentrations such that circulating levels remain within the therapeutic range and its effect prolonged. Tolcapone inhibits COMT both peripherally and centrally whereas entacapone acts only peripherally (Kurth *et al.,* 1998). It could be demonstrated that tolcapone reduces “off” time by an average of 40% and increases “on” time by about 25% in fluctuating Parkinson patients. Doses of L-dopa could thus be significantly reduced. The results of a double blind, multicentre, randomised trial of tolcapone carried out by Kurth *et al.*, (1997) showed that, it reduced the wearing-off time and the requirements for L-dopa in patients with fluctuating condition. Rinne et al., (1997) also showed that entacapone reduced patients' daily "off" time by approximately 1.3 hours. Side effects of COMT inhibitors include potentiation of dyskinesias, nausea, orange discoloration of the urine, and sleep disturbances. Chronic tolcapone use can result in diarrhea that can be severe. Elevations of liver enzymes have been associated with tolcapone. Because of two cases of fatal toxic hepatitis, tolcapone was recently withdrawn from the market in Europe. Currently entacapone is currently the only available COMT inhibitor in the UK. No association between the drug and liver toxicity has been shown and elevations of liver enzymes have not been reported. The dosage should be reduced in patients with hepatic impairment in whom the bioavailablility of entacapone is increased. Overall, entacapone appears to be a safe drug. It also did not cause significant changes in biochemical or haematological parameters in clinical studies of up to six months' duration in the report of a 12 month tolerability study carried out by Holm and Spencer, (1999).

2.3.2 Alzheimer's Disease

ALZHEIMER's disease, which is characterized by progressive loss of memory and cognitive function, affects 15 million people worldwide. The incidence increases steadily from 0.5 percent per year at the age of 65 years to nearly 8 percent per year after the age of 85 years (Evans *et al.*, 1989). The prevalence increases from 3 percent at the age of 65 years to 47 percent after the age of 85 years because survival for a decade is common. Mutations in the gene for the amyloid precursor protein and the genes for presenilin (Evans et al., 1989) cause rare, dominantly inherited forms of the disease occurring before the age of 60 years, and the ϵ 4 variant of apolipoprotein E is associated with the sporadic form and some familial forms with onset after the age of 60 years. Definite cases are those confirmed by the postmortem findings of dense plaques containing the β -amyloid peptide and elements of degenerating neurons, neurofibrillary tangles composed of abnormally phosphorylated tau protein, and loss of neurons and synapses in the brain. Degeneration in the basal forebrain profoundly reduces the content of acetylcholine and the activities of choline acetyltransferase (Price *et al.*, 1998). Although other neurotransmitters can be involved, the loss of acetylcholine occurs early and correlates with the impairment of memory. Symptomatic treatment for Alzheimer's disease has focused on augmenting cholinergic neurotransmission (Francis *et al.*, 1999).

Pathophysiology

The classic pathological features in AD include the presence of senile plaques, which develop between neurones in the brain and neurofibrillar y tangles, which develop within neurones. These microscopic changes are believed to be an integral part of the cause, development and course of the illness (Francis *et al.*, 1999). Senile plaques comprising of insoluble β-amyloid polypeptides appear to form as a result of disorders in processing β-amyloid and its precursor protein, amyloid precursor protein (APP). It is believed that inflammation around the plaques destroys neighbouring neurones and leads to the formation of neurofibrillary tangles. Healthy neurones have an internal support structure partly made up of structures called microtubules, which act like tracks, guiding nutrients and molecules from the body of the cell down to the ends of the axons. A special kind of protein called tau makes these microtubules stable. In AD, tau is changed chemically and undergoes phosphorylation. Neurofibrillary tangles consist partly of phosphorylated tau, which then links together to form filaments, the density of which is thought to be directly related to the severity of dementia. The resulting effect of these neurofibrillary tangles is to compromise microtubular function, with the eventual destruction of the neurone. These processes in cholinergic neurones lead to a decline in levels of acetylcholine (ACh) within synapses. This is followed by a drop in acetylcholinesterase (AChE) activity, perhaps in order to compensate for this loss. In response to the reduced AChE activity, it is thought that the activity of another cholinesterase enzyme, butyrylcholinesterase (BuChE), increases and as the disease progresses, it becomes the main metabolising enzyme for acetylcholine, until the neurone is completely destroyed.

Acetylcholinesterase inhibitors (AChEI*)*

The cholinergic hypothesis of AD was put forward following the observation that cognitive deterioration results from a progressive loss of cholinergic neurons and a decrease in levels of acetylcholine in the brain (Francis *et al.*, 1999). Drugs that inhibit the degradation of acetylcholine within the synapse and so enhance cholinergic neurotransmission are the mainstay of treatment for AD. Tacrine was the first AChEI approved for the treatment of mild-to moderate AD over 15 years ago. It is no longer prescribed because it was associated with poor tolerability and risk of hepatotoxicity (Fallow *et al.*, 2008). In the UK, three second-generation AChEIs were approved for the treatment of mild-to-moderate dementia in AD. These are donepezil, rivastigmine and galantamine. These agents differ in their pharmacological actions, particularly in relation to enzyme specificity. Donepezil selectively inhibits AChE, rivastigmine inhibits both AChE and BuChE and galantamine selectively inhibits AChE but also has activity as an allosteric modulator of nicotinic acetylcholine receptors (nAChRs) (Weinstock, 1999). The decrease in the expression and activity of nicotinic acetylcholine receptors which also contributes to the overall reduction in central cholinergic neurotransmission is considered important in the treatment of AD (Lilienfeld, 2002).

Memantine

Memantine is also licensed in the UK for treatment of AD but in moderately severe to severe disease unlike AChEIs. It acts as an antagonist at N-methyl-D-aspartate receptors, an action believed to be neuroprotective and disease-modifying (Danysz *et al.,* 2000). Excitotoxicity occurs when there is excessive exposure to the neurotransmitter glutamate or overstimulation of glutamate receptors, resulting in injury or death of neurones. This neuronal death is partly mediated by overactivation of N-methyl-D-aspartate (NMDA)-type glutamate receptors resulting in an excessive influx of calcium ions (Ca^{2+}) through the receptor's associated ion channel. However, because physiological NMDA receptor activity is essential for normal neuronal function, the total block of NMDA receptor activity would be clinically unacceptable. Fortunately adamantine, a derivative of memantine selectively blocks excessive NMDA receptor activity without disrupting normal activity (Lipton, 2005)

2.4 **Epilepsy**

The word 'epilepsy' is derived from the Greek, meaning 'to take hold of, seize'. For centuries, there has been the ancient fear that the sufferer of epilepsy has been possessed, literally taken hold of, by some malign external force. For this reason people with epilepsy have always encountered as much prejudice as those suffering from psychiatric disorders. The lack of self-control evident during a seizure was feared, and the sufferer was therefore spurned. Yet only rarely does epilepsy directly cause psychiatric symptoms: it is predominantly a neurological condition involving disorders of movement or consciousness; moreover, patients are asymptomatic for most of the time. Epilepsy has been defined as a chronic, paroxysmal, non progressive disorder of intermittent disorganized electrical activity in the brain, which causes the seizure. Seizures are characterized most commonly by impairment of motor activity (convulsions), consciousness, perception or behaviour. The term 'fit' is now avoided, as is 'epileptic' in reference to a patient, for whom the term 'person with epilepsy' (PWE) is preferred. Epilepsy may also be defined as a permanent, recurrent seizure disorder. The terms **convulsion** and seizure are often used interchangeably and basically have the same meaning. A **seizure** may be defined as a periodic attack of disturbed cerebral function. A seizure may also be described as an abnormal disturbance in the electrical activity in one or more areas of the brain. Seizure disorders are generally categorized as idiopathic or acquired. Idiopathic seizures have no known cause; acquired seizure disorders have a known cause, including high fever, electrolyte imbalances, uremia, hypoglycemia, hypoxia, brain tumors, and some drug withdrawal reactions. Once the cause is removed (if it can be removed), the seizures theoretically cease.

2.4.1 **Epidemiology of epilepsy**

Epilepsy is the most common non- infectious neurologic disease in developing African countries, including Nigeria (Senanayake and Roman, 1993, Osuntokun, 1978) and remains a major medical and social problem (Senanayake and Roman, 1993). At a conservative estimate, 50 million people worldwide have epilepsy with an annual incidence of 20 to 70 cases per 100,000 (Shavon, 1999) and the point prevalence of 0.4 to 0.8 percent (Hauser *et al.,* 1991). The incidences are highest in childhood, plateaus from the age of 15 to 65years, and rose again among the elderly (Shovon, 1990; Hauser *et al.,* 1991).

The prevalence of epilepsy is particularly high in developing countries especially in Latin America and several African countries, notably Liberia, Nigeria and United Republic of Tanzania (Sananayake and Roman, 1993) compare with the prevalence rates of between 4 and 6 per 1000 which have been reported among caucasians (Hauser *et al.,* 1991). In Nigeria, its prevalence based on defined communites, varies from 15 to 37 per 1000 (Reynolds, 1988). Osuntokun *et al* (1987) reported a prevalence rate of 5.3 per 1000 among the inhabitants of Igbo-Ora, a community with the highest age specific prevalence ratio occuring below the age of 20 years. In another community- based study, Longe and Osuntokun (1989) reported a prevalence of 6.2 per 1000 among the residents of Udo, a rural community of Edo- speaking people in Nigeria.The age distribution in Nigerians appears similar to that described among the Caucasians, between 75% and 85% of epileptic people have onset of seizures below 30 years of age (Osuntokun, 1978). Osuntokun *et al.,* (1974) noted that the onset in 68% of patients was in the first and second decades of life while it was 82% in children over 5years of age (Obembe and Ahmed, 1988). Ogunniyi *et al.,* (1998) reported a mean age of 21years with dominance of partial sezures (53.3%) among adult Nigerians.

Most patients suffering from epilepsy in African countries prefer anonymity and are reluctant to disclose their condition because of the stigma attached to the disease (Osuntokun, 1978). This factor affects the prevalence rates; hence there is likelihood that most of the reported prevalence rates represent a ‘tip of the iceberg’ as the chances of under reporting are high.

Epilepsy tends to be more common in the lower socio-economic groups (Danesi *et al.*, 1981). Showmansky and Glaser (1979) found higher incidence of epilepsy among the poor blacks in United States of America. Several studies in Africa also confirmed prevalence of epilepsy among rural dwellers (Osuntokun, 1978; Watts, 1992). These epidemiological findings are of importance as they showed that, part of the burden of epilepsy can be solved by preventive methods to improve social health because of risk factors associated with increase in prevalence of epilepsy. In a case control study in which 155 Nigerian epileptic patients where compared with age and sex matched controls, febrile convulsions and head trauma were significant risk factors for epilepsy, while childhood immunization was significantly associated with decreased prevalence (Ogunniyi *et al.*, 1987). Other studies have confirmed the importance of birth asphyxia, intracranial infection, hereditary factors and head injuries as important risk factors (Danesi, 1985; Senanayake and Roman, 1993; Osuntokun, 1978). Many of these factors are preventable or modifiable and the introduction of appropriate measures to achieve this may lead to a substantial decrease in the incidence of epilepsy in developing countries.

2.4.2 Pathophysiology of epilepsy

Seizure activity is characterized by paroxysmal discharges occurring synchronously in a large population of cortical neurons. This is characterized on the EEG as a sharp wave or “spike”. The basic physiology of a seizure is traceable to an unstable cell membrane or its surrounding supportive cells.

The Seizure originates from the gray matter of any cortical or perhaps sub- cortical areas. Initially, a small number of neurons fire abnormally. Normal membrane conductance and inhibitory synaptic currents breakdown, and excess excitability spreads, either locally to produce a focal seizure or more widely and produce a generalized seizure (Delorenzo *et al.*, 1992). The clinical manifestation depends on the site of the focus, the degree of irritability of the surrounding of the brain and the intensity of the impulse. An abnormality of potassium conductance, a defect in voltage sensitive ion channels or a deficiency in the membrane ATPase linked to ion transport may result in neuronal membrane instability or a seizure. Selected neurotransmitters (e.g. glutamate, aspartate, acetylcholine, norepinephrine, histamine corticotropin releasing factor, purines, peptides, cytokines and steriod hormones) enhance the excitability and propagation of neuronal activity propagation. A relative deficiency of inhibitory neurotransmitters such as glutamate would promote abnormal neuronal activity. Normal neuronal activity also depends on an adequate supply of glucose, oxygen, sodium, Potassium chloride, and amino acids. Systemic P^H may also precipitate seizures. The different kinds of epilepsies probably arise from different neurophysiologic abnormalities.

2.4.3 **Causes of epilepsy**

The known causes of epilepsy are as follow:

Genetic factors: This increases the risk of idiopathic epilepsy (epilepsy of unknown cause)

Antenatal and birth factors: Neonatal asphyxia, birth trauma and congenital abnormalities can lead to brain damage which gives rise to epileptiform disorders.

Infection: Infection of the nervous system (e.g. meningitis, brain abscess, virus encephalitis) may be accompanied by epilepsy, or epilepsy may be a later consequence of brain damage caused by the infection. The damage may be localized as is usually the case with brain abscess, or it may be diffuse as can arise from virus encephalitis.

Cerebral injury: An accident involving head injury is one of the primary causes of epilepsy, especially if the injury involves a depressed fracture or haematoma.

Metabolic and nutritional disorders: There is a strong connection between metabolic disorders and epileptic fits, although the mechanisms involved are poorly understood. Conditions such as electrolyte and water imbalance, hypoglycaemia, phenylketonuria, porphyria, lipid storage disease and pyridoxine (vitamin B_6) deficiency may trigger attacks.Glutamate decarboxylase, the enzyme that catalyses the formation of gamma amino butyric acid (GABA) from glutamic acid, uses pyridoxal phosphate as a coenzyme.Thus pyridoxine deficiency is accompanied by impaired synthesis of GABA and a decrease in its brain content.This may explain the precipitation of epilepsy by pyridoxine deficiency.

Circulatory disturbance, neoplasm and degenerative ageing disease: These factors may produce cerebral damage that results in epilepsy of late onset in older age group.

Toxic factors: Epileptic attacks often occur as a consequence of lead or mercury poisoning. High doses of neuroleptic phenothiazines, tricyclic antdepressants or antihistamines may produce convulsion

2.4.4 **Etiology of epilepsy**

Seizures occur because small numbers of neurons discharge abnormally. Anything that disrupts the normal homeostasis of the neuron and disturbs its stability may trigger abnormal activity and seizure. In the elderly, 67% of seizures are partial in onset. The causes of seizures in elderly maybe multifactorial and include cerebrovascular disease (both ischaemic and haemorrhagic stroke), neurodegenerative disorders, tumor, head trauma, metabolic disorders, and CNS infections (Ramsey, 1991). The incidence of idiopathic epilepsy is higher in children (Cascino, 1993).

Many factors have been shown to precipitate seizures in susceptible individuals (Hauser, 1990). Hyperventilation may precipitate seizures. Sleep, sleep deprivation, sensory stimuli and emotional stress may initiate seizures. Hormonal changes occurring around the time of menses, puberty or pregnancy have been associated with the onset of or an increased frequency of seizures. The most clearly established risk factors for epilepsy in all age groups are head trauma especially in cases where the *dura mater* has been breached and where there is evidence of loss of consciousness, CNS infection and stroke. Immunizations have not been associated with an increased risk of epilepsy except pertussis with consequent increase in febrile seizures (Ramsey, 1993).

2.4.5 **Classification of epileptic seizures**

The International League Against Epilepsy (ILAE) proposed two major schemes for classification of seizures and epilepsy. These are the International Classification of Epileptic Seizures (Black, 1993) and the International Classification of the Epilepsies and Epileptic Syndromes (Delorenzo *et al.*, 1995). The former classification combines clinical description with electrophysiological (EEG recordings) features of epilepsy. Seizures are thus divided into two main pathophysiologic groups – partial seizures and generalized seizures (Table 1).

Table 2. 1: International classification of epileptic seizures

1. Partial seizures (seizures begin locally)
   - A. Simple (without impairment of consciousness)
     1. with motor symptoms
     2. with special sensory or sematosensory symptoms
     3. with psychic symptoms
   - B. Complex (with impairment of consciousness)
     1. simple partial onset followed by impairment of consciousness- with or without automation
     2. Impaired consciousness at onset- with or without automation
   - C. Secondarily generalized (partial onset evolving into generalized tonic- clonic seizures)

II. Generalized seizures (bilaterally symmetrical and without local onset)

(A) Absence

(B) Myoclonic

(C) Clonic

(D) Tonic

(E) Tonic- clonic

(F) Atonic

(G) Infantile spasms

III. Status Epilepticus (adapted from Delorenzo *et al.*, 1995)

Partial (focal) seizures: These begin in one hemisphere of the brain and unless they become generalized, result in an asymmetrical clinical manifestation. Partial seizures manifest as alterations in motor functions, sensory or sematosensory symptoms. The symptoms (aura) often experienced prior to generalized tonic clonic (GTC) seizures maybe a simple partial seizure that secondarily generalizes. Partial seizures with an alteration of consciousness are described as complex partial. With complex partial seizures, the patient may have automatisms, periods of memory loss, or aberrations of behaviour (Hauser, 1990). Partial epilepsy may begin in infancy and may be difficult to recognise in an elderly population. Patients with complex partial seizures are typically unconscious of these events.

Generalized seizures possess clinical features that indicate involvement of both hemispheres. Motor manifestations are bilateral and there is a loss of consciousness. Generalized seizures may be further subdivided by EEG and clinical manifestations (Delorenzo, 1995) into generalized absence seizures and generalized tonic – clonic seizures.

Generalized absence seizures are manifested by a sudden onset, interruption of ongoing activities, a blank stare and possibly a brief upward rotation of the eyes. They generally occur in young children through adolesence.The EEG during the seizure has a characteristic 2 to 4 cycle/s spike and slow – wave complex (Hauser, 1990).

Generalized tonic – clonic seizures, also known as grand mal. The seizure results in a sudden sharp tonic contraction of muscles followed by a period of rigidity and clonic movements. The patient may fall and be injured. During the seizure the patient may cry or moan, lose splinter control, bite the tongue or develop cyanosis. After the seizure, the patient may be unconscious for a variable period of time and frequently goes into a deep sleep.

Tonic and clonic seizures may occur separately (Delorenzo, 1995). Brief shock like muscular contraction of the face, trunk, and extremities are known as myoclonic jerks.

They may be isolated events or rapidly repetitive. A sudden loss of muscle tone is known as atonic seizure. This may be described as head drop, the dropping of a limb or slumping to the ground (Meldrum, 1990).

The International classification of epilepsies or epilepsy syndromes (Delorenzo *et al.*, 1995), adds components such as age of onset, intellectual development, findings on neurological examination, and results of neuro imaging studies to more fully define epilepsy syndromes.

Status epilepticus

This may be defined as recurrent seizures without an intervening period of consciousness before the next seizure or any seizure lasting longer than 30minutes whether or not consciousness is impaired (Ramsey, 1993; Treiman, 1993). The syndrome most commonly associated with the term status epilepticus is tonic clonic or generalized convulsive status epilepticus (GCSE). Status epilepticus is characterized by repeated primary or secondary generalized seizures that are associated with a persistent post-ictal state. Non convulsive status epilepticus (NCSE) is characterized by a fluctuating or continous “twilight” state that produces altered consciousness (i.e absence status epilepticus or conscious status epilepticus) or repeated simple partial seizures. Simple partial seizures are manifested as focal motor convulsions, focal sensory symptoms or focal impairment of function not associated with altered consciousness (Casino, 1993; Njam *et al.,* 2001).

The exact mechanisms responsible for GCSE are unknown, it appears that there is an activated cascade of changes in an excitatory amino acid neurotransmission, gamma – amino butyric acid (GABA) inhibition, and N - methyl - D- aspartate (NMDA) receptor mediated channel events (Dichter and Ayala, 1987).

It is unlikely that a single mechanism is responsible for SE but multiple interrelated events occur simultaneously at the cellular, brain, and systemic levels.Neurotransmitters released from the presynaptic terminal may cause either an excitatory or inhibitory effect on neuronal discharge. An increase in excitatory (e.g. glutamate and acetylcholine) or a decrease in inhibitory (e.g. GABA) neurotransmitters can cause sustained seizures with subsequent neuronal death (Lipton and Rosenberg, 1994).

The ionic events that occur during SE are associated with opening of ion channels coupled with excitatory amino acid receptors. During GCSE, glutamate activation of the NMDA receptor causes processes that normally suppress the NMDA receptor – coupled channels to be overcome, resulting in opening of the gated calcium channels. This causes cell depolarization and calcium entry, which further depolarizes the cell. Sustained depolarization may not only sustain GCSE but may eventually cause normal death. However, because the NMDA receptor antagonists are no more effective than traditional anticonvulsants, it is unlikely that glutamate is the sole mechanism for GCSE (Berthram and Lothman, 1990).

2.4.6 **Treatment of epilepsy**

The treatment of epilepsy is (or arguably should be) inseparably bound to the underlying cause. In the majority of cases this is still unknown and are labelled as ‘idiopathic’, however there are a significant proportion (up to a third) that are due to identifiable precipitants. Common known causes include cerebrovascular, ischaemic or haemorrhagic events, trauma, malignancy, infections, neurodegenerative diseases or toxins (Banerjee and Hauser, 2008). It is important to identify these cases as they may not fulfill the diagnostic criteria of epilepsy and there may be specific and sometimes curative treatments that will often differ markedly from the idiopathic varieties.

2.4.6.1 *Therapeutic management of epilepsy*

The therapeutic goal in epilepsy treatment is complete seizure control without excessive side effects. The prognosis depends in part upon the type of seizure disorder, but overall, only about 40 to 60% of patients become totally seizure free with available drugs.These agents are

Chemically and pharmacologically diverse, having in common only their ability to inhibit seizure activity without impairing consciousness (Berg, 2008).The choice of drug or drugs used depends on seizure classification, since a particular drug may be more or less specific for a particular type of seizure; patients having a mixture of seizure types present particular therapeutic difficulties. It is not always clear when to treat with one drug (monotherapy) or more than one drug (polytherapy) in a particular patient (French and Pedley, 2008). Approximately 25% of patients given a single anticonvulsive agent do not achieve successful seizure control because of an unacceptable level of side effects (Kwan and sander, 2004). Therefore, two or more drugs may be combined in an attempt to provide better seizure control.

Anti epileptic drugs

Anticonvulsant drugs may be divided into four classes, based on their mechanism of action. Although it may be premature to assign a mechanism of action to some of these compounds, the proposed classes are a convenient way to group the drugs.

Sodium channel bocking agents

Drugs sharing this mechanism include phenytoin (*Dilantin*), carbamazepine (*Tegretol*), oxcarbazepine (*Trileptal*), topiramate (*Topamax*), valproic acid (*Depakene*), zonisamide

(*Zonegran*), and lamotrigine (*Lamictal*). All of these agents have the capacity to block sustained high-frequency repetitive firing (SRF) of action potentials. This is accomplished by reducing the amplitude of sodium-dependent action potentials through an enhancement of steady-state inactivation (Duncan *et al.*, 2006).

The sodium channel exists in three main conformations: a resting (R) or activatable state, an open (0) or conducting state, and an inactive (I) state. The anticonvulsant drugs bind preferentially to the inactive form of the channel. Because it takes time for the bound drug to dissociate from the inactive channel, there is time dependence to the block. Since the fraction of inactive channels is increased by membrane depolarization as well as by repetitive firing, the binding to the in active (I) state by antiepileptic drugs can produce voltage-, use-, and time dependent block of sodium-dependent action potentials(French and Pedley,2008). These agents differ primarily in their pharmacokinetic properties, their adverse reactions, and their interactions with other drugs.

Phenytoin

Phenytoin is a valuable agent for the treatment of generalized tonic–clonic seizures and for the treatment of seizures with complex symptoms (Sander, 2004). The approval of phenytoin for management of epilepsy revolutionized the search for new anticonvulsant drugs as well as immediately improving the day-to-day functioning of epileptic patients.

Adverse effect

Adverse effects of phenytoin include nystagmus, ataxia, vertigo, and diplopia (cerebellovestibular dysfunction), skin rashes, usually morbilliform in character, exfoliative dermatitis or toxic epidermal necrolysis (Lyellís syndrome), blood dyscrasias and hepatic necrosis. It causes gingival hyperplasia, or overgrowth of the gums in children receiving long-term therapy is (occurs in up to 50% of patients). Hirsutism also is an annoying side effect of phenytoin, particularly in young females. Thickening of subcutaneous tissue, coarsening of facial features, and enlargement of lips and nose (hydantoin facies) are often seen in patients receiving long-term phenytoin therapy. Peripheral neuropathy and chronic cerebellar degeneration have been reported, but they are rare. There is evidence that phenytoin is teratogenic in humans, but the mechanism is not clear (Marson *et al.*, 2007). However, it is known that phenytoin can produce a folate deficiency, and folate deficiency is associated with teratogenesis.

Carbamazepine

Carbamazepine is an effective agent for the treatment of partial seizures and generalized tonic–clonic seizures; its use is contraindicated in absence epilepsy. Carbamazepine is also useful in the treatment of trigeminal neuralgia and is an effective agent for the treatment of bipolar disorders.

Adverse effects

Carbamazepine administration is associated with drowsiness, nausea, headache, dizziness, in coordination, vertigo, and diplopia like most of the agents that block sodium channels,. These effects occur particularly when the drug is first taken, but tolerance often develops over a few weeks. There appears to be little risk of cognitive impairment with carbamazepine. It also causes a variety of rashes and other allergic reactions including fever, and lymphadenopathy, but the incidence of serious hypersensitivity reactions is rare. Systemic lupus erythematosus can occur, but discontinuation of the drug leads to eventual disappearance of the symptoms.

Oxcarbazepine

Oxcarbazepine is chemically and pharmacologically closely related to carbamazepine, but it has much less capacity to induce drug-metabolizing enzymes. This property decreases the problems associated with drug interactions when oxcarbazepine is used in combination with other drugs. The clinical uses and adverse effect profile of oxcarbazepine appear to be similar to those of carbamazepine.

Lamotrigine

Lamotrigine is effective in generalized and partial epilepsies. Its primary mechanism of action appears to be blockage of voltage dependent sodium channels, however its effectiveness against absence seizures indicates that additional mechanisms may be involved.

Adverse effect

Skin rashes appear to be the major concern with lamotrigine use. The incidence of rash is greater in children than in adults. Other adverse effects are similar to those of drugs with the same mechanism of actionand these include cerebellovestibular changes leading to dizziness, diplopia, ataxia, and blurred vision. Disseminated intravascular coagulation has been reported.

Topiramate

Topiramate is most useful in patients with generalized tonic–clonic seizures and those with partial complex seizures. Topiramate causes a higher incidence of CNS related side effects (primarily cognitive slowing and confusion) than other AEDs. It does not appear to cause a significant incidence of rashes or other hypersensitivity reactions; however, a significantly higher incidence of kidney stones has been observed in persons receiving topiramate than in a similar untreated population.

Zonisamide

It is effective in partial complex and generalized tonic–clonic seizures and also appears to be beneficial in certain myoclonic seizures. It has a long half-life (about 60 hours) and requires about 2 weeks to achieve steady-state levels.

Adverse effects

It causes cerebello-vestibular side effects similar to those of most other AEDs sharing its mechanism of action. In addition, it appears to cause an increased incidence of kidney stones.

Valproic Acid (Sodium Valproate)

Although it is marketed as both valproic acid (*Depakene*) and as sodium valproate (*Depakote*), it is the valproate ion that is absorbed from the gastrointestinal tract and is the active form (Perucca, 2002). As with several other AEDs, it is difficult to ascribe a single mechanism of action to valproic acid.

This compound has broad anticonvulsant activity, both in experimental studies and in the therapeutic management of human epilepsy. Valproic acid has been shown to block voltage-dependent sodium channels at therapeutically relevant concentrations. In several experimental studies, valproate caused an increase in brain GABA; the mechanism was unclear (Perucca, 2002). There is evidence that valproate may also inhibit T-calcium channels and that this may be important in its mechanism of action in patients with absence epilepsy.

Adverse effects

The most serious adverse effect associated with valproic acid is fatal hepatic failure. Fatal hepatotoxicity is most likely to occur in children under age 2 years, especially in those with severe seizures who are given multiple anticonvulsant drug therapy. The hepatotoxicity is not dose related and is considered an idiosyncratic reaction; it can occur in individuals in other age groups, and therefore, valproic acid should not be administeredto patients with hepatic disease or significant hepatic dysfunction or to those who are hypersensitive to it. Valproic acid administration has been linked to an increased incidence of neural tube defects in the fetus of mothers who received valproate during the first trimester of pregnancy. Patients taking valproate may develop clotting abnormalities. Valproic acid causes hair loss in about 5% of patients, but this effect is reversible. Transient gastrointestinal effects are common, and some mild behavioral effects have been reported. Metabolic effects, including hyperglycemia, hyperglycinuria, and hyperammonemia, have been reported. An increase in body weight also has been noted. Valproic acid is not a CNS depressant, but its administration may lead to increased depression if it is used in combination with phenobarbital, primidone, benzodiazepines, or other CNS depressant agents.

Drugs That Primarily Enhance the Action GABA

Drugs that act via enhancement of GABA action include Phenobarbital and benzodiazepines, Vigabatrin, tiagabine and Gabapentin.

Benzodiazepines

The primary action of the benzodiazepines as anticonvulsants is to enhance inhibition through their interaction with the GABAA receptor at the benzodiazepine binding site. However, there appears to be an additional action of benzodiazepines: blocking voltage-dependent sodium channels. This effect is not seen at usual doses but is likely a factor in their use in the treatment of status epilepticus. As AEDs, they have their major usefulness in the treatment of absence, myoclonic, and atonic seizures and in the emergency treatment of status epilepticus.

Adverse effects

Drowsiness occurs readily at therapeutic doses. The other limiting side effect of the benzodiazepines is the rapid development of tolerance to their anticonvulsant effects. Drooling and hyper salivation may be troublesome in children and in infants.

Tiagabine

Tiagabine (*Gabitril*) blocks the reuptake of GABA into neurons and glia, thereby resulting in higher levels of GABA in the synaptic cleft. The ability to increase GABA concentrations is presumed to be involved in the effectiveness of tiagabine in the treatment of seizure disorders (Loscher, 2002). It is primarily used in the treatment of partial complex seizures.

Adverse effects

Dizziness, somnolence, nervousness, nausea, and confusion

Vigabatrin

Vigabatrin (*Sabril*) is a relatively specific irreversible inhibitor of GABA-transaminase (GABA-T), the major enzyme responsible for the metabolism of GABA in the mammalian CNS. As a result of inhibition of GABA-T, there is an increase in the concentration of GABA in the brain and consequently an increase in inhibitory neurotransmission (Brill *et al.,* 2006).

At present, the primary indication for vigabatrin is in the treatment of patients with partial seizures, but it appears to be an effective and generally well tolerated antiepileptic medication for other seizure types as well (Weisler *et al.,* 2007). It should not be used in patients with absence epilepsy or with myoclonic seizures. Vigabatrin is not approved as an AED in the United States, although it is approved in many other countries.

Phenobarbital and Primidone (Mysoline)

Phenobarbital and primidone are quite similar both chemically and pharmacologically, and much of the anticonvulsant activity of primidone may be ascribed to its metabolic conversion to phenobarbital.As would be expected in such a case, the clinical indications for the two compounds are very similar. There is some indication that primidone may be more effective in the treatment of partial seizures with complex symptoms, but the evidence is not compelling. The primary mechanism of action of Phenobarbital is related to its effect of facilitating GABA inhibition. By binding to an allosteric site on the GABA-benzodiazepine receptor, it initiates structural conformation changes that prolonged the opening of the chloride channels resulting in enhancement of GABA’s inhibitory activity (Stafstrom, 2006). At somewhat higher concentrations, phenobarbital can block sodium channels and may block excitatory glutamate responses. Primidone is metabolized to phenobarbital and phenylethylmalonamide. The latter metabolite has anticonvulsant activity, but most of the anticonvulsant efficacy of primidone is due to the phenobarbital that is produced. At present, phenobarbital and primidone are considered as alternative drugs for the treatment of partial seizures and for generalized tonic–clonic epilepsy.

Adverse effects

Sedation and disturbance in cognitive function particularly in children.

Agents That Block T-Calcium Channels

Ethosuximide

It is now generally accepted that the specific antiepileptic action of ethosuximide (and the older agent trimethadione, no longer employed) against absence epilepsy is its ability to reduce the low-threshold calcium current (LTCC) or T (transient) current.

These currents underlie the 3-Hz spike wave discharges that are characteristic of absence epilepsy. A blockade of T-calcium current is likely also to be a mechanism used by valproic acid. The only clinical use for ethosuximide (*Zarontin*) is in the treatment of absence epilepsy (Gomora *et al.*, 2001). If absence attacks are the only seizure disorder present, ethosuximide alone is effective. If other types of epilepsy are present, ethosuximide can be readily combined with other agents.

Adverse effect

Most of the side effects of ethosuximide are dose related and consist of nausea, gastrointestinal irritation, drowsiness, and anorexia. A variety of blood dyscrasias have been reported, but serious blood disorders are quite rare.

Idiopathic generalized epilepsy

When considering pharmacological actions, a popular first line agent for use in idiopathic generalized adult epilepsy is *sodium valproate*. The benefits of this drug were serendipitously discovered in 1963 when valproic acid was used as a solvent to dissolve various compounds that were being investigated for antiepileptogenic properties in electrical seizure models of mice The exact mode of action has never been fully elucidated but what is known is that it seems to inhibit the action of *succinic semialdehyde dehydrogenase* via reducing *succinic acid* concentration and thus removing inhibition of *L-glutamic acid decarboxylase (GAD).* The GAD is then free to convert *L-glutamic acid* to the inhibitory neurotransmitter *gamma amino butyric acid (GABA)* (Loscher, 2002). Brill *et al.,* (2002) have shown that *sodium valproate* could also act via an increase in neuropeptide Y in the thalamus and temporal lobe and therefore reducing epileptiform oscillations. The concept of antiepileptic agents simply increasing inhibitory factors and reducing paroxysmal epileptiform discharges is almost certainly a gross simplification. Although sodium valproate is useful in a wide variety of epilepsy types including tonic-clonic, myoclonic and absence seizures, the main limitations are its teratogenicity and the wide side effect profile. Dose related effects include tremor, thrombocytopenia and weight gain while idiosyncratic reactions include hair loss and impaired liver function.

2.4.6.2 *The ketogenic diet*

Dietary manipulations have been used to treat epilepsy since ancient times (Temkins, 1994). In the 5th century B.C., Hippocrates reported an epileptic man whose seizures were completely cured by total abstinence from food and drink. Galen felt strongly that diet played a major role in the etiology of epilepsy. He even suggested that all epilepsy beginning in adolescence was due to a "dietary error." In general, Galen favored a healthy, balanced diet, but occasionally recommended an "attenuating diet" for some patients. He once advised the father of a boy with epilepsy to feed him only a very light diet. In biblical times, Jesus reportedly cast demons out of several epileptic children; afterwards, he would inform his disciples that the cure came about by prayer and fasting. The renowned 8th-century physician Avicenna recommended abstinence from certain foods in persons with epilepsy. In more recent times, dietary manipulations became popular early in this century, when several physicians reported temporary remission of seizures during absolute fasting.

The basis of the therapeutic effectiveness of the ketogenic diet is thought to be the ketosis that develops when the brain is relatively deprived of glucose as an energy source and must shift to utilization of ketone bodies as the primary fuel (Owen *et al.*, 1967). Ketone bodies are derived from fat, whereas glucose, derived from carbohydrate, would tend to counteract the effect of ketones. Dietary fats would thus be "ketogenic" while proteins and carbohydrates would be "antiketogenic." Schwartz *et al.,* (1989) and subsequent studies showed that seizure control was optimized with a ketogenic potential ratio of 3 or greater, thus giving rise to the current nomenclature of a 3: 1 or 4: 1 ketogenic diet. Modifications of these "classic" diets have been devised in recent years, and include the medium-chain triglyceride (MCT) diet, substitution of corn oil for MCT oil and a "modified" MCT diet consisting of a mixture of long- and medium chain triglycerides (Schwartz *et al.,* 1989).

Indications for use

Two broad indications for the use of the ketogenic diet have found general acceptance among neurologists and their patients, namely (a) seizures refractory to AED therapy and (b) unacceptable AED toxicity. The diet seems to have the greatest beneficial effects in young children, who produce and utilize ketones more rapidly and whose dietary intake can be more closely supervised. In older children, it is more difficult (though not impossible) to prepare a palatable diet with the appropriate ratio of fat, carbohydrate and protein necessary to produce the desired level of ketosis. Also, older children usually have well-defined dietary likes and dislikes and compliance may become a limiting factor. The ketogenic diet has traditionally been employed for refractory seizures, in rather desperate cases in which no medication seems to work. Whether the diet might be useful in less severe seizure disorders has not been evaluated.

2.4.6.3 *Non-pharmacological management of epilepsy*

Epilepsy Surgery

The classic aim of epilepsy surgery was a complete removal of the epileptogenic focus without neurological deficit (Rosenow and Luders, 2001). This goal fails to consider more complex psychosocial and quality of life issues inherent in most patient encounters. There have been various outcome measures applied to surgical management of epilepsy syndromes. Classically seizure frequency and overall mortality were the markers used, but over time the importance of morbidity and patient quality of life have become more recognised. The efficacy of surgery, like antiepileptic drugs, must take these complex factors into account (Tellez-Zenteno *et al.*, 2007). There are two main divisions of epilepsy surgery; that taken with curative intent and that performed as a palliative procedure.The selection criteria vary between centres but patients are generally considered for surgical intervention when they fulfil the core criteria of:

- Focal seizures with a lesion identifiable on imaging (in most cases) that is amenable to surgical intervention
- Supportive electrophysiological data, often in the form of an scalp or invasive electroencephalogram (EEG)
- Refractory to medical therapy or intolerable side effects
- No contraindications to surgery

2.5 Role of trace elements in health and epilepsy

Trace elements (e.g., zinc, selenium, and copper) are minor building components in tissues including the nervous system. The very complex balance of trace elements is crucial for maintaining human health and overcoming health problems (AMA, 1989). The brain barrier system (the blood-brain and blood cerebrospinal fluid (CSF) barriers) is important for trace element homeostasis in the brain (Takeda, 2001). The concentration of trace elements in cerebral tissue is not equal in all parts of the brain (Donaldson *et al.*, 1974). Trace elements play important functional roles in peripheral and central nervous systems (Westbrook and Mayer, 1987). Zinc, selenium, and copper are indispensable components for certain enzymes responsible for various metabolic processes in different tissues including the brain (Barbeau and Donaldson, 1974). They are important parts of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) as well as transport protein with antioxidant properties, ceruloplasmin (Crl) (a copperbinding protein), and providing protection against damage by peroxidative superoxide radicals.

Through enzymatic or indirect action, they block destructive alteration of lipids, proteins, and nucleic acids by oxygen-derived free radicals, radiation, certain heavy metals, and other toxic substances (McCord and Weswig, 1969). Vitamin E and GSH-Px have similar and complementary physiological roles in protecting cells from damage caused by endogenous peroxides (Rotruck, 1973). Trace elements are also important for the development of the nervous system, myelination of nerve fibers (Prasad, 1979), and neuronal excitability (Westbrook and Mayer, 1987). Alteration of homeostasis by some trace elements in the brain may be involved in the susceptibility, development, and termination of seizures in animal models of genetically determined epilepsy (Hirate, 2002). Theoretically, trace elements may play a role in the production of seizures and their control in humans. The relation between epilepsy and trace elements has however not been convincingly documented in human studies because the data in the literature are conflicting and thus poorly understood (Verrotti *et al.*, 2002).Many studies suggested that the body electrolytes, the level of some trace elements, and the membrane lipid peroxidation due to increase in free radicals or decrease in activities of antioxidant defense mechanisms have been causally involved in some forms of epilepsies and seizure recurrence (Abbott *et al.*,1991). Some antiepileptic drugs (AEDs) have also been shown to alter trace element metabolism and free radical scavenging enzyme activities in humans and experimental animals (Iihan *et al.*, 1999).

Zinc

In the human brain, Zinc (Zn^{2+}) is abundant, having, after iron, the highest concentrations among all transition metals. Most of the brain zinc (approximately 90%) is bound up in metal-protein complexes. Many neurons contain a significant amount of reactive ionic zinc while in the telencephalon, zinc-containing fiber systems form a vast association network that reciprocally interconnects isocortical, allocortical, and limbic structures. Large amounts of chelatable Zn^{2+} are also concentrated in the limbic region, notably in the hippocampus formation (Frederickson, 1989). Approximately 10% of the total zn^{2+} in the brain exists in the synaptic vesicles of what is known as zinc-containing neurons (a subclass of glutaminergic neurons) and is released in a calcium and impulse-dependent manner (Frederickson and Moncrieff, 1994). Because the hippocampal, amygdalar, and perirhinal regions are prominent nodes in this glutaminergic network, it is presumed that vesicular Zn^{2+} is involved in modulation of neuronal excitability and in the synaptic plasticity of developmental and experiential learning (Frederickson and Moncrieff, 1994). Zn^{2+} is a potent modulator of amino acid receptors (especially the NMDA receptor) and co-release of zinc along with glutamate would provide a mechanism for modulating postsynaptic excitability with little or no effect at physiological firing rates, but selectively depressing excitability (by NMDA-receptor depression) when firing rates reach dangerous, paroxysmal levels (Frederickson and Moncrieff,1994). The precise mechanism by which zinc interferes with NMDA function is unknown, but appears to act as a non-competitive antagonist whose major site of action is outside the channel pore in contrast to the action of divalent cations, notably magnesium (Mg^{2+}), which binds within the pore to block ion permeation. In addition, glutaminergic synapses may have differential sensitivities to zinc inhibition and might even be capable of modulating their sensitivity by altering NMDA subunit composition

(Huang, 1997). It has been reported that vesicular zinc enriched regions, for example, the hippocampus, are responsive to dietary zinc deprivation, which also causes brain dysfunction such as learning impairment and olfactory dysfunction (Takeda, 2001). Zinc homeostasis in the brain is closely related to neuronal activity. It has been reported that susceptibility to epileptic seizures, which may decrease vesicular zinc, is also enhanced by zinc deficiency (Takeda *et al.*, 1999). Experimental studies revealed that the concentration of zinc was decreased in the piriform cortex and amygdaloid nuclei complex during convulsions. The decrease in activity of zinc in the brain may be associated with the increase in the susceptibility to seizures in El mouse (a genetically animal model of epilepsy) (Takeda *et al.*, 1999). In contrast, zinc ions were found to inhibit the activity of Na+-K+ ATPase enzyme, which is known to concentrate in the hippocampus (Donaldson, 1971). It was claimed that this situation increased the neuronal excitability and led to seizure (Barbeau and Donaldson, 1974). In addition, Zn^{2+} can inhibit glutamic acid decarboxylase activity, thereby resulting in decreased levels of GABA or an increase in carbonic anhydrase activity (Ebadi *et al.*, 1984). Several lines of evidence point to the possible role of zinc in the pathological changes occurring in the hippocampus in temporal lobe epilepsy, death of hippocampal neurons, and aberrant sprouting of hippocampal mossy fibers so that they form recurrent synapses onto the dentate gyrus granule cells. The distribution of zinc in the brain was altered under certain pathological conditions including epilepsy.

Selenium

Selenium (Se^{2+}) is another essential trace element known to reduce lipid peroxides. The best known biological activity of Se^{2+} is in the seleno-enzyme GSH-Px complex (which in the form of selenocystine) with catalase and SOD is part of the cellular antioxidant defense system against free radical peroxides (Burk *et al.*, 1980; Turan *et al.*, 1991).

Cellular Se-GPX is ubiquitous in both prokaryotes and eukaryotes (Burk *et al.*, 1980). Selenium depletion in the brain amongst patients with epilepsy may constitute an important triggering factor for the origin of intractable seizures and subsequent neuronal damage (Ramaekers *et al.*, 1994). According to Savaskan *et al.* (2003) Se^{2+} deficiency in vivo results in a massive increase in susceptibility to kainate-induced seizures and cell loss. Primates and other animals made Se^{2+}-deficient by dietary restriction develop alopecia, weight loss, epilepsy, and degeneration of the liver, pancreas, and kidney. Patients with systemic Se^{2+} deficiency develop liver disease, depigmented hair, osteoarthropathy, neuro impairments including progressive neuronal degeneration, severe mental retardation, and intractable epilepsy (Muth *et al.*, 1971). In neuronal cell culture, addition of Se^{2+} in the form of selenite within a physiological range protects against excitotoxic insults and even attenuates primary damage. The neuroprotective effect is not mediated via a direct antioxidant effect of selenite but requires de novo protein synthesis. Gel shift analysis demonstrates that the effect is connected to the inhibition of glutamate induced NF-Kappa B and AP-I activation (Savaskan et al., 2003).

Calcium

Alterations in the levels of serum Ca^{2+} have been shown to be responsible for initiation of convulsions (Kiviranta *et al.*, 1996). Neonatal hypocalcaemia and hypomagnesaemia have also been reported to cause convulsions. Serum Ca^{2+} levels were reported to be unaltered in generalized and unspecified seizures (Shah *et al.*, 2001). Rutter et al. (1976) observed normal serum levels in children with febrile convulsion while Hamed et al. (2004) reported insignificant difference in the levels of Ca^{2+} in the untreated group of patients and normal healthy group, but a significant increase in the levels of serum Ca^{2+} in the CBZ- and VPA-treated groups of patients compared to controls ($P<0.05$) and untreated group of patients (P<0.001). They concluded that the increased serum Ca^{2+} levels among the treated group of patients could be a marker for better seizure control by AEDs. A rise in $Ca^{2+/}Mg^{2+}$ ratios compared to control mean value, with unaltered Ca^{2+} serum levels and significantly lowering in Mg^{2+} levels were reported by Shah et al. (2004) , and concluding that the $Ca^{2+/}Mg^{2+}$ ratio is a new concept and may help to judge enhancement of neuronal excitability. Leaver et al. (Leaver *et al.*, 1987) suggested that decline in both Ca^{2+} and Mg^{2+} concentrations results in frequent seizures.

Magnesium

Mg^{2+} is an essential element with a role in neuronal excitability. It inhibits the facilitating effect of calcium (Ca^{2+}) on synaptic transmission, Mg^{2+} alters Ca^{2+} mobilization and may stabilize excitable membranes and also exerts a voltage-dependent blockage of the NMDAreceptor channel. Serum levels of Mg^{2+} were reported by Sha et al., (2001) to be decreased in epileptic patients. A variety of pathological conditions are associated with low serum Mg^{2+} levels in humans. Hypocalcemia and hypokalemia frequently accompany magnesium deficiency in humans. It has been shown previously that low Mg^{2+} level-induced epileptiform activity in rat entorhinal cortex slices changes with time from a pattern of serial seizure-like events (SLEs) to a state of continuously recurring epileptiform activity. Magnesium sulfate was observed to be effective in the prevention and control of eclamptic convulsions in randomized controlled trials performed worldwide by Lewis and Sibai (1997). Steidl *et al*., (1987) stated that various AEDs result in deficiency of red blood cells (RBCs) Mg^{2+} and serum Zn^{2+} while therapy with magnesium lactate produces significantly higher RBC Mg2+ and serum Zn^{2+} with improved clinical EEG and biochemical findings (Steidl *et al*., 1987). Iihan et al., (1999) reported reduced serum level of Mg^{2+} in epileptic patients whether treated or not and regardless of the type of AEDs utilized. Shah et al. (Sha *et* *al.*, 2001) reported a significant decrease in serum Mg^{2+} levels in all seizure groups in comparison to controls, and attributed it to be the hyperexcitability observed as uncontrolled seizures with administration of AEDs. Many studies however reported unchanged levels of Mg^{2+} in patients receiving AEDs (Steidl *et al.*, 1987). Hamed *et al.*, (2004) reported normal levels of serum Mg^{2+} among all groups of epileptics (treated or untreated) and irrespective of the degree of control with AEDs.

Potassium

The deficiency in K^+ is generally correlated to hypomagnesaemia and hypocalcaemia and other disturbances in electrolyte homeostasis. Natelson *et al.*, (1979) and Shah *et al.*, (2001) reported reduced K+ levels in untreated group of epileptics but normal levels in the treated group. The authors concluded that hypokalemia that is observed in untreated epileptics may be expressed as an increase in the ratio of intracellular to extracellular K^+ concentrations, which may result in serious neurological symptoms.

Sodium

In general, no abnormality was noticed in serum Na^+ level in epileptic patients during and after seizure activity (Sha *et al.*, 2001; Natelson *et al.*, 1979). The observed hyponatraemia resulting in grand mal seizures in some cases of epileptic children treated with CBZ was associated with water intoxication, a rare side effect of CBZ (Koivikko and Valikangas, 1983). Shah *et al.*, (2001) reported a rise in Na^+/K^+ ratio as compared to control mean value, with unaltered Na+ level and hypokalemia in patients leading to increase in recurrence of seizures. Natelson *et al.*, (1979) reported unaffected Na^+ and low K^+ levels during seizures while Biochonski, (1976) had reported hypernatremia and hypokalemia in epileptic children.

2.6 Medicinal plants used in management of central nervous disorders

African traditional healers treat mental illnesses and disorders of the central nervous system, using a variety of indigenous plants (Watt and Breyer-Brandwijk, 1962). These include anxiety, fits, convulsions, epilepsy, hysteria, nightmares and mental disturbances. Many authors have documented the effectiveness of traditional medicines in treating these disorders in other parts of the world (Bergman, 1973). The success of traditional medicines is often attributed to the 'placebo effect' rather than through active principles producing predictable physiological responses (Hutchings, 1992). Sramek *et al.,* (2000) showed that the plants used by traditional healers play more active roles than those of placebos or mystical symbols.

2.6.1 Memory, Alzheimer's disease and adaptogenics

Alzheimer's disease is characterised by a progressive impairment of cognitive function including loss of memory and inability to perform basic daily life activities (Francis *et al.,* 1999). Recently, the Amaryllidaceae alkaloid galanthamine has been approved in a number of European countries for the treatment of Alzheimer's disease (Wilkinson and Murray, 2001). Although galanthamine was originally isolated from European amaryllids, it is also found in several African Amaryllidaceae used traditionally (Viladomat *et al.,* 1997). Galanthamine and other acetylcholinesterase enzyme (AChE) inhibitors that are plant based alkaloids alleviate the symptoms of Alzheimer's disease by inhibiting the activity of AChE and hence maintain or elevate the levels of acetylcholine in the brain (Sramek *et al*., 2000). Twenty-two Amaryllidaceae alkaloids of various ring types from *Crinum moorei* Hook.f. (Amaryllidaceae), *Crinum macowanii* bark (Amaryllidaceae), *Crinum bulbispermum* (Burm.) Milne-Redhead and Schweickerdt (Amaryllidaceae) and *Cyrtanthus falcatus* R.A. Dyer (Amaryllidaceae) were screened and found to possess AChE inhibition activity (Elgorashi *et al.,* 2004). Plant adaptogens promote a non-specific increase in the body's resistance to various naturally occurring harmful agents (Wagner *et al*., 1994; Rege *et al*., 1999). Various studies have shown that adaptogenic plants (*Baccharis trimera, Davilla rugosa, phyllantus niruri*) affect the nervous system, improving cognitive functions by slowing down the deterioration of cognitive processes observed in elderly people. The Southern African amaryllid *Brunsvigia grandifolia* Linn, and members of the genus *Crinum*, are used traditionally as such 'tonics' (Hutchings *et al*., 1986). Both contain galanthamine (Viladomat *et al*., 1997). Other traditionally used tonics, *Clausena anisata* and *Catha edulis* (Vahl) Endl. (Celastraceae), are recognized scientifically for their psychotropic activity.

2.6.2 Depression

Depression is a major disease affecting 13–20% of the population worldwide (Licinio and Wong, 1999). Despite availability of drugs with different pharmacological activities for its treatment, depression continues to be a major medical problem. Extracts of *Hypericum perforatum* L. (Hypericaceae), popularly called St. John'sWort, exhibited antidepressant effects in many animal experiments and clinical studies (Linde *et al*., 1996) and are used as such. Related species, indigenous to the region, are yet to be screened for similar activity and chemical composition. The mood elevating properties of *Sceletium tortuosum* (L.) N.E. Br. (Mesembryanthemaceae) has been attributed to mesembrine, an alkaloid with potent selective serotonin (5-HT) re-uptake activity. Selective serotonin (5-HT) re-uptake inhibitors (SSRIs), are a group of drugs used in the treatments in the treatment of depression in orthodox medicine (Van Ameringen *et al*., 2000). They include citalopram, fluoxetine, fluvoxamine, paroxitine and sertraline.

2.6.3 Anticonvulsant activity and epilepsy treatment

Epilepsy is clinically characterized by recurrent episodes of convulsions which may be brief and associated with other stereotyped motor behaviour including disordered perception and impairment or actual loss of consciousness (Pedersen et al., 2008). In Nigeria, a large number of medicinal plants are used in ethnomedicinal management of epileptic seizures. Some of the plants that that have been scientifically screened for efficacy include: *Alstonia boonei, Holarrhena floribunds, Cerebra odellam* (Akah & Nwabie, 1983) and *Samseviera liberica* (Adeyemi *et al.*, 2007). Other plants used traditionally in Nigeria for the management of seizure but have not been scientifically evaluated include; *Brumsvigia grandiflora* (leaves), *Rhus Chirindensiss* (leaves and roots), *Cussonia spicata* (roots), *Grewia bicolor* (leaves), *Tamarindus indica* (roots) and *Crinum zeylanicum* (bulb).

2.6.4 Crinum species in ethnomedicine

Crinum species produce tunicate bulbs which are dormant at ertain times of the year. But ‘‘few plants possess a striking feature as the Amaryllid’’especially when the heavy umbels of lily-like flowers appear. With this apparent disappearance and then reappearance, it is no wonder that the plants entered into folklore. The 130 species of *Crinum* (Verdoorn, 1973) have a pan tropical distribution, with the centre of diversity south of the Sahara (Fangan and Nordal, 1993). They are, therefore, found in regions where there is a long history of traditional plant usage. They were esteemed by herbalists who used them for all manner of illnesses. While the knowledge survived for centuries through apprenticeship to traditional healers, early missionaries, colonial botanists and Victorian plant hunters were among the first to document it (Watt and Breyer-Brandwijk, 1962).

One of the earliest recorded uses of *Crinum* is as a violent emetic. The preparation was made from the mucilage of the bulb and was combined with a bitter gum resin (Le

Maout and Decaisne, 1873). *C. asiaticum* is known throughout Southeast Asia and Polynesia as an emetic and diaphoretic (Ahmad, 1996). According to Roberts (1990), *C. bulbispermum* is a favourite medicinal herb. The southern Sotho people use the leaves and sliced or crushed bulbs to make a strong brew for treating colds, coughs, and as an external application or wash for wounds, scrofula and haemorrhoids. A drawing poultice for abscesses and suppurating sores is also made from the bulbs. These may be roasted by the Zulu and Tswana and applied to aching joints. Rheumatism and backache are treated in a similar way. The Zulus bind the roasted bulb to varicosities using the leaves which, because of their strap-like shape, hold the dressings in place. The flowers are bound over swollen joints and sprains to soothe and help reduce swelling. Several tribes are reported to use the juice squeezed from the base of the leaves to cure ear ache (Watt and Breyer-Brandwijk, 1962). In India, the roasted bulb is used as a rubefacient in the treatment of rheumatism. Sometimes pieces of roasted bulb are placed behind the ear or over the ear to ease the pain. The latter also reported that some tribes make a brew of the leaves which they believe to be an effective treatment for malaria. This same brew is drunk by the Zulu as a treatment for rheumatic fever (usually half a cup chopped leaves in one cup boiling water, strained after standing for 5 min). The Tswana drink a brew of crushed leaf bases and stalks to increase the flow of urine in bladder and kidney infections. The sliced bulb is also warmed and applied over the kidneys to ease discomfort. In Lesotho, *C. bulbispermum* is used as a medicine to increase milk flow. It is also planted outside huts to act as a charm against evil. *Crinum delagoense* bulbs are used in Zulu and Xhosa traditional medicine, to prepare a decoction for treating swellings and urinary tract problems (Hutchings et al., 1986). The starch obtained from the bulbs of *C. flaccidum* is eaten by the Austrialian aborigines as a kind of gruel.

C. giganteum Andr. is a Congo leprosy remedy. In Mexico it is known by the common name Lirio and is used as a ceremonial species during festivities of the Sierra Norte (Leszczynska-Borys, 1995). *C. jagus* is used as a veterinary medicinal plant in Cameroon. In Nigeria, however, it is used to treat open sores and as an ingredient in anticonvulsant preparations (Adesanya *et al.*, 1992). The reason why Crinums are used for medicinal purposes is possibly due to their alkaloidal constituents, which in some instances are common to a variety of species. The alkaloids are classically grouped as the Amaryllidaceae alkaloids because of their limited taxonomic distribution. Waller and Nowacki (1978) report that only the Amaryllidaceae have alkaloids of the norbelladine type and that derivatives of norbelladine-, a biosynthetic intermediate of the majority of Amaryllidaceae alkaloids can be used as taxonomic characters for the determination of plant relationships among the Amaryllidaceae (Waller and Nowacki, 1978). The Amaryllidaceae alkaloids exhibit a range of biological activity, both pharmacological and microbiological (Pham et al., 1998). Among the most noted effects are: analgesic, central nervous system, antitumour, antiviral (Lewis, 1990) and anticholinergic (Pham *et al.*, 1998). This has stimulated further pharmacological screening of the alkaloids (Lewis, 1994) which more recently showed activity against HIV (Pham *et al.,* 1998).

Alkaloids of this family act on the central nervous system (Wildman, 1960). Their structural resemblance to the morphine and codeine skeletons (Ghosal et al., 1985) may account for their analgesic activity, e.g. caranine ($C_{16}H_{17}NO_3$), crinine ($C_{16}H_{17}NO_3$), galanthamine ($C_{17}H_{21}NO_3$) (Wildman, 1960). The mode of action and duration of effect of galanthine ($C_{18}H_{23}NO_4$) effect are, comparable to that of morphine e.g. Narwedine ($C_{17}H_{19}NO_3$) was reported to potentiate the pharmacological effects of caffeine, carbazole, arecoline and nicotine, and narwedine and vittatine ($C_{16}H_{17}NO_3$) the analgesic effect of morphine. Haemanthidine ($C_{17}H_{19}NO_5$) and lycorine ($C_{16}H_{17}NO_4$) are analgesics with activity greater than that of aspirin (Lewis, 1998). The alkaloids of the phenenthridine, lycorenine and pretazettine groups have the advantage over other alkaloidsof being less toxic (Ghosal et al., 1985). Galanthamine ($C_{17}H_{21}NO_3$), one of the more common alkaloids in the Amaryllidaceae family, has been prominent in the popular and scientific press (Lewis, 1996). The alkaloid shows reversible anticholinesterase (Ghosal *et al.,* 1985; Martin, 1987) and muscarinic activity (Lewis, 1996) and is showing promise as a treatment for nervous diseases (Harborne and Baxter, 1993; Greenblatt *et al*., 1999), paralysis syndrome (Lewis, 1999; Greenblatt *et al.,* 1999), schizophrenia and other forms of dementia (Sramek *et al*., 2000) as well as Alzheimer's disease (Greenblatt *et al.,* 1999). Galanthamine acts in a similar manner to other Alzheimer's drugs by replenishing acetylcholine levels in brain areas lacking cholinergic neurones. This it does by binding to the active site of the brain enzyme acetylcholinesterase (Greenblatt *et al.,* 1999). In addition, it stimulates pre- and postsynaptic nicotinic receptors which can, in turn, increase the release of neurotransmitters like acetylcholine and glutamate (Sramek *et al.*, 2000), thus directly stimulating neuronal function. The stimulation of nicotinic receptors is also suggested to protect against β-amyloid toxicity (Sramek et al., 2000). In theory, the release of additional acetylcholine is of benefit to the patient, although its usefulness has yet to be clinically proven (Sramek *et al*., 2000). Its dual mode of action (Greenblatt *et al.,* 1999), coupled with the evidence that galanthamine has few and tolerable side effects, make it a promising candidate for the treatment of Alzheimer's disease (Greenblatt *et al.,* 1999) and designing more effective drugs. However, given that galanthamine, at present, does not offer advantages in efficacy, safety or convenience over related products, it is its stimulation of nicotinic receptors and potential benefits thereof,that offer exciting prospects for further clinical research (Sramek *et al*., 2000). In early clinical trials galanthamine stabilized patients' symptoms for up to one year (Bonner, 1995). It was shown by Sramek *et al.,* (2000) to improve cognitive function with a beneficial effect on behavioural symptoms, provided that treatment is instituted early. Improvement in daily living is also associated with its usage (Sramek *et al.,* 2000). As a consequence, the United States, EU and Switzerland have approved the use of galanthamine (Reminyl®) for the symptomatic treatment of mild-to moderate Alzheimer's disease (Sramek *et al.,* 2000) . Galanthamine also has the ability to amplify the nerve-muscle transfer (Ghosal *et al.,* 1985), by reversing non depolarizing neuromuscular block and restoring synaptic transmission (Martin, 1987). In Eastern Europe, it has long been used as a reversal agent in anaesthetic practice. It inhibits traumatic shock (Martin, 1987) and was patented for use in the treatment of nicotine dependence (Lewis, 1996).

2.6.4.1 *The plant Crinum zeylanicum (Linn.) Amaryllidaceae*

Crinum zeylanicum (Linn.), (Family; Amaryllidaceae) is a bulbous plant, widely distributed from Senegal to western Cameroon and throughout much of tropical Africa. Its leaves are about 75 cm long and 6 cm wide and bear 4-6 white flowers with a broad purple band along the centre. It is mostly found in damp sites of savannah region. The Hausas of Northern Nigeria calls it "Albasaar Kwaadii' (frog's onion), while Yorubas of the Southern part of Nigeria calls it "Isumeri". In Sierra Leone, the leaves cold infusion are used as a stimulant for bathing young children suffering from general debility, rickets etc. The bulb is used in Ghana as a vermifuge. In Western part of Nigeria, the bulb is used externally for skin trouble, injuries and on refractory ulcers (Adesanya *et al.*, 1992). In Southern part of Nigeria the Ibinis use juice obtained from the bulb for management of seizures (Jaiyeoba, personal communication). There is no documented data on the neuropharmacological effects of this plant. Considering its widespread use in traditional medicine, the present study is designed to carry out neuropharmacological evaluation of the crude bulb extract and active fractions of *Crinum zeylanicum*. In addition oral acute and 90-days chronic toxicity studies will be carried out to evaluate the safety.

Plate 2.1 ***Crinum zeylanicum*** plant

Chapter 3.0 **MATERIALS AND METHODS**

3.1 **Plant material**

The whole plant (bulb, leaves and flower) of *Crinum zeylanicum* was collected by Mr. Goodluck Jaiyeoba, a traditional herbal medicine practitioner from Rafin Sayan, a village in Suleja, Niger State of Nigeria. The plant was identified and authenticated by Mrs. Jemilat Ibrahim of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu- Abuja where a voucher sample (NIPRD/H/6258) was prepared and deposited for future references.

3.2 **Chemicals and Drugs**

(a)Methanol (Hopkins and Williams England), ethyl acetate (Hopkins and Williams England), n-Hexane (Hopkins and Williams England)

(b) Acetic acid (Hopkins and Williams England) were supplied in 2.5 L bottles and appropriate dilutions were made with distilled water.

(c) Methanolic extract, solid residue fraction (SRF), pentobarbitone sodium (Sigma chemical Co., USA), pentylenetetrazole (Sigma chemical Co., USA), picrotoxin (Sigma chemical Co., USA), pilocarpine (Sigma chemical Co., USA), Apomorphine (Sigma chemical Co., USA), Chlorpromazine (Sigma chemical Co., USA), strychnine (Sigma chemical Co., USA), Diazepam (Sigma chemical Co., USA), scopolamine (Sigma chemical Co., USA), 4-aminopyridine (Sigma chemical Co., USA), morphine (Sigma chemical Co., USA). Each of these was prepared by dissolving the powder in normal saline prior to administration. Phenytoin (Roche chemicals) was supplied in ampoules and appropriate dilution was made with normal saline. The drug solutions were usually prepared fresh for each day's experiment to maintain stability of the drugs used. The solutions were kept in air tight, amber coloured bottles and stored in the refrigerator until ready for use.

3.2.1 **Route of Drug Administration**

The methanolic extract, the solid residue fraction and piroxicam were administered orally throughout the studies. Pentobarbitone sodium, apomorphine, chlorpromazine, Pentylene tetrazole, picrotoxin, pilocarpine hydrochloride, strychnine, diazepam, 4-aminopyridine and morphine were administered intraperitoneally while phenytoin was administered intramuscularly.

3.3 **Animals**

Wistar albino mice (18 - 20 g) of both sexes and Wistar albino rats of both sexes (180-200) obtained from Animal Facility Centre of National Institute for Pharmaceutical Research and Development, Abuja, Nigeria while one day old chicks obtained from NAPRI, Zaria, Kaduna state, Nigeria were used in the study. The Mice were fed standard laboratory diet (Ladokun feeds Limited Ibadan), given water *ad libitum* and maintained under laboratory conditions of temperature 22 ± 1°C, relative humidity 14 ± 1% and 12 h light and 12 h dark cycle. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985) and NIPRD Standard Operating Procedures for neuropharmacological studies involving whole Animal.

3.4 **Preparation of extract**

The bulb of *Crinum zeylanicum* was crushed and air-dried at room temperature. About 100g of the dry plant material was macerated in 70% methanol for 48 hours. The resulting mixture was filtered using muslin cloth followed by Whatman filter paper (No.

1). The aliquots obtained was dried on water bath at 100 ^{0} Cand stored at -4°C until required for use.The proportion of the yield obtained was 23%.

3.4.1 **Fractionation of Crinum zeylanicum bulb Crude methanolic extract**

Ten grams of the methanolic extract of Crinum zeylanicum was dispersed in 50 ml of distilled water. The resulting colloidal solution was then partitioned with 4× 50ml portion of ethyl acetate. The mixture obtained gave the ethyl acetate soluble portion, the aqueous soluble portion and a solid residue portion. The Solid residue fraction (SRF) was obtained after decanting the ethyl acetate layer and centrifuging the aqueous phase at 2500rpm for 15 minutes. The ethyl acetate soluble fraction (ESF) and the solid residue fraction were air –dried at room temperature while the aqueous soluble fraction (ASF) was freezed dried to prevent from being spoilt byfungal growth (water evaporates slowly). All the fractions were kept at -4^{0} C until required for use.

3.5 **Phytochemical analysis.**

The dark brown crude extract and its fractions, ESF, ASF and SRF were screened phytochemically for the presence of secondary metabolites using standard conventional protocols (Sofowora 1993).

3.5.1 **Test for saponins.**

To a small quantity of each of the powdered samples was added 95% ethanol and boiled.The mixture was filtered and 2.5mls of the filtrate was added to 10mls of distilled water in a test tube. The filtrate was added to 10mls of distilled water in a test tube and was shaken vigorously for about 30mins, and allowed to stand for over 30mins. Honey comb froth indicates the presence of saponins (Sofowora 1993).

3.5.2 **Test for flavonoids (Lead acetate test for Flavonoids).**

Five grams (5 g) of the powdered sample was completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath, the mixture was filtered and the filterate was used (Trease and Evans, 1989). To 5mls of the filterate (detanned water extract) was added 10% lead acetate solution; a colored precipitate indicates the presence of flavonoids.

3.5.3 **Test for glycoside**

The extract was hydrolysed with HCl for few hours on a water bath. To the hydrolysate, 1ml of pyridine was added and a few drops of sodium nitroprusside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

3.5.4 **General test for alkaloids**.

Small quantity of the plant extract was added to each of 5 test tubes. To each of the sampled test tube a few drops of the following reagents were added and shaken.

Mayer's solution (potassium mercuric iodine solution)

Dragendroffs reagent (potassium bismuth iodine solution)

Wagner's (solution of iodine in potassium iodide)

Hager's reagent (saturated solution of picric acid)

The appearance of precipitate or turbid sediment indicates the presence of alkaloids (Trease and Evans 1989)

3.5.5 **Test for tannins**.

Extraction:

An extract was prepared by boiling 3g of the bulb powder in 50 ml of distilled water for 3 minutes on a hot plate. The mixture was filtered and the resulting filtrate was used to carry out the following test for tannins.

Ferric chloride test:

A portion of the water extract was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution was added. A blue or green color indicates the presence of tannins (Trease and Evans 1989).

Lead sub -acetate test:

Few drops of lead sub-acetate solution was added to 1 ml of the water extract and shaken. Production of a brown precipitate indicates the presence of tannins (Trease and Evans, 1989).

3.5.6 **Test for resins.**

Fifteen milliliter (15mls) of petroleum ether extract was made from 0.1g of each of the powdered samples and filtered into a test tube. An equal volume copper acetate solution was added, shaken vigorously, allowed to separate, and appearance of a green color indicates the presence of resins.

Powdered sample (0.5g) was dissolved in acetic anhydride and 1 drop of concentrated sulphuric acid added. A purple or violet color indicates the presence of resins.

3.5.7 **Test for Balsams**

2 drops of alcoholic ferric chloride solution were added to 5 ml of 90% ethanol extract of the powdered bulb. A dark green color indicates the presence of balsams.

3.5.8 **Test for Terpenes and Sterols**

Extraction:

5g of each of the powdered samples was extracted by maceration with 50mls of 95% ethyl acetate, filtered and filtrate was evaporated to dryness. The residues were dissolved in 10mls of anhydrous chloroform and then filtered. The filtrate was divided into two and was used for the following tests.

3.5.8.1 *Liebermann Burchard test*.

One portion of the filterate above was mixed with 1ml acetic anhydride, followed by 1ml concentrated sulphuric acid down the wall of the test tube to form a layer underneath, the formation of a reddish brown solution indicates the presence of terpenes (Sofowora 1993).

3.5.8.2 *Salkowski's test for sterols*.

The second portion of the solution was mixed with 2mls of concentrated sulphuric acid; the formation of a reddish brown color indicates the presence of steroidal rings (Sofowora, 1993).

3.5.9 **Ferric chloride test for phenols**.

2mls of detanned water extract was diluted with distilled water and a few drops of 10% ferric chloride solution were added. A green or blue solution indicates the presence of phenolic nucleus.

3.5.10 **Test for carbohydrates**

Extraction:

The powdered sample (3g) was boiled separately in 50ml of distilled water for 3mins on a hot plate and the mixture was filtered using Whartman filter paper. A few drops of molisch reagent was added to 2ml of the filterate, followed by a small quantity of sulphuric acid and was allowed to form a lower layer a purple ring at the interface indicates the presence of carbohydrates, the test is known as molisch test (Trease and Evans 1989).

3.6 **Elemental analysis**

The elemental analysis of the *Crinum zeylanicum* bulb powder and its methanolic (bulb) extract was carried out in the Department of chemistry of Science and Technology Complex, SHEDA-Abuja, using the atomic Absorption spectrophotometer (AAS) methods described by by Kaneez *et al*., (2001). The bulb material and the methanolic bulb extract were dried in moisture extraction oven for 48 h at a temperature of 65^0 C.

The samples were pulverized into powder and kept in a dessicator. The samples were then digested using the wet-ashing microwave assisted method. Thereafter an Analysengerate GmbH model 200A (Buck scientific, Germany) was used for the determination of metals using as a radiation source cathodeon (England) hollow cathode lamps for Zn, Mn, K, Mg, Ca; Buck scientific hollow cathode lamps for Cu, Na and Pb, and Fischer scientific hollow cathode lamp for Fe. The elements were measured under optimum operating conditions with an air-acetylene flame.

3.7 **Acute toxicity study of crude extract in mice and rat**

The oral acute toxicity of methanolic bulb extract of *Crinum zeylanicum* in mice and rats was evaluated using modified Lorke's method (1983). The study was carried out in two phases. In the first phase, nine mice and nine rats were randomized into three groups of three mice and three rats each and were given 10, 100 and 1000 mg extract/kg body weight orally (via a cannula), respectively. The mice and rats were observed for signs of adverse effects and death for the first four critical hours and thereafter daily for 7 day before the second phase. In the second phase of the study, another set of nine mice and nine rats were randomized into three groups of three mice and three rats each and were given 1600, 2900 and 5000 mg extract/kg body weight, respectively based on the result of the first phase. The experimental animals (mice and rats) were kept under same conditions and observed for toxic signs and mortality for the first critical four hours and thereafter daily for 7 day. A similar experiment was carried out in mice and in rat using intraperitoneal route of drug administration. The number of deaths in each group within 24 h was recorded and the median lethal dose (LD_{50}) values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred)

$LD_{50} = \sqrt{MLD \times MTD}$

Where LD_{50} = median lethal dose

MLD = minimum lethal dose

MTD = maximum tolerated dose

3.7.1 **Acute toxicity study of solid residue fraction in mice**

The oral acute toxicity of solid residue fraction of *Crinum zeylanicum* in mice was evaluated using modified Lorke's method (1983). The study was carried out in two phases. In the first phase, nine mice and nine rats were randomized into three groups of animals and were given 10, 100 and 1000 mg extract/kg body weight orally (via a cannula), respectively. They were observed for signs of adverse effects and death for the first four critical hours and thereafter daily for 7 day before the next phase. In the second phase of the study, another set of nine animals were randomized into three groups of three animals and were given 100, 140 and 225, 370 and 600 mg extract/kg body weight orally, respectively. All the animals were observed for toxic signs and mortality for the first four critical hours and thereafter daily for 7 day. The number of deaths in each group within 24 h was recorded and the LD_{50} values were again calculated as above.

3.8 **Gross behavioural effects of *Crinum zeylanicum* methanolic bulb extract in mice**

This study was carried out according to the method described by Irwin (1968) and modified by Perez-Saad and Buznego (2008). Adult mice of both sexes were randomized into four groups of five mice each. Group I mice received 10 ml distilled water/kg body weight. Mice in groups II, III, IV and V received 50, 100, 250, 500 and

1000 mg extract/kg body weight orally respectively. The mice were placed in separate transparent cages after 1 hour of extract administration and were observed behaviourally for a period of one hour;

A) CNS stimulant effects such as excessive jumping, biting, sniffing, scratching, etc.
B) CNS depressant effects indicated by excessive reduced motor activity reduced startle response and reduced response to manual manipulation.
C) Autonomic effect such as pupillary size, lacrymation, salivation, defeacation and urination were also observed.

3.9 **Pentobarbitone - induced hypnosis in mice**

The method of Roland et al (1991) was used. Adult mice of both sexes were randomized into five groups of six mice each. Group I mice received 10ml distilled water/kg body weight orally while those in groups II, III and IV were given 250, 500 and 1000 mg extract/kg body weight respectively orally. Mice in group V received 2mg diazepam/kg body weight intraperitoneally. One hour after extract and thirty minutes after diazepam administration respectively, 25mg pentobarbitone sodium /kg body weight was administered to each mouse intraperitoneally. Each mouse was placed individually in a transparent cage and then observed for onset and duration of sleep, with the criterion for sleep being loss of right reflex on all four limbs after being gently rolled sideways (Miya et al., 1973). The interval between loss and recovery of righting reflex was used as the index of hypnotic effect (duration of sleep) (Ramirez et al., 1998).

3.10 **Neuro-behavioural studies**

3.10.1 **Studies on spontaneous motor activity**

The study was carried out using the method described by Amos et al., (2001). Adult mice of both sexes were randomized into five groups of six mice each. Groups I mice received 10ml distilled water /kg body weight orally. Groups II, III and IV mice received 250, 500 and 1000 mg extract/kg body weight respectively orally while group V mice received 2mg chlorpromazine/kg body weight intraperitoneally. One hour and thirty minutes after extract and chlorpromazine administration, the mice were transferred individually into Letical activity cages (LE 3806) consisting of four ventilated motor cages connected to a multi-counter. Activities were automatically recorded after a 1-min latency period for 6 min at 30 min intervals for a period of 120 min.

3.10.2 **Test for motor co-ordination** (Rotarod Test)

The study was carried out according to the method described by Perez et al (1998). Rota rod treadmill device (Ugo Basile no. 7680, Italy) was used for this experiment. Mice trained to remain on slowly moving (16rpm) rods of 5cm diameter for 180seconds or longer were selected and randomised into 4 groups of 6 mice each. Group I mice received 10ml distilled water/ kg orally. Groups II, III and IV received 250, 500 and 1000mg extract/kg orally respectively. One hour after administration of extract, the mice were placed singly on the rod for 3 minutes, at 30 minutes intervals for 3 h. If an animal failed more than once to remain on the rod for 3 minutes, the test was considered positive, meaning that there is lack of motor co-ordination.

3.10.3 **Hole-board test for exploratory behavior in mice**

The method described by Ozturk et al (1996) was adopted for this study. A Letica board (LE 3333) of 60CM ×30cm with 16 evenly distributed holes was used. Thirty adult mice of both sexes were randomized into five groups of six mice each. Group 1 received 10 ml distilled water/kg body weight orally. Groups II, III and IV received 250, 500 and 1000 mg extract/kg body weight orally, while the mice in group V received 2 mg diazepam/kg body weight intraperitoneally. One hour after administration of the extract and thirty minutes after diazepam administration, each mouse was placed singly on an automatic Letica board with 16 evenly spaced holes connected to a counter (Letica LE3333). The instrument automatically counted the number of times the mouse dipped its head into the holes during a 5 minutes period.

3.10.4 **Apomorphine –induced stereotype behavioural studies in mice**

The method described by Randrup and Munkvad (1967) was used for the stereotype behavioural studies. Adult mice were randomized into five groups of six mice each. Group I received 10ml normal saline/kg body weight orally. Groups II, III and IV received 250, 500 and 1000mg extract/kg body weight orally respectively. The group V mice were given 2 mg chlorpromazine/kg body weight intraperitoneally. One hour after administration of saline and extract and thirty minutes after chlorpromazine administration, all the mice were given 2 mg apomorphine/kg intraperitoneally. The signs of stereotype behaviour that included circling, jumping, sniffing and general locomotion were recorded for a period of 2 h using a hand held tally counter (Irwin, 1968).

3.11 **Seizure studies**

3.11.1 **Pentylene tetrazole (PTZ) - induced seizures**

The anti-convulsant method described by Mahomed and Ojewole (2006) was used for this study. Adult mice of both sexes were randomised into five groups of seven mice each. Group1 received 10 ml distilled water/kg. Groups II, III and IV received 250, 500 and 1000 mg extract/kg body weight orally respectively while group V mice received 4mg diazepam/kg intraperitoneally. After 1 hour all the mice were given 85mg PTZ/kg body weight intraperitoneally. The mice were then observed for onset and duration of tonic hind limb extension for 30 minutes. The ability of the extract to prevent tonic hind limb extension or prolong its onset was considered as an indication of anticonvulsant activity (Amabeoku et al., 1998).

Following fractionation of the crude extract, each fraction including aqueous soluble fraction (ASF), Ethylacetate soluble fraction (ESF) and solid residue fraction (SRF) were also evaluated separately for anti-seizure activity. For each test, thirty mice were randomized into five groups of six mice each. Group 1 mice received 10ml normal saline/kg body weight orally. Groups II, III and IV mice received 25, 50 and 100 mg of the fraction/kg body weight orally respectively, while group V mice received 4 mg diazepam/kg intraperitoneally. After 1 hour all the mice were given 85mg PTZ/kg body weight intraperitoneally. The mice were then observed for onset and duration of tonic hind limb extension for 30 minutes. The ability of the fraction(s) to prevent tonic hind limb extension or prolong its onset was considered as an indication of anticonvulsant activity.

3.11.2 **Maximal electric shock-induced seizures in chicks**

The method described by Swinyard, (1969) was used for this study. Eighty (day-old) white cockerels were randomly divided into five groups of 10 chicks each. Group I received 5 ml normal saline/kg body weight, chicks in groups II, III and IV received 250, 500 and 1000 mg extract/kg body weight while group V chicks received 25 mg phenytoin/kg body weight respectively. One hour after treatment with the extract, the normal saline and thirty minutes after intramuscular administration of phenytoin, maximal electroshock was administered to induce seizure in the chicks using Ugo Basile electroconvulsive machine (model 7801) connected to Claude 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 1.6 s, 150 pulse/s, 100 mA and 0.8 ms, respectively. The current of 100 mA, produced tonic seizures in 70-90% of the control chicks. Seizures were manifested as tonic hind-limb extension (Swinyard, 1969).The ability of the extract to prevent or prolong the latency or onset of tonic hind-limb extension was taken as an indication of anticonvulsant effect. The chicks were also monitored for occurrence of death up to 24 h after the experiment. The study was repeated with the solid residue fraction at 25, 50 and 100 mg/kg body weight orally respectively.

3.11.3 **Picrotoxin - induced seizures**

The anticonvulsant method described by Salih and Mustafa (2008) was used. Adult mice of both sexes were randomized into five groups of seven mice each. Group I mice received 5 ml normal saline/kg body weight. Mice in groups II, III and IV received 250, 500 and 1000 mg extract/kg body weight respectively, while mice in group V received 4mg diazepam/kg body weight intraperitoneally. One hour after extract treatment and thirty minutes after diazepam administration, 10 mg Picrotoxin/kg body weight was given to all the mice intraperitoneally. They were then observed for hindlimb tonic seizures for thirty minutes. Delay/prolongation of the latency or onset of the hind limb tonic extension was considered as an indication of anticonvulsant activity (Navarro-Ruiz *et al.*, 1995). Similar experiment was carried out using 25, 50 and 100 mg SRF/kg body weight orally respectively.

3.11.4 **Strychnine -induced seizures in mice**

The method described by Adeyemi *et al*., (2007) was used. Adult mice of both sexes were randomized into five groups of seven mice each. Group I received 10ml distilled water/kg body weight orally. Groups II, III and IV received 250, 500 and 1000 mg extract/kg body weight orally while group V received 4mg diazepam/kg body weight intraperitoneally. At 30min and 1h after diazepam and extract administration, all the mice were given 1mg Strychnine/kg body weight intraperitoneally. The animals were observed for 30 minutes for hind-limbs tonic seizures or convulsions. The ability of the extract to prevent tonic hind limb extension or prolong its onset when compared with the normal saline treated group was considered as an indication of anticonvulsant activity. Similar experiment was carried out using 25, 50 and 100 mg SRF/kg body weight orally respectively.

3.11.5 **Pilocarpine- induced seizures in mice**

The method described by Navarro-Ruiz *et al.*, (1995) was used for the study. Adult mice of both sexes were randomised into five groups of seven mice each. Group I received 10 ml distilled water/kg body weight orally. Groups II, III and IV received 250, 500 and 1000 mg extract/kg body weight orally. Group V was given 10 mg phenobarbitone/kg body weight intraperitoneally. Thirty minutes after extract and drug treatment, 2 mg scopolamine/kg body weight was given to all the mice subcutaneously to minimize peripheral manifestation of cholinergic stimulation. One hour after extract treatment and thirty minutes after diazepam administration, 350mg pilocarpine/kg body weight was administered to all the mice. Each mouse was then observed for thirty minutes for tonic-hind limb extension. The ability of the extract to prevent tonic hind limb extension or prolong its onset when compared with the distilled water treated group was considered as an indication of anticonvulsant activity. Mice that did not convulse 30 minutes after Pilocarpine administration were considered protected. Similar experiment was carried out using 25, 50 and 100 mg SRF/kg body weight orally respectively.

3.11.6 **4-Aminopyridine (4-AP) induced seizures in mice**

The method described by Mihaly et al (1990) was adopted for the study. Adult mice were randomized into five groups of seven mice each. The Group I mice were given 10ml distilled water/kg body weight orally, 1h before administration of 5 mg 4-AP/kg body weight. Groups II, III and IV were given 250, 500 and 1000 mg extract/kg body weight respectively orally while group V mice were given 4mg diazepam/kg body weight intraperitoneally. One hour after extract and thirty minutes after diazepam administration, 5mg 4-AP/kg body weight was given to all the mice intraperitoneally. The mice were then observed for tonic hind limb extension for 30 minutes. The ability of the extract to prevent tonic hind limb extension or prolong its onset was considered as an indication of anticonvulsant activity. Mice that did not convulse 30minutes after 4-Aminopyridine administration were considered protected. Similar study was carried out with the solid residue fraction of methanolic bulb extract of *Crinum zeylanicum* at doses 25, 50 and 100 mg/kg body weight orally respectively.

3.12 **Analgesic studies**

3.12.1 **Acetic acid induced writhing in mice**

The modified method of Koster *et al.*, (1959) was used for the study. Adult mice of both sexes were randomized into five groups of six mice each. Group I received 10 ml distilled water /kg body weight. Groups II, III, and IV received 250, 500 and 1000mg extract/kg body weight orally while the mice in group V received 10 mg piroxicam/kg body weight orally. Thirty minutes after treatment, each mouse was given 10ml of 0.7% solution of acetic acid/kg body weight intraperitoneally and then placed in a transparent Perspex observation box. Five minute after administration of acetic acid, the number of writhes for each mouse was counted for 5 minutes and counting was repeated twice at 15 minutes interval, i.e., at 20th and 35th minutes.

The percentage inhibition of writhes for the extract and piroxicam treated groups was calculated as follows:

$$\{(\text{Control mean} - \text{Test mean}) / \text{control mean}\} \times 100$$

3.12.2 **Formalin test in rat**

The study was carried out according to the method described by Tjolsen et al., (1992). Adult Wistar rats were randomized into five groups of 6 rats each. Group I was given 10ml distilled water/kg body weight orally. Groups II, III and IV received 250, 500 and 1000mg extract/kg body weight orally while group V rats received 4mg Morphine/kg body weight intraperitoneally. Thirty minutes after morphine and 1 h after extract treatment, each rat was given 50µl of a 2.5% solution of formalin subcutaneously under the plantar surface of the left hind paw. Each rat was placed in an observation chamber and monitored for pain response for one hour. Severity of pain responses was assessed as:

Score	Observed response
0	rats walked or stood firmly on injected paw.

1 The injected paw was favored or partially elevated.

2 The injected paw was clearly lifted off the floor.

3 The rat licked, chewed, or shook the injected paw.

Rats were placed in such a way as to ensure a non-obstructed view of the injected paw. Anti - nociceptive effect was determined in 2 phases: the early (first) phase of the nociceptive responses which normally peaked at 5 min after formalin injection and the second phase 15-30 min after formalin injection, represented the neurogenic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987).

Percentage reduction in the pain score was calculated as:

{(Control mean-Test mean) /control mean}×100

3.12.3 **Hot plate test in mice**

The 'hot-plate' (thermal) test method used in this study was modified from those described in detail Williamson *et al.,* (1996). A 600 mL glass beaker was placed on a 'Heidolph® MR 2002' hot-plate (with adjustable temperature). The temperature of the hot-plate was then regulated to 55± 1 °C. Each mouse was placed in the glass beaker (on the hot-plate) in order to obtain the animal's response to electrical heat-induced pain (licking of the forepaws and eventually jumping out of the glass beaker). Jumping out of the beaker was taken as an indicator of the animal's response to electrical heat-induced pain. The time taken for each mouse to jump out of the beaker (i.e. reaction time) was noted and recorded in seconds. Each mouse served as its own control. Thus, before treatment, its reaction time was determined thrice at 1 h intervals. The mean of these three determinations constituted the 'initial reaction time' – that is, the reaction time before treatment of the mouse. The mean reaction time of all the mice used was pooled to obtain the final, ‘control’ mean reaction time (*T*b). Each of the test mice was thereafter treated with either distilled water, 250, 500 and 1000 mg extract/kg body weight or 4mg morphine/kg body weight (MPN). At 30, 60, 90 minutes after treatment with distilled water, the extract, or the reference drug (MPN), the reaction time was again evaluated, but only once on each occasion. This value was pooled for the mice used in each treatment and time window, and the final ‘test’ mean value (*T*a) for each treatment group was calculated. This final ‘test’ mean value (*T*a) represented the ‘after-treatment reaction time’ for each group of treated mice. This ‘test’ mean value (*T*a) was subsequently used to determine the percentage thermal pain stimulus relief or protection, by applying the formula:

% protection against thermal pain stimulus = Test mean − Control mean × 100/Control mean

= $Ta - Tb \times 100/Tb$

Graded doses of the extract (250, 500 and 1000 mg/kg body weight) were tested orally, while reference drug, morphine (MPN), was used at a dose of 4 mg/kg i.p. only. Treated ‘control’ mice received 5 mL distilled water /kg orally only.

3.13 **Chronic toxicity study**

Chronic toxicity study was carried out in accordance to WHO (1992) and OECD 407 (1998) guidelines. Ninety six rats separated into 48 males and 48 females were randomized into four groups of 12 rats each. The first group served as control and received 10 ml normal saline/kg body weight orally while the rats in groups 2, 3 and 4 received 250, 500 and 1000 mg extract/kg body weight orally respectively daily for 90 day.

3.13.1 **Clinical symptoms**

The rats were observed daily before, during and after treatment for physical signs of clinical intoxication and mortality.

3.13.2 **Feed Intake**

The amounts of feed consumed by rats in each group were measured daily as the difference between the quantity of feed supplied and the amount remaining after 24 hours respectively.

Daily feed intake (DFI) was expressed as amount of feed consumed by the rats in a group per 100 g body weight.

$$\text{DFI/100g body weight} = \frac{\text{Daily Feed Intake (g)}}{\text{Body weight per group}} \times \text{100g body weight}$$

3.13.3 **Water Intake**

Daily water intake (DWI) was also expressed as the amount of water taken by the rats in a group per 100g body weight.

$$\text{DWI/100g body weight} = \frac{\text{Daily Water intake (g)}}{\text{Body Weight per group}} \times \text{100 g Body weight}$$

2.13.4 **Body Weight Changes**

Rats in all the groups were weighed twice every week during the period of treatment and on the day of sacrifice.

3.13.5 **Haematology and Serum Biochemistry**

On the 43rd and 91st day of the experiment, six rats from each treatment groups was euthanized with diethyl ether and blood samples were collected by cardiac puncture after opening up the rats surgically. One portion was collected into K^+ EDTA bottles for estimation of haematological parameters {Packed cell volume(PCV), haemoglobin concentration(Hb), red blood cell count (RBC), platelets, white blood cell count (WBC) and differentials, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)} using an automated haematological machine(Cell-Dyn™ Abbot,US). Another portion was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 minutes. The sera were separated, stored at -4°C and used for evaluation of biochemical parameters {alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, total and conjugated bilirubin, urea and creatinine levels } using commercial kits obtained from Randox Laboratories, UK.

3.13.6 **Relative organ-body weight ratio**

Different organs namely the brain, heart, spleen, liver, lungs, kidneys, brain, intestine and testis or uterus were removed from each rat, weighed (absolute organ weight) and observed macroscopically. The relative organ weight (ROW) was calculated as follows:

$$ROW = \frac{\text{Absolute Organ Weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

3.13.7 **Histopathology**

At the end of 42 and 90 day, six rats per group were removed randomly from each of groups 1 to 4. Each rat was then weighed, euthanized and gross pathologic examinations were conducted. On each of the posted rat selected organs (brain, heart, spleen, liver, lungs, kidneys, brain, intestine and testis or uterus) were removed, weighed and preserved in 10% formal saline for histopathologic analysis. Tissue slices of 3 to 4 cm thick were cut and kept in the automatic tissue processor where they were fixed in 10% formalin-saline solutions for 2 hours. They were then dehydrated for 2 hours. They were then dehydrated for 2 hours in each of the following ascending grades of alcohol: 85%, 90%, and 100% v/v. The dehydrated tissues were then cleared in toluene for 2 hours, after which the tissue slices were embedded in paraffin wax and left to cool. The blocks were trimmed on the microtome at 5 microns. The ribbon of sections was dehydrated in xylene and rehydrated in the following descending grades of alcohol 100%, 90%, and 70% v/v. They were then stained inhaematoxylin for about 5 minutes, differentiated in 1% acid alcohol, blued in scotch's tap water and stained in eosin for 3 minutes. They were later rinsed, dehydrated in ascending gardes of alcohol: 70%, 90% and 100%v/v; finally they were cleared in xylene and mounted in a pox. The slides were then examined microscopically for pathological lesions. The lesions observed were assessed for the following: mucosal atrophy, the presence of inflammatory cells in the wall, eosinophils, lymphocytes and plasma cells. These were graded according to mild (+), moderate (++) or severe (+++). Photomicrographs of representative lesions were taken at various magnifications.

STATISTICAL ANALYSIS

Graph pad prism version 5.02 was used to analyse data obtained and these were expressed as mean ± standard error of mean.The differences between means were compared using One way analysis of variance (ANOVA) followed by Dunnet's post hoc test. $P \leq 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 **Yield of extracts**

4.1.1 Yield of Crude extract

The crude methanolic bulb extract of *Crinum zeylanicum* obtained was 23% of the original bulb material.

4.1.2 **Yield of Fractions**

The yield of the three fractions obtained were; 45% for aqueous soluble fraction (ASF), 9.26% for solid residue fraction (SRF) and 1.44% for the ethyl acetate soluble fraction (ESF).

4.2 **Phytochemical analysis**

4.2.1 **Phytochemical analysis of crude extract**

Phytochemical analysis of the crude methanolic bulb extract of *Crinum zeylanicum* revealed presence of moderate quantities of balsam, sterols, terpenes and resins while carbohydrate, tannins, phylobatannins, saponins, flavonoids, alkaloids and volatile oils were present in small amounts. Anthraquinones, phenols and glycoside were absent in the crude extract (4.1).

4.2.2 **Phytochemical analysis of fractions**

Solid residue fraction (SRF) contained moderate quantities of alkaloids, saponins, flavonoids, sterols and terpenes, the aqueous soluble fraction (ASF) contained saponins and tannins in moderate quantities with carbohydrate and phylobatannins in small amounts. The ethyl acetate soluble fraction contained small quantities of volatile oils, balsams and resins (4.1)

4.3 **Elemental analysis**

4.3.1 Elemental analysis of *Crinum zeylanicum* bulb

Sodium, potassium, calcium, magnesium, manganese, lead, iron, chromium and nickel were present in the raw bulb while copper and cadmium were absent (Table 4.2).

4.3.2 Elemental analysis of the methanolic extract

Sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), lead (Pb), iron (Fe) and nickel (Ni) were present in the smaller proportion compared to the raw bulb while copper (Cu), chromium (Cr) and cadmium (Cd) were absent (Table 4.2).

Table 4.1: Results of phytochemical screening of crude methanolic bulb extract of *Crinum zeylanicum*, the SRF, ESR and ASF

Secondary metabolite	Crude methanolic extract	SRF	ESR	ASF
Balsam	++	-	+	-
Sterols	++	++	-	-
Terpenes	++	++	-	-
Resin	++	-	+	-
Carbohydrates	+	-	-	+
Tannins	++	++	-	++
Phylobatannin	+	-	--	+
Saponins	+	++	-	++
Flavonoids	+	++	-	-
Alkaloids	+	++	-	-
Anthraquinones	-	-	-	-
Volatile oil	+	-	+	-
Glycosides	-	-	-	-
Phenol	-	-	-	-

+ Slightly present; ++ moderately present; - Absent
SRF: Solid residue fraction, ESR: Ethyl acetate fraction, ASF: Aqueous soluble fraction

Table 4.2: Elemental constituents of the bulb and methanolic bulb extract of *Crinum zeylanicum*

Elements	Bulb(mg/L)	Methanolic bulb extract of CZ(mg/L)
Na	0.81 ± 0.01	0.78 ± 0.01
K	1.50 ± 0.01	0.84 ± 0.01
Ca	49.10 ± 0.01	39.68 ± 0.01
Mg	227.92 ± 0.01	30.99 ± 0.01
Mn	2.18 ± 0.01	0.63 ± 0.01
Pb	0.018 ± 0.01	0.011 ± 0.01
Fe	33.99 ± 0.01	6.69 ± 0.01
Ni	2.40 ± 0.01	1.10 ± 0.01
Cu	-	-
Cr	0.69 ± 0.01	-
Cd	-	-

4.4 **Acute toxicity study of the methanolic bulb extract in mice and rats**

In the first phase of the acute toxicity study, the extract at 10 mg /kg body weight did not produce any remarkable behavioural change in the mice and rats. At 100 and 1000 mg extract/kg body weight respectively, erect fur, reduced physical activity and sedation were observed. In the second phase of the study, 1600 and 2900 mg extract/kg produced moderate paw licking, erect fur, reduced physical activity and sedation. At 5000 mg extract/kg body weight however, severe paw licking, erect fur, reduced activity and sedation were seen. No mortality was recorded at all the doses used in this phase. The oral median lethal dose (LD_{50}) was estimated to be greater than 5000 mg extract/kg body weight.

In the intraperitoneal route of administration, dose- dependent signs of toxicity were observed. the first phase, mild paw-licking, stretching of the body, erect fur, abdominal contraction, lying flat on belly and sedation were observed in mice and rats given 10 mg extract /kg body weight respectively. At 100 and 1000 mg extract/kg body weight, moderate paw-licking, stretching, erect fur, abdominal contraction, lying flat on belly and sedation were observed. No mortality was recorded. In the second phase, at 1600 and 2900 mg extract/kg, no mortality was recorded but there was severe paw-licking, stretching of the body, erect fur, abdominal contraction, lying flat on belly and sedation. Severe paw-licking, stretching, erect fur, sedation, coma and convulsion were seen in mice and rats at 5000 mg extract/kg body weight. A hundred percent mortality was also recorded. The maximum tolerated dose was 2900 mg/kg while 5000 mg/kg was the minimum toxic dose. The intraperitoneal median lethal dose of *Crinum zeylanicum* in mice and rats was calculated to be $\int 2900 \times \int 5000 \approx 3807.88$ mg/kg body weight.

4.4.1 **Acute toxicity of the Solid residue fraction (SRF) in mice**

In the first phase of the study, no abnormal behavioural signs of toxicity were observed at 10 mg/kg body weight while paw licking, reduced locomotion and sedation were observed at 100 mg/kg body weight. At 1000 mg SRF/kg body weight, sedation, difficulty in breathing, coma, convulsion and 100% mortality were recorded. In the second phase, no abnormal behavioural signs of toxicity were observed at 100, 140, 225 and 370. However, at 600 mg SRF/kg body weight, decreased locomotor activity, sedation, coma, convulsion and death were observed. The minimum toxic dose was 600 mg/kg body weight and the maximum tolerated dose was 370 mg/kg body weight. The oral median lethal dose was calculated to be $\int 370 \times \int 600 \approx 470$ mg/kg body weight. Intraperitoneally, dose- dependent signs of toxicity and mortality were observed. In the first phase, no abnormal behavior was observed and no mortality was recorded at 10 mg SRF/kg body weight. At 100 and 1000 mg SRF/kg salivation, paw licking, defeacation, difficulty in breathing, inactivity, sedation, coma, convulsion and 100% death were recorded. In the second phase, at doses of 10, 15, 35 mg SRF/kg body weight intraperitoneally, no abnormal behavior was seen but at 60 mg SRF/kg body weight calmness, writhing, lying flat on the stomach were observed. Inactivity, sedation, difficulty in breathing, coma, convulsion and death were observed in mice given 100 mg SRF/kg. The minimum toxic dose was 100 mg/kg body weight and the maximum tolerated dose was 60 mg/kg body weight. The intraperitoneal median lethal dose of SRF was calculated to be $\int 100 \times \int 60 \approx 77.49$ mg/kg body weight.

4.5 **Gross behavioural effects of *Crinum zeylanicum* bulb methanolic extract in mice**

There was significant dose- dependent decrease in motor activity. At 250 mg extract/kg body weight, the extract produced mild paw licking and erect fur. At 500 mg extract/kg body weight, moderate paw licking, salivation, urination and defeacation were observed, while intense paw licking, salivation, urination and defeacation were observed at 1000 mg extract/kg body weight. These signs were seen approximately fifteen minutes after extract administration and lasted for 1h (Table 4.3).

4.6 **Effect of methanolic bulb extract, solid residue fraction of *Crinum zeylanicum* extract and diazepam on pentobarbitone- induced sleeping time in mice**

There was no significant difference in the pentobarbitone-induced sleeping time between the group treated with 250 mg extract/kg body weight and the group that received pentobarbitone alone. The extract produced significant ($P<0.05$) and dose-dependent shortening of on-set and prolongation of the sleeping time at 500 and 1000 mg extract/kg body weight respectively. Diazepam also produced highly significant ($p<0.01$) shortening and prolongation of the sleeping time in the mice. There was no significant difference in sleeping time of the group that received 1000 mg extract/kg body weight and 2 mg diazepam/kg body weight groups. The solid residue fraction (SRF) significantly ($P<0.05$) and dose- dependently shortened the onset of sleep and highly significantly ($P<0.01$) prolonged the duration of sleep. There was no significant difference in the onset and duration of sleep at 100 mg SRF/kg body weight and 2 mg diazepam/kg body weight (Table 4.4).

Table 4.3: Gross behavioural effects of oral administration of methanolic bulb extract of *Crinum zeylanicum* in mice.

Treatment	Control	50 mg extract /kg	250 mg extract/kg	500 mg extract/kg	1000 mg extract/kg
Autonomic effect	Normal behaviour	None	Mild paw licking, Erect fur	Moderate paw-picking, salivation, urination and defeacation	Intense paw-licking, salivation, urination and defeacation
Central nervous effects	Normal behaviour	None	Slight reduction in motor activity	Moderate reduction in motor activity	Significant reduction in motor activity, sedation.
Death	Normal behaviour	None	None	None	None

Table 4.4: Effect of oral administration of *Crinum zeylanicum* methanolic bulb extract, SRF and diazepam on Pentobarbitone-induced sleeping time in mice

Treatment	On-set of sleep (min)	Duration of sleep (min)
25mg Pentobarbitone/kg	11.29±0.81	42.60±3.52
250mgextract/kg±25mg Pentobarbitone/kg	11.50±1.92	43.36±2.02
500mgextract/kg±25mg Pentobarbitone/kg	7.88±1.01*	79.80±5.37*
1000mgextract/kg±25mg Pentobarbitone/kg	3.57±0.47**	107.60±10.69**
25mg SRF/kg+25mgPentobarbitone/kg	5.20±0.86*	58.80±3.76**
50mgSRF/kg+25mgPentobarbitone/kg	3.60±0.51*	70.60±4.05**
100mgSRF/kg+25mgPentobarbitone/kg	2.80±0.80**	112.60±2.94**
2mgdiazepam/kg+25mgPentobarbitone/kg	2.80±0.37**	113.40±4.52**

*Significantly different from the control at $P<0.05$, and **at $P<0.01$, n= 6

SRF: Solid Residue Fraction

4.7 **Neuro-behaviourial studies**

4.7.1: **Effect of methanolic bulb extract of *Crinum zeylanicum* and chlorpromazine on spontaneous motor activity in mice**

The extract at all doses used produced highly significant (P<0.01) and dose- dependent decrease in spontaneous motor activity in the mice 30 minutes after treatment and highly significant (P<0.01) dose- dependent decrease at 500, 1000 and 2mg chlorpromazine/kg body weight respectively, 60 minutes post treatment (Table 4.5).

4.7.2 **Effect of methanolic bulb extract *Crinum zeylanicum* on motor co-ordination (Rota-rod) in mice**

The extract-treated mice were able to maintain their posture on the rotating rod without falling for over 180 seconds, the cut off time on the tread mill at all doses used (Table 4.6).

4.7.3 **Effect of *Crinum zeylanicum* methanolic bulb extract on exploratory behaviour of mice**

The extract caused very highly significant (P<0.001), dose- dependent decrease in the number of head dips (exploratory activity) in hole board apparatus in the mice. There was no significant difference in mean number of head dips in mice given 1000mg extract/kg body weight and those given 2mg diazepam/kg body weight (Table 4.7).

Table 4.5: Effect of oral administration of methanolic bulb extract of *Crinum zeylanicum*
on spontaneous motor activity of mice

Time/ min	control	250 mg extract/kg	500 mg extract/kg	1000 mg extract/kg	2 mg CPZ/kg
0	403.00±1.33	419.00±1.76	406.00±1.56	482.00±11.67	439.50±1.41
30	413.00±1.67	385.00±2.76*	313.00±1.78*	395.00±3.73*	398.50±1.78*
60	420.00±2.09	380.00±2.06*	308.00±1.65**	298.00±2.06**	182.70±9.99**
90	435 ± 1.52	376 ±2.48*	305 ± 1.52**	281 ± 3.06**	135 ±1.60**
120	445 ± 1.76	367 ± 2.57*	296 ± 1.59**	276 ±2.17**	106.30±8.20**

*****Significantly differently from the control at P≤0.05, and****** at P≤0.01, n=6

Table 4.6: Effect of *Crinum zeylanicum* bulb methanolic extract on motor co-ordination of mice

Treatment	Pre-treatment (Sec)	Time on rod 30 min	Time on rod 60 min	Time on rod 90 min	Time on rod 120 min	Time on roc 180 min
10 ml distilled water/kg	180.00±0.00	180.00±0.00	180.00±00	180.00±0.00	180.00±0.00	180±0.00
250 mg extract/kg	180.00±0.00	306.00±0.14	326.00±0.18	329.00±0.44	334.00±0.54	336.00±0.2
500 mg extract/kg	180.00±0.00	318.14±0.22*	328.14±0.52*	330.14±0.22*	348.14±0.32*	388.14±0.2
1000 mg extract/kg	180.00±0.00	354.28±0.08*	365.28±0.38*	370.28±0.42*	384.28±0.08*	454.28±0.0

*Significantly different from the control at P<0.05

Table 4.7: Effect of *Crinum zeylanicum* bulb methanolic extract on exploratory behaviour of mice

Treatment	Number of head dip	Number of head dip

	Pre-treatment	Post-treatment
10 ml distilled water/kg	8.60±0.51	8.80±0.37
250 mg extract/kg	8.20±0.37	5.40±0.51*
500 mg extract/kg	8.20±0.20	3.20±0.37**
1000 mg extract/kg	8.40±0.51	2.00±0.71***
2mg diazepam/kg	8.40±1.03	1.20±0.37***

*significantly different from the control at $P<0.05$, ** $P<0.01$ and*** $P<0.001$, n=6

4.7.4 **Effect of *Crinum zeylanicum* methanolic bulb extract on apomorphine-induced stereotype behaviour in mice**

The extract at all doses used and 2 mg chlorpromazine/kg body weight highly significantly ($P<0.0001$) and dose-dependently attenuated apomorphine-induced stereotype behaviour (sniffing, jumping/climbing, limb licking and cycling). There was no significant difference in the effect of the extract at 1000mg kg body weight and 2 mg chlorpromazine/kg body weight (Table 4.8).

Table 4.8: Effect of *Crinum zeylanicum* methanolic bulb extract on Apomorphine-induced stereotype behaviour in mice

Stereotype behaviour	control	250 mg/kg	500 mg/kg	1000 mg/kg	2 mg CPZ/kg
Sniffing	1168.00±17.00	966.00±6.50***	810.00±4.80***	694.00±3.80***	667.00±14.00**
Jumping/ Climbing	543.30±16.06	404.00±1.71***	395.80±1.80***	382.00±3.27***	369.30±3.18***
Limb Licking	502.00±1.90	478.00±3.70***	462.00±2.50***	417.00±4.60***	347.00±19.00**
Cycling	319.00±1.50	284.00±4.20***	275.00±5.40***	269.00±5.50***	264.00±5.70***

Very highly significantly different from the control at ***P<0.0001, n=6

CPZ=Chlorpromazine

4.8 **Seizure studies**

4.8.2 **Effect of *Crinum zeylanicum* methanolic bulb extract, SRF and diazepam on PTZ-induced seizures in mice**

The extract produced dose- dependent prolongation of onset of seizures in the mice which was significant ($p<0.05$) in the 500 and 1000 mg extract/kg body weight groups respectively. The extract produced 16.67%, 33.33% and 83.33% protection at 250, 500 and 1000 mg extract/kg body weight respectively. The solid residue fraction significantly ($P<0.01$) prolonged the latency of hind limb tonic extension (HLTE) at 50 and 100 mg extract/kg body weight respectively. It produced 66.67 and 100 % protection at 50 and 100 mg/kg body weight respectively. Diazepam at 4mg/kg also produced 100% protection in treated mice (Table 4.9).

4.8.2 **Effect of *Crinum zeylanicum* methanolic bulb extract, SRF and phenytoin on maximal electric shock (MES)–induced seizures in chicks**

The methanolic bulb extract of *Crinum zeylanicum* at 250, 500 and 1000 mg/kg body weight significantly and dose-dependently delayed the onset of maximal electric shock-induced seizure in the chicks. The solid residue fraction at 25 and 50 mg/kg body weight respectively significantly delayed the onset of seizure, while 100 mg SRF/kg and 20 mg phenytoin/kg body weight protected 90 and 100 percent of the chicks respectively (Table 4.10).

4.8.3 **Effect of *Crinum zeylanicum* methanolic bulb extract, SRF and diazepam on picrotoxin -induced seizures in mice**

The extract of Crinum zeylanicum did not protect the mice against picrotoxin-induced seizures at the dose levels used (250-1000 mg/kg). Diazepam at the dose of 4mg/kg body weight very highly significantly ($p<0.001$) protected all the treated mice. SRF produced significant ($P<0.01$), dose-dependent prolongation of onset of seizures, latency of hind limb tonic extension (HLTE) and produced 16.67%, 50 % and 100% protection at 25, 50 and 100 mg/kg respectively (Table 4.11).

Table 4.9: Effect of *Crinum zeylanicum* methanolic bulb extract, SRF and diazepam on PTZ- induced seizures in mice

Treatment	Onset of seizure (Sec)	Latency of HLTE (Sec)	Number of mice that convulsed	% protected
10ml distilled water/kg	36.00 ± 2.56	145.20±27.46	6	0.00
Extract 250 mg/kg	137.00 ± 55.66	714.80±35.70*	5	16.67
Extract 500 mg/kg	202.00 ± 38.16*	0.00±0.00**	4	33.33
Extract 1000 mg/kg	991.00 ± 35.70*	0.00±0.00**	1	83.33
25 mg SRF/kg	0.43 ± 0.05	3.79±1.10	6	0.00
50 mg SRF/kg	187.00 ± 0.37	1820.00±4.68**	2	66.67
100 mg SRF/kg	198.00 ± 1.10	0.00±0.00**	0	100
4 mg diazepam/kg	0.00 ± 0.00	0.00 ± 0.00**	0	100

* and ** are significantly different from the control at P≤0.05 and P≤0.01 respectively, n=6

SRF: Solid Residue Fraction

HLTE: Hind limb tonic extension

Table 4.10: Effect of *Crinum zeylanicum* methanolic bulb extract, SRF and phenytoin on maximal electric shock –induced seizures in chicks

Treatment	Mean onset of seizure (sec)	Number of chicks that convulsed	% Protection against seizure
5 ml normal/kg	2.20 ± 0.25	10	0
250 mg extract/kg	5.20 ± 0.35	10	0
500 mg extract/kg	17.00 ± 3.00*	10	0
1000 mg extract/kg	25.30 ± 0.44**	10	0
25 mg SRF/kg	48.00 ± 3.20**	10	0
50 mg SRF/kg	314.00 ± 1.48***	10	0
100 mg SRF/kg	0.00±0.00	10	90
25 mg Phenytoin/kg	0.00±0.00	10	100

*Significantly different from the control at $P \leq 0.05$, ** at $P \leq 0.001$ and *** at $P \leq 0.0001$, n=10

SRF: Solid Residue Fraction

Table 4.11: Effect of *Crinum zeylanicum* methanolic bulb extract, SRF and diazepam on picrotoxin- induced seizures in mice

Treatment	Onset of seizures (min)	Latency of HLTE(min)	No of mice that convulsed	Protection%
10ml distilled water/kg	4.20±0.92	4.51±1.03	6	0
250 mgextract/kg	5.14±0.02	6.13±0.25	6	0
500 mg extract/kg	5.42±0.21	7.49±0.48	6	0
1000 mg extract/kg	6.14±0.58	10.29±0.76*	6	0
25 mg SRF /kg	4.60±0.68	12.40±1.17**	5	16.67
50 mg SRF/kg	25.80±13.70**	0.00±00***	3	50.00
100 mg SRF/kg	0.00±0.00***	0.00±0.00***	0	100.00
4 mg diazepam/kg	0.00±0.00***	0.00±0.00***	0	100.00

* Significantly different from the control at$P \leq 0.05$, ** at $P \leq 0.01$ and *** at $P \leq 0.001$, n=7

SRF: Solid Residue Fraction

HLTE: Hind limb tonic extension

4.8. 4 **Effect of *Crinum zeylanicum* bulb methanolic extract, SRF and diazepam on strychnine-induced seizures in mice**

The extract at all doses used, produced significant (P<0.05), dose-dependent prolongation of onset of seizures and latency of hind limb tonic extension (HLTE) but did not protect the treated mice. The solid residue fraction significantly (P<0.001) and dose-dependently prolonged the onset of seizures, delayed HLTE in the mice and gave 50 and 100% protection at 50 and 100 mg /kg body weight respectively. Diazepam also protected all the mice (100%) against strychnine –induced seizures (Table 4.12).

4.8.5 **Effect of *Crinum zeylanicum* bulb methanolic extract, SRF and phenobarbitone on Pilocarpine –induced seizures in mice.**

The extract produced significant (P<0.001) dose-dependent prolongation of onset of seizures, hind limb tonic extension (HLTE) and protected 33.33, 50 and 100 % of the mice at 250, 500 and 1000 mg extract/kg body weight respectively. The SRF also produced significant (P<0.001), dose -dependent delay in onset of seizures and prolongation of the latency of HLTE. It gave 16.67, 83.33 and 100% protection at 25, 50 and 100 mg /kg body weight respectively. Phenobarbitone also significantly antagonized pilocarpine-induced seizure (Table 4.13).

4.8.6 **Effect of *Crinum zeylanicum* bulb methanolic extract, SRF and diazepam on 4-Aminopyridine induced seizures in mice**

4-aminopyridine produced hind limb tonic seizures in all the mice used for the study. The extract produced significant (P<0.001), dose-dependent prolongation of onset and latency of hind limb tonic extension (HLTE). The SRF also produced significant (P<0.001) and dose-dependent prolongation of onset and latency of HLTE in mice. The extract and SRF did not protect the mice from the 4-aminopyridine–induced seizures. Diazepam at 4 mg/kg body weight completely abolished the HLTE phase and protected all the mice (Table 4.14).

Table 4.12: Effect of *Crinum zeylanicum* bulb methanolic extract, SRF and diazepam on strychnine- induced seizures in mice

Treatment	Onset of seizure (Sec)	Latency of HLTE (Sec)	No of mice that convulsed	% protected
10ml distilled water/kg	186.00 ± 0.40	186.00±0.40	6	0.00
250 mg extract/kg	214.12 ± 0.13*	316.80 ±0.40*	6	0.00
500 mg extract/kg	383.24 ±0.93*	385.28±0.21*	6	0.00
1000mg extract/kg	389.12±0.50*	450.00±0.55*	6	0.00
25 mg SRF/kg	275.18 ±0.28*	949.83±0.92**	6	0.00
50 mg SRF/kg	397.34 ± 0.45*	1168.80±5.83**	3	50.00
100mg SRF/kg	1094.4±1.33*	0.00±0.00***	0	100.00
4 mg diazepam /kg	1440.00±1.01*	0.00±0.00***	0	100.00

*Significantly different from the control at $P \leq 0.05$, ** at $P \leq 0.001$ and *** at $P \leq 0.0001$, n=7
SRF: Solid Residue Fraction
HLTE: Hind limb tonic extension

Table 4.13: Effect of *Crinum zeylanicum* bulb methanolic extract, SRF and Phenobarbitone on Pilocarpine-induced seizures in mice

Treatment	Onset of seizures (Sec)	Latency of HLTE (Sec)	Number of mice that convulsed	Protection %
10ml distilled water	209.40±0.14	232.20±0.15	6	0
250mg extract/kg	414.00±1.80**	1065.60±2.08*	4	33.33
500mg extract/kg	600.00±0.00**	1320.00±0.90**	3	50
1000mg extract/kg	1544.40±1.00**	0.00±0.00**	0	100
25 mg SRF/kg	414±2.34**	1245.60±2.08**	5	16.67
50 mg SRF/kg	600.20±0.85**	1740.00±0.90**	1	83.33
100 mg SRF/kg	1500.74±1.78**	0.00±0.00**	0	100
10mgPhenobarbitone/kg	0.00±0.00**	0.00±0.00**	0	100

*Significantly different from the control at $P<0.001$ and ** at $p\leq 0.0001$, n=7

SRF: Solid Residue Fraction

HLTE: Hind limb tonic extension

Table 4.14: Effect of *Crinum zeylanicum* methanolic bulb extract, SRF and diazepam on 4-Aminopyridine- induced seizures in mice

Treatment	Onset of seizures(Sec)	Latency of HLTE (Sec)	Number of mice that convulsed	Protection%
10 ml distilled water/kg	174.00±0.10	219.60±0.29	6	0
250 mg extract/kg	216.00±0.40**	354.09±0.60**	6	0
500 mg extract/kg	228.00±0.37**	415.20±0.36**	6	0
1000 mg extract/kg	288.00±0.58**	744.00±0.81**	6	0
25 mg SRF/kg	211.80±0.13**	428.40±0.37**	6	0
50 mg SRF/kg	231.60±0.75**	458.40±0.22**	6	0
100 mg SRF/kg	352.80±0.75**	483.78±0.42**	6	0
4 mg diazepam/kg	0.00±0.00**	0.00±0.00**	0	100

**Significantly different from the control at $P<0.001$, n=7,

SRF: Solid Residue Fraction

HLTE: Hind limb tonic extension

4.9 Analgesic studies

4.9.1 Effect of *Crinum zeylanicum* methanolic bulb extract and piroxicam on acetic acid- induced abdominal constriction in mice.

The extract at doses of 250, 500 and 1000mg /kg body weight significantly (P<0.05-0.001) and dose-dependently decreased the number of acetic acid-induced abdominal constrictions in mice in a time-dependent manner. Piroxicam highly significantly (p<0.001) decreased the number of acetic acid-induced abdominal constrictions in mice in a time-dependent manner (Table 4.15).

4.9.2 Effect of *Crinum zeylanicum* methanolic bulb extract and morphine on formalin–induced pain in rats.

The extract caused significant (P<0.05) and dose-dependent reduction in responses to formalin induced nociception in both early and late phases**.** Maximal percentage (48.67%) inhibition of nociception was produced in the early phase at 1000 mg extract/kg body weight. Morphine, at the dose of 4 mg /kg body weight produced maximal percentages of (95%) and (91.67%) inhibition of nociception in both early and late phases of the study respectively (Table 4.16).

4.9.3 Effect of methanolic bulb extract of *Crinum zeylanicum and* morphine on hot plate test in mice

The extract significantly (P<0.05-0.001) and dose-dependently increased the reaction time of the mice to heat stimulus from the hot plate. The maximum analgesic effect of the extract was observed at 60 minutes after treatment. The increase in reaction time obtained with 4 mg morphine /kg body weight was highly significant (p<0.001) and higher than that recorded at1000 mg extract/kg body weight (Table4.17).

Table 4.15: Effect of methanolic bulb extract of *Crinum zeylanicum* and piroxicam on acetic acid- induced abdominal constriction in mice.

Duration (Min)	10 ml distilled water/kg	250 mg extract/kg	500 mg extract/kg	1000 mg extract/kg	10 mg Piroxicam/kg
0-5	15.20±2.18	11.20±1.77*	5.00±0.71**	3.80±0.86***	2.60±0.98***
10-15	13.40±1.83	10.40±1.63*	5.20±0.37**	2.80±0.58***	2.40±0.68***
20-25	14.20±1.02	9.00±1.73*	5.00±0.89**	1.40±0.32***	1.00±0.21***
30-35	12.80±1.50	9.60±2.34*	5.60±0.51**	1.24±0.18***	0.80±0.58***
40-45	15.40±1.47	7.80±0.86**	5.00±0.95***	0.80±0.17***	0.40±0.02***

*Significantly different from the control at $P<0.05$, ** at $P<0.01$ and *** at $P<0.001$,

n=6

Table 4.16: Effect of bulb methanolic extract of *Crinum zeylanicum* and morphine on formalin –induced pain in rats

Group	Early phase /Mean pain score	%inhibition	Late phase/ Mean pain score	%inhibition
10 ml distilled water/kg	3.00±00	0.00±0.00	3.00±0.00	0.00
250mgextract/kg	2.00±0.18	33.33	2.00±0.12	33.33
500mgextract/kg	1.80±0.21*	40.00	1.95±0.25*	35.00
1000mgextract/kg	1.54±0.16*	48.67	1.81±0.18*	39.67
4mg morphine/kg	0.15±0.02**	95.00	0.25±0.05**	91.67

*Significantly different from the control at P<0.05 and ** at P< 0.01, n=6

Table 4.17: Effect of *Crinum zeylanicum* methanolic bulb extract and morphine on the latency of reaction time of mice placed on hot plate.

Treatment	0 Min	30 Min	60 Min	90 Min
10 ml distilled water/kg	4.17±0.71	4.67±0.71	4.67±0.56	4.83±0.48
250 mg extract/kg	4.17±0.48	8.17±0.31*	19.17±1.64**	20.17±1.49**
500 mg extract/kg	4.00±0.37	13.17±1.08*	22.50±0.76**	24.83±0.60**
1000 mg extract/kg	4.33±0.61	21.00±0.73**	38.50±1.06**	38.67±1.54**
4 mg morphine/kg	4.67±0.49	40.83±2.55**	53.67±1.43**	55.00±1.69**

*Significantly different from the control at $P \leq 0.05$, ** and at $P \leq 0.001$, n=6

4.10 Chronic oral toxicity study

4.10.1 **Clinical Signs and Mortality**

Adverse clinical manifestations were not observed before, during and post treatment. No mortality was recorded at the doses used.

4.10.2 **Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on feed intake of female and male rats**

There was no significant change in feed intake of rats in the first six weeks of the study, while significant decrease ($p<0.05$ - 0.01) in feed intake was seen at 500 and 1000 mg extract/kg body weight respectively in the 12th and 13th weeks of the study. However in male rats there was no significant change in feed intake when compared with the control at all doses used throughout duration of study (Table 4.18).

4.10.3 **Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on water intake of female and male rats**

There was no significant difference in water intake between control and treated groups in the first six weeks of the study, while significant increase ($p<0.05$ - 0.01) was seen at 500 and 1000 mg extract/kg body weight respectively in the 12th and 13th weeks of the study. In male rats however, no significant change in water intake at all doses used for study (Table 4.19).

4.10.4 **Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb *extract* on body weight of female and male rats**.

The extract did not produce significant change in body weight of the rats in the first six weeks of the study. Significant ($P<0.001$) decrease in body weight was observed in the 10^{th}, 11^{th}, 12^{th} and 13^{th} weeks of the study at 500 and 1000 mg /kg body weight respectively when compared with control. In male rats, the extract did not produce significant change in body weights at all doses used throughout the duration of the study when compared with the control (Table 4.20).

Table 4.18: Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on feed intake of female and male rats in g/100g body weight/24h

Female rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Week I	14.98±1.31	14.54±1.57	14.48±1.01	14.77±1.01
Week II	13.50±0.67	13.41±1.19	13.05±1.16	13.91±0.98
Week III	12.34±0.85	12.30±1.13	12.95±0.82	12.30±0.84
Week IV	12.50±0.75	12.16±0.75	12.46±1.29	12.67±0.43
Week V	11.31±1.60	11.26± 1.45	11.42±1.81	11.88±1.95
Week VI	12.37±0.87	12.88±0.47	12.12±0.30	12.96±0.49
Week VII	14.30±0.47	13.84±0.71	12.30±0.49	12.43±0.49
Week VIII	13.61±0.34	13.26±0.46	12.67±0.39	12.50±0.35
Week IX	12.66±0.83	11.06±0.64	11.73±0.24	11.65±0.39
Week X	14.10±0.61	13.93±0.63	12.05±0.36	12.04±0.49
WeekX1	13.73±0.75	13.55±0.70	11.89±0.67	10.23±0.48
Week XII	12.73±0.75	12.81±0.11	10.79±0.14*	9.09±054*
Week XIII	12.66±0.32	12.46±0.85	8.49±0.89**	6.84±0.49**
Male rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Week I	10.63±1.48	11.09±2.77	10.88±2.25	11.65±2.58
Week II	11.02±1.01	11.54±1.25	11.02±0.88	11.96±1.20
Week III	11.25±0.42	11.26±0.06	11.33±0.84	11.34±0.98
Week IV	13.75±0.77	13.65±0.85	13.60±0.71	13.95±0.92
Week V	11.87±0.98	11.33± 0.31	11.43±0.02	11.49±0.40
Week VI	11.73±1.27	11.55±01.32	11.99±1.34	11.69±1.33
Week VII	12.63±1.41	12.09±1.79	12.62±1.25	12.66±1.62
Week VIII	12.82±1.01	12.54±1.25	12.427±1.48	12.96±1.20
Week IX	13.25±0.42	13.26±0.12	12.33±0.88	12.34±0.58
Week X	13.75±0.77	13.57±0.92	13.89±0.73	13.95±0.82
WeekX1	13.67±0.98	13.33±1.31	13.34±1.27	13.49±1.36
Week XII	13.79±1.27	13.56±1.36	13.99±1.33	13.69±1.32
Week XIII	14.57±1.08	14.33±1.31	14.48±1.20	14.94±1.36

*and ** significantly different from the control at P<0.05 and P<0.01 respectively, n=6

Table 4.20: Effect of 90 - day oral administration of *Crinum zeylanicum* methanolic bulb extract on water intake in ml/100g body weight of female and male rats

Female rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Week I	19.40±2.80	19.20±2.70	19.00±2.80	19.65±2.90
Week II	19.35±1.30	19.60±1.50	19.60±1.60	19.45±1.20
Week III	18.45±0.86	18.00±0.99	18.75±0.75	18.95±1.20
Week IV	18.00±0.55	18.00±0.57	19.20±0.87	18.00±0.68
Week V	19.50±1.70	19.40± 1.50	19.75±1.80	20.40±2.00
Week VI	19.27±0.22	19.51±0.43	18.90±0.14	20.17±0.54
Week VII	18.27±0.70	18.59±0.75	18.84±0.52	18.01±0.87
Week VIII	17.17±0.77	17.04±0.84	17.47±0.54	17.84±0.67
Week IX	17.37±0.18	17.34±0.73	17.24±0.42	17.49±0.62
Week X	16.76±0.51	15.89±0.54	16.49±0.69	16.34±0.65
Week XI	16.58±0.39	15.98±0.84	16.42±0.84	16.93±0.59
Week XII	18.77±0.33	18.67±0.58	29.73±0.60**	30.70±0.12**
Week XIII	18.48±0.53	18.45±0.26	28.54±0.26**	31.30±0.38**
Male rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Week I	14.36±0.48	14.90±0.76	14.68±0.25	14.56±0.58
Week II	14.23±1.10	14.43±0.55	14.20±0.58	14.66±0.20
Week III	15.25±0.22	15.62±0.36	15.53±0.44	15.74±0.48
Week IV	15.75±0.77	15.65±0.85	15.60±0.71	15.95±0.92
Week V	15.87±0.98	15.33± 0.31	15.43±0.02	15.49±0.40
Week VI	15.73±1.27	15.55±01.32	15.99±1.34	15.69±1.33
Week VII	16.63±1.41	16.09±1.79	16.62±1.25	16.66±1.62
Week VIII	16.82±1.01	16.54±1.25	16.27±1.48	16.96±1.20
Week IX	16.25±0.42	16.26±0.12	16.33±0.88	16.34±0.58
Week X	15.75±0.77	15.57±0.92	15.89±0.73	15.95±0.82
WeekX1	16.67±0.98	16.33±1.31	16.34±1.27	16.49±1.36
Week XII	16.79±1.27	16.56±1.36	16.99±1.33	16.69±1.32
Week XIII	15.27±1.28	15.53±1.21	15.68±1.20	15.34±1.16

** Significantly different from the control at $p<0.01$, $n=6$

Table 4.20: Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on body weight of female rats

Female rats	control	250 mg/kg	500 mg/kg	1000 mg/kg
Week I	139.00 ± 1.20	138.00 ± 1.26	139.00 ± 1.13	137.00 ± 1.30
Week II	147.00 ± 1.12	148.00 ± 1.80	148.00 ± 1.70	148.00 ± 2.40
Week III	155.00 ± 2.10	156.00 ± 2.90	155.00 ± 1.90	140.00 ± 0.50
Week IV	166.00 ± 1.20	165.00 ± 1.34	165.00 ± 2.50	166.00 ± 2.40
Week V	170.00 ± 2.12	171.00 ± 1.40	171.00 ± 0.80	172.00 ± 1.12
Week VI	173.00 ± 2.42	173.00 ± 1.53	174.00 ± 1.80	173.00 ± 1.22
Week VII	157.00 ± 1.20	158.00 ± 2.20	159.00 ± .13	160.00 ± 3.42
Week VIII	165.00 ± 2.12	164.00 ± 0.80	166.00 ± 0.70	168.00 ± 2.60
Week IX	168.00 ± 0.10	167.00 ± 1.90	166.00 ± 1.70	167.00 ± 1.50
Week X	176.00 ± 1.20	175.00 ± 1.10	174.00 ± 1.50	175.00 ± 1.40
Week XI	177.00 ± 2.32	178.00 ± 0.40	176.00 ± 1.20	177.00 ± 1.34
Week XII	176.80 ± 0.12	176.10 ± 0.40	174.00 ± 0.80*	169.00 ± 0.72**
Week XIII	178.75 ± 1.42	177.00 ± 1.20	175.00 ± 0.80*	168.00 ± 1.12**
Male rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Week I	156.00 ± 1.20	158.00 ± 1.26	157.00 ± 1.13	157.00 ± 1.30
Week II	157.00 ± 1.12	158.00 ± 1.80	158.00 ± 1.70	158.00 ± 2.40
Week III	162.00 ± 2.10	163.00 ± 2.90	163.00 ± 1.90	162.00 ± 0.50
Week IV	167.70 ± 2.30	167.00 ± 1.34	166.80 ± 2.50	168.00 ± 2.40
Week V	173.00 ± 2.12	172.67 ± 2.40	172.76 ± 2.80	172.00 ± 1.12
Week VI	176.00 ± 2.42	175.00 ± 1.53	176.00 ± 1.80	177.00 ± 1.22
Week VI I	178.21 ± 1.20	178.00 ± 2.20	179.00 ± 0.13	160.00 ± 3.42
Week VIII	165.00 ± 2.12	164.00 ± 0.80	166.00 ± 0.70	168.00 ± 2.60
Week IX	168.00 ± 0.10	167.00 ± 1.90	166.00 ± 1.70	167.00 ± 1.50
Week X	176.00 ± 1.20	175.00 ± 1.10	174.00 ± 1.50	175.00 ± 1.40
Week XI	177.90 ± 2.32	178.67 ± 0.40	176.54 ± 1.20	177.32 ± 1.34
Week XII	176.80 ± 0.12	176.10 ± 0.40	176.43 ± 0.80	176.00 ± 0.72
Week XIII	178.75 ± 0.24	177.00 ± 0.12	177.00 ± 0.32	178.00 ± 1.43

*significantly different from the control at p<0.05 and ** at p<0.01, n=6

4.10.5 **Effect of 42-day oral administration of *Crinum zeylanicum* methanolic bulb extract on haematological parameters of female rats.**

In female rats extract significant (P<0.05) decrease in white blood cell counts at 250 and 500 mg/kg body weight and haematocrit at 500 mg/kg body weight respectively was observed. The lymphocyte count was significantly (P<0.01) decreased at 250 mg extract/kg body weight. The extract significantly (P<0.0001) and dose-dependently increased platelet and neutrophil counts at 250 mg /kg body weight respectively. No statistically significant change was observed in other haematological parameters. Similarly in male rats, the extract produced significant (P<0.05) decrease in white blood cell count at 250 and 500 mg/kg body weight respectively. Significant (P<0.05) increase was observed in neutrophil count at 250 mg extract/kg body weight with significant (P<0.001), and dose-dependent increase in platelet count (Table 4.21).

4.10.6 **Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on haematological parameters of female rats.**

In female rats, there was significant (P<0.05) increase in white blood cell, haematocrit counts at all doses used and lymphocyte counts at 250 and 1000 mg/kg body weight respectively compared to the control. The platelet count was significantly (P<0.0001) and dose-dependently increased while neutrophil count was significantly (P<0.05) decreased at 250 and 1000 mg extract/kg body weight respectively. Other haematological parameters remained normal. In male rats, the extract at 500 and 1000 mg/kg body weight produced significant (P<0.05) increase in white blood cell counts. At 250 mg extract/kg body weight, the platelet count was significantly (P<0.01) increased and decreased at 500 and 1000 mg extract/kg body weight respectively. Other haematological parameters were normal (Table 4.22)

Table 4.21: Effect of 42-day oral administration of *Crinum zeylanicum* methanolic bulb extract on haematological parameters of female and male rats

Female rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
WBC (10^9/L)	13.00±1.70	7.70±1.40*	8.40±0.45*	13.00±1.80
RBC (10^{12}/L)	6.30 ± 1.10	7.20 ± 0.28	7.40 ± 0.45	7.70 ±0.15
HB (g/dL)	13.00 ± 0.77	13.00 ± 0.50	12.00 ± 0.69	14.00 ± 0.17
HCT (%)	46.00 ± 0.80	42.00 ±0.60	41.00±0.90*	48.00± 0.20
MCV (μm^3)	60.20 ± 0.65	60.00 ± 0.82	59.83 ± 1.45	62.83 ± 1.54
MCH (pg)	17.20 ± 0.16	17.17 ± 0.17	18.83 ± 0.60	18.33 ± 0.42
MCHC (g/dL)	28.80 ± 0.31	28.83 ± 0.31	31.50 ± 1.41	28.83 ± 0.17
PLT (10^3/μl)	445.34±2.34	457.00±1.78**	506.00±1.54***	530.00±2.54***
LYM %	72.00±0.46	58.00±0.98**	71.00±3.50	70.00±2.80
MXD %	7.60±1.10	6.80±1.50	7.00±1.60	6.00±1.00
NEUT %	20.00±0.45	43.00±0.44**	22.00±0.31	23.00±0.37
Male rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
WBC (10^9/L)	13.00 ± 0.41	7.70 ± 0.14*	6.40 ± 0.45*	13.00 ± 0.03
RBC (10^{12}/L)	6.30 ± 1.10	7.20 ± 0.28	6.40 ± 0.45	7.70 ± 0.15
HB (g/dL)	13.00 ± 0.77	13.00 ± 0.50	12.00 ± 0.69	14.00 ± 0.17
HCT (%)	46.00 ± 2.8	42.00 ± 1.6	41.00 ± 2.9	48.00 ± 1.20
MCV (μm^3)	54.86 ± 0.16	55.14 ± 0.63	56.47 ± 1.03	56.86 ± 1.35
MCH (pg)	28.29 ±0.11	28.71 ± 0.30	28.00 ± 0.16	21.29 ± 0.63
MCHC (g/dL)	29.96 ± 0.64	29.05 ± 0.24	29.73 ± 0.26	31.20 ± 0.54
PLT (10^3/μl)	571.00±7.37	711.00±8.34**	745.20±2.10**	773.60±4.83**
LYM %	72.00±4.60	58.00±9.80	71.00±3.50	70.00±2.80
MXD %	7.60±1.10	6.80±1.50	7.00±1.60	6.00±1.00
NEUT %	20.00±4.50	43.00±4.40*	22.00±3.10	23.00±3.67

*significantly different from the control at $P < 0.05$, ** $P<0.01$ and ***at $P<0.0001$, n=6

WBC: white blood cells, RBC: red blood cells, HB: haemoglobin, HCT: haematocrit, PLT: platelets, LYM: lymphocyte, MXD: monocyte and NEUT: neutrophil

Table 4.22: Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on haematological parameters of female and male rats

Female rat	Control	250 mg/kg	500 mg/kg	1000 mg/kg
WBC (10^9/L)	10.74 ± 0.41	14.08 ± 0.14*	12.20 ± 0.17*	16.96 ± 0.03*
RBC (10^{12}/L)	6.50 ± 0.35	7.20 ± 0.22	7.60 ± 0.25	7.60 ± 0.17
HB (g/dL)	11.78 ± 0.55	12.88 ± 0.41	12.86 ± 0.05	12.98 ± 0.37
HCT (%)	38.80 ± 0.08	43.80 ± 0.39*	43.60 ± 0.68*	44.00 ± 0.30*
MCV (µm^3)	62.40 ± 0.45	61.86 ± 0.82	62.33 ± 0.45	62.63 ± 0.44
MCH (pg)	19.10 ± 0.26	19.17 ± 0.27	19.15 ± 0.20	19.63 ± 0.22
MCHC (g/dL)	29.80 ± 0.45	29.83 ± 0.51	30.50 ± 0.94	28.42 ± 0.47
PLT (10^3/µl)	671.00±7.37	811.00±8.34**	845.20±2.10**	873.60±4.83**
LYM %	84.80±0.11	88.60±0.44*	85.60±2.54	88.60±0.08*
MXD %	4.20±0.86	3.20±0.37	3.20±0.37	3.20±0.58
NEUT %	11.00±0.63	8.20±0.20*	11.20±2.44	8.20±0.36*
Parameters	Control	250 mg/kg	500 mg/kg	1000 mg/kg
WBC (109/L)	12.60 ± 0.77	10.30 ± 1.36	18.16 ± 2.35*	16.10 ± 2.46*
RBC (1012/L)	7.32 ± 0.27	7.72 ± 0.43	7.60 ± 0.13	7.88 ± 0.14
HB (g/dL)	12.22 ± 0.32	12.90 ± 0.79	12.80 ± 0.08	12.98 ± 0.40
HCT (%)	42.60 ± 1.32	44.80 ± 2.04	46.00 ± 0.71	46.40 ± 1.44
MCV (µm3)	60.20 ± 0.65	60.00 ± 0.82	59.83 ± 1.45	62.83 ± 1.54
MCH (pg)	17.20 ± 0.16	17.17 ± 0.17	18.83 ± 0.60	18.33 ± 0.42
MCHC (g/dL)	28.80 ± 0.31	28.83 ± 0.31	31.50 ± 1.41	28.83 ± 0.17
PLT (10^3/µl)	736.20± 1.52	770.40±1.60**	654.00±1.40**	602.20±1.76**
LYM %	82.00±1.82	82.80±0.63	85.80±1.36	80.60±2.14
MXD %	5.60±0.25	4.40±0.68	4.20±0.66	4.20±0.49
NEUT %	12.60±1.94	12.80±3.37	10.00±1.67	13.20±0.07

*significantly different from control at $P<0.05$ and**at $P<0.001$, n=6

WBC: white blood cells, RBC: red blood cells, HB: haemoglobin, HCT: haematocrit, PLT: platelets, LYM: lymphocyte, MXD: monocyte and NEUT: neutrophil

4.10.7 **Effect of 42-day -oral administration of *Crinum zeylanicum* methanolic bulb extract on liver function parameters in female and male rats.**

The extract significantly ($P<0.05$, 0.001) and dose-dependently decreased the serum levels of ALP, AST and ALP, while serum levels of total bilirubin, total cholesterol, triglyceride, high density and low density lipoproteins were normal in female rats. Similarly, in male rats the extract produced significant ($P<0.001$) and dose-dependent increase in serum levels of ALP, ALT, AST and LDL. There was significant ($P<0.001$) increase in serum levels of total cholesterol and triglyceride which was not dose-dependent. The serum levels of total bilirubin and high density lipoprotein were not statistically changed (Table 4.23).

4.10.8 **Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on liver function parameters of female and male rats.**

The extract produced highly significant ($P<0.001$), dose-dependent increase in serum levels of ALP, AST, ALT, total cholesterol, triglyceride, and low density lipoprotein of the rats compared to control. No statistically significant changes were observed in the total bilirubin and high density lipoprotein levels in female rats. While in the male rats significant ($P<0.001$) dose-dependent increase in serum levels of ALP, ALT, AST and LDL was observed. The significant ($P<0.001$) increases in serum levels of total cholesterol and triglyceride were not dose-dependent. The serum levels of total bilirubin and HDL were normal (Table 4.24).

4.10.9 **Effect of 42-day oral administration of *Crinum zeylanicum* methanolic bulb extract on electrolytes, urea and creatinine levels in female and male rats.**

Serum levels of electrolytes, urea and creatinine were not adversely affected by the extract at all doses used and thus remained normal (Table 4.25).

4.10.10 **Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on electrolytes, urea and creatinine levels in female and male rats.**

Serum levels of urea, creatinine and electrolytes in the treated rats were not statistically different from the control (Table4.26).

Table 4.23: Effect of 42-day oral administration of *Crinum zeylanicum* methanolic bulb extract on liver function parameters of female and male rats

Female rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
ALP (IU/L)	96.00±0.22	84.00±0.49*	76.00±0.55**	76.00±0.51**
AST (IU/L)	344.00±0.37	335.00±0.25**	329.00±0.19**	317.00±0.33**
ALT (IU/L)	80.00±0.13	78.00±0.11*	76.50±0.60*	69.00±0.11**
T/Bil (mg/dL)	5.10±0.28	5.30 ± 0.37	5.20 ± 0.37	5.30±0.70
T/Chol(mg/dL)	83.00±0.51	84.00±0.44	82.40±0.63	85.10±0.45
Trig (mg/dL)	134.00±0.24	135.00±0.24	133.00±0.76	133.21±0.21
HDL (mg/dL)	38.00±0.25	40.00±0.26	40.00±0.39	40.00±0.40
LDL (mg/dL)	19.00±0.60	20.00±0.68	20.44±0.24	20.00±0.59
Male rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
ALP (IU/L)	109.20±3.96	140.40±1.43**	153.00±1.33**	165.80±2.30**
AST (IU/L)	277.80±4.64	279.20±3.63**	394.20±1.77**	418.00± 0.15**
ALT (IU/L)	84.80±0.33	89.20±0.18**	125.84±1.24**	129.60±0.89**
T/Bil (mg/dL)	4.94±0.36	4.84±0.34	5.40 ±0.21	5.60 ±0.57
T/Chol(mg/dL)	63.40±0.30	70.80±0.58**	71.60±0.50**	71.40±0.30**
Trig (mg/dL)	71.60±0.18	78.87±0.27**	91.00±0.15**	84.40±0.66**
HDL (mg/dL)	43.80±0.72	42.80±0.13	45.00±1.67	45.00±1.83
LDL (mg/dL)	4.20±0.83	7.60±0.31*	8.20±0.22*	9.12±0.52*

*significantly different from the control at P<0.05 and ** at P<0.001, n=6

ALP: Alkaline phosphatase, AST: Aspartate transaminase, ALT: Alanine transaminase

T/Bil: Total bilirubin, HDL: High density lipoprotein, LDL: Low density lipoprotein,

Trig: Triglyceride, T/Chol: Total cholesterol

Table4.24: Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on liver function parameters of female and male rats.

Female rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
ALP (IU/L)	109.20±3.96	140.40±1.43**	153.00±1.33**	165.80±2.30**
AST (IU/L)	277.80±4.64	279.20±3.63**	394.20±1.77**	418.00± 0.15**
ALT (IU/L)	84.80±0.33	89.20±0.18**	125.84±1.24**	129.60±0.89**
T/Bil (mg/dL)	4.94±0.36	4.84±0.34	5.40 ±0.21	5.60±0.57
T/Chol(mg/dL)	63.40±0.30	70.80±0.58**	71.60±0.50**	71.40±0.30**
Trig (mg/dL)	71.60±0.18	78.87±0.27**	91.00±0.15**	84.40±0.66**
HDL (mg/dL)	43.80±0.72	42.80±0.13	45.00±1.67	45.00±1.83
LDL (mg/dL)	4.20±0.83	7.60±0.31*	8.20±0.22*	9.12±0.52*
Male rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
ALP (IU/L)	156.00 ±0.28	166.60±0.38**	174.00±.066**	178.40±0.60**
AST (IU/L)	241.00 ±0.69	297.20±0.20**	345.20±1.77**	402.60± 0.40**
ALT (IU/L)	100.20 ±0.44	97.00±0.36**	111.80±0.16**	140.40±0.50
T/Bil (mg/dL)	4.26 ±0.31	4.80 ±0.50	4.32 ±0.38	4.56±0.82
T/Chol(mg/dL)	68.60 ±0.23	76.60 ±0.71**	76.20±0.40**	80.40±0.40**
Trig (mg/dL)	37.80 ±0.18	58.80 ±0.74**	44.60±0.99**	63.20±0.42**
HDL (mg/dL)	36.80 ±0.16	38.60 ±0.21	39.00±0.07	35.40±0.043
LDL (mg/dL)	24.00 ±0.12	26.20 ±0.06*	28.40±0.74*	32.40±0.47*

*significantly different from the control at P<0.05 and ** at P<0.001, n=6

ALP: Alkaline phosphatase, AST: Aspartate transaminase, ALT: Alanine transaminase

T/Bil: Total bilirubin, HDL: High density lipoprotein, LDL: Low density lipoprotein,

Trig: Triglyceride, T/Chol: Total cholesterol

Table 4.25: Effect of 42-day oral administration of *Crinum zeylanicum* methanolic bulb extract on serum electrolytes, urea and creatinine levels in female and male rats

Female rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Na^+ (mEq/L)	124.00±0.76	123.00±0.55	126.00±1.97	127.00±2.73
K^+ (mEq/L)	13.00±0.67	12.00±0.72	12.00±0.63	13.60±0.60
CL^- (mEq/L)	97.00±0.20	99.00±1.58	97.00±0.11	96.00±0.86
$HCO3^-$ (mEq/L)	24.60±0.51	24.20±0.97	24.20±0.58	23.60±0.13
Urea (mg/dl)	8.50±0.85	9.60±0.56	8.30±0.73	7.70±0.10
Creatinine(mg/dl)	73.00±0.31	72.90±0.34	73.00±0.31	74.00±0.31
Male rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Na+ (mEq/L)	134.00±0.76	133.00±0.55	135.00±1.97	135.00±2.73
K+ (mEq/L)	14.00±0.27	13.98±0.12	14.00±0.13	13.60±0.60
CL- (mEq/L)	97.40±0.20	99.20±1.58	97.85±0.11	96.40±0.86
HCO3- (mEq/L)	24.60±0.51	24.20±0.97	24.20±0.58	23.60±0.13
Urea (mg/dl)	10.50±0.25	8.60±0.56	8.65±0.73	8.70±0.10
Creatinine(mg/dl)	54.00±0.31	52.90±0.34	53.00±0.31	54.00±0.31

n=6

Table4.26: Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on electrolytes, urea and creatinine levels in female and male rats.

Female rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Na^+ (mEq/L)	141.80 ±1.46	144.20±0.49	142.60±2.00	140.80±0.97
K^+ (mEq/L)	7.50±0.25	8.00±0.28	8.56±0.65	8.72±0.94
CL^- (mEq/L)	101.40±1.54	103.40±0.51	101.60±1.29	100.60±0.81
$HCO3^-$ (mEq/L)	23.00±0.71	24.20±0.97	25.60±0.51	24.60±0.60
Urea (mg/dl)	20.24±3.42	22.20±2.67	22.26±1.60	19.08±2.23
Creatinine(mg/dl)	65.80±3.29	68.80±3.81	70.80±1.99	71.60±5.78
Male rats	**Control**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Na^+ (mEq/L)	145.20±0.66	146.20±0.58	143.80±0.37	143.80±0.86
K^+ (mEq/L)	7.30±0.51	7.52±0.20	8.00±0.48	8.84±0.34
CL^- (mEq/L)	101.40±0.95	105.20±0.37	103.60±0.51	103.20±0.80
$HCO3^-$ (mEq/L)	24.00±0.32	25.00±0.45	23.80±0.86	24.60±0.75
Urea (mg/dl)	20.40±0.75	22.16±0.26	20.54±0.84	18.74±0.24
Creatinine(mg/dl)	64.60±0.23	71.20±0.25	67.20±0.29	64.00±0.75

n=6

4.10.11 **Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on relative organ- body weight ratio of female and male rats**

The relative organ weight ratios were not adversely affected by the extract at all doses used and thus remained normal (Table 4.27).

4.10.12 **Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on relative organ- body weight ratio of female and male rats**

The relative organ weight ratios were not adversely affected by the extract at all doses used and thus remained normal (Table 4.28).

Table 4.27: Effect of 42-day oral administration of *Crinum zeylanicum* methanolic bulb extract on relative organ-body weight ratio of female and male rats

Female rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Liver	3.20±0.09	3.40±0.64	3.45±0.29	3.48±0.23
Stomach	1.20±0.01	1.23±0.09	1.20±0.01	1.30±0.05
Lungs	0.76±0.05	0.74±0.04	0.75±0.02	0.77±0.08
Kidneys	0.63±0.02	0.62±0.02	0.67±0.02	0.65±0.01
Spleen	0.38±0.01	0.31±.06	0.38±0.05	0.43±0.05
Brain	0.60±0.05	0.67±0.04	0.67±0.07	0.69±0.06
Ovaries	0.75±0.27	0.60±0.13	0.61±0.16	0.90±0.21
Intestine	0.51±0.11	0.65±0.06	0.39±0.07	0.48±0.04
Heart	0.35±0.04	0.40±0.04	0.38±0.03	0.41±0.02
Male rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Liver	3.20±0.09	3.40±0.64	3.45±0.29	3.48±0.23
Stomach	1.20±0.11	1.23±0.39	1.20±0.71	1.30±0.65
Lungs	1.76±0.15	1.74±0.34	1.75±0.22	1.77±0.58
Kidneys	0.73±0.52	0.72±0.92	0.77±0.42	0.75±0.11
Spleen	0.98±0.01	0.91±.06	0.98±0.05	0.93±0.05
Brain	0.80±0.35	0.87±0.44	0.67±0.57	0.69±0.36
Testes	0.75±0.27	0.60±0.13	0.61±0.16	0.90±0.21
Intestine	0.51±0.11	0.65±0.06	0.39±0.07	0.48±0.04
Heart	0.45±0.04	0.42±0.07	0.48±0.03	0.48±0.08

N=6

Table 4.28: Effect of 90-day oral administration of *Crinum zeylanicum* methanolic bulb extract on relative organ-body weight ratio of female and male rats

Female rats	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Liver	8.38±0.41	9.86±0.43	9.35±0.29	9.27±0.73
Stomach	3.91±0.79	4.11±0.42	5.19±0.33	3.62±0.39
Lungs	2.67±0.31	3.44±0.93	2.10±0.36	2.61±0.25
Kidneys	1.71±0.09	1.60±0.12	1.68±0.13	1.95±0.18
Spleen	1.18±0.09	1.05±0.11	1.02±0.04	1.14±0.23
Brain	1.96±0.14	1.69±0.22	1.80±0.08	1.89±0.20
Ovaries	1.46±0.36	1.40±0.22	1.47±0.24	1.52±0.33
Intestine	2.97±0.40	2.36±0.60	2.72±0.44	3.22±0.76
Heart	1.01±0.10	0.97±0.10	0.84±0.07	1.07±0.11
Male	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Liver	8.38±0.41	9.86±0.43	9.35±0.29	9.27±0.73
Stomach	3.90±0.79	4.00±0.42	3.82±0.33	3.92±0.05
Lungs	2.67±0.05	3.44±0.93	2.10±0.93	0.77±0.08
Kidneys	1.71±0.09	1.59±0.12	1.68±0.13	1.95±0.18
Spleen	1.18±0.09	1.05±0.11	1.01±0.04	1.14±0.23
Brain	1.96±0.14	1.69±0.22	1.80±0.08	1.88±0.20
Testes	1.46±0.35	1.40±0.22	1.27±0.24	1.72±0.33
Intestine	2.97±0.40	2.86±0.06	2.72±0.44	2.98±0.764
Heart	1.05±0.14	0.97±0.10	0.84±0.07	1.07±0.11

N=6

4.10.24: **Histopathology results of organs treated with *Crinum zeylanicum* methanolic bulb exract for 42 day in female and female rats**

No remarkable changes were seen in all the organs studied at 250, 500 and 1000 mg extract/kg body weight administered orally daily (Plates 4.01-4.20).

4.10.25: **Histopathology results of organs treated with *Crinum zeylanicum* methanolic bulb extract for 90 day in female and male rats**

Liver congestion was seen in female rats while lymphocytic infiltration at 1000 mg extract/kg body weight. The other organs studied were normal.

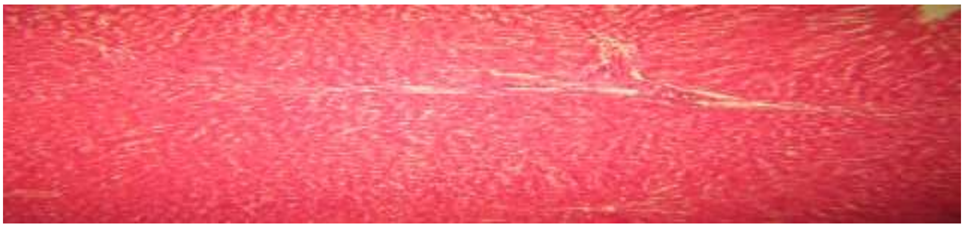

Plate I: Histotological findings in liver from control groups treated with 10 ml normal saline/kg body weight orally for 42 and 90 days of treatment showing **normal lobular architecture of the liver (HE x40)**

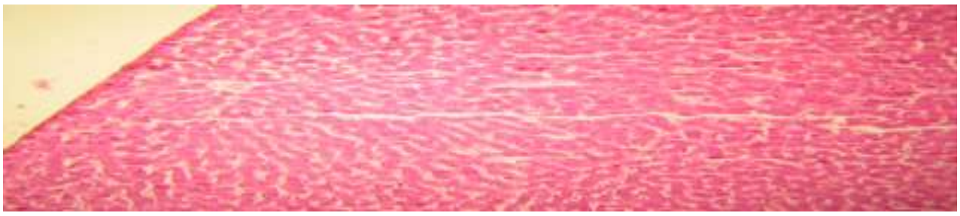

Plate II: Histotologic findings in liver of rats treated with 250 and 500 mg/kg of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90 days showing **normal lobular architecture of the liver** (HE x40)

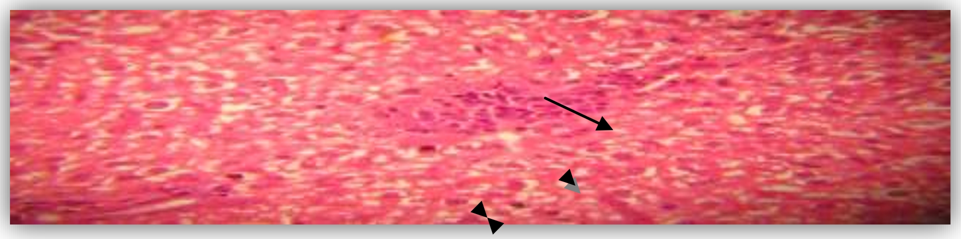

Plate III: Histopathologic findings in liver of male rats treated with 1000 mg /kg of methanolic bulb extract of *Crinum zeylanicum* orally for 90 days showing **liver cell necrosis and lymphocyte infiltration (HE x40)**

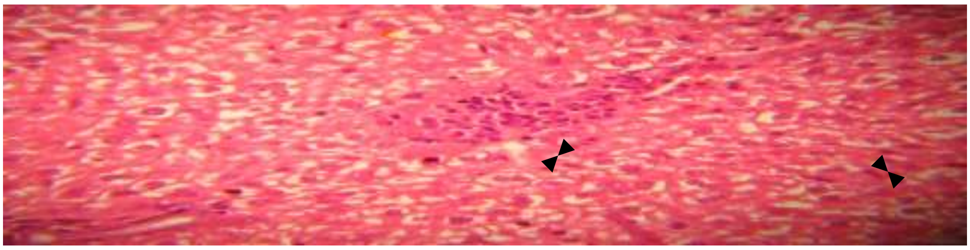

Plate IV: Histopathologic findings in liver of female rats given 1000 mg /kg of methanolic bulb extract of *Crinum zeylanicum* orally for 90 days showing **liver congestion (HE x40)**

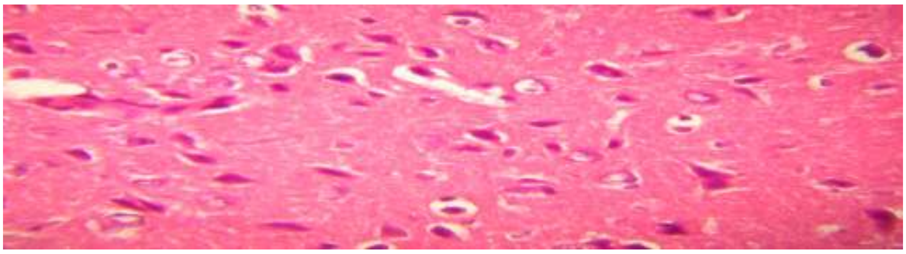

Plate V: Histotologic findings in brain of control group rats treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **normal brain architecture (HE x40)**

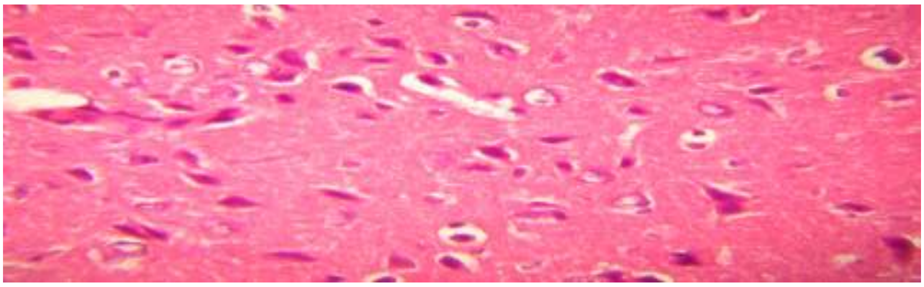

Plate VI: Histotologic findings in brain from groups treated with 250, 500 and 1000 mg/kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90 days showing **normal brain architecture (HE x40)**

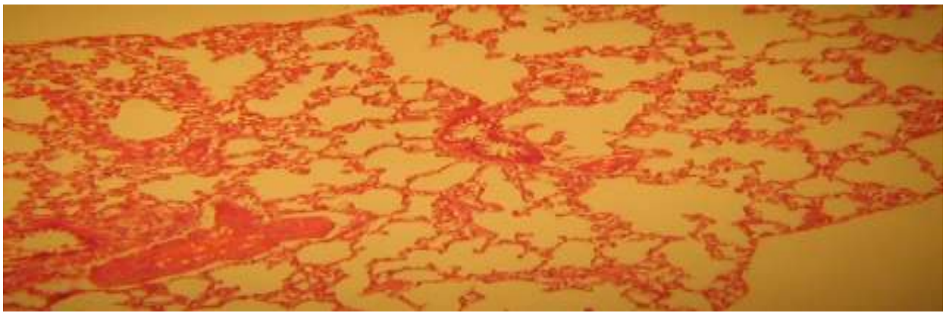

Plate VII: Histopathologic findings in lungs of control rats treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **normal normal lung architecture (HE x40)**

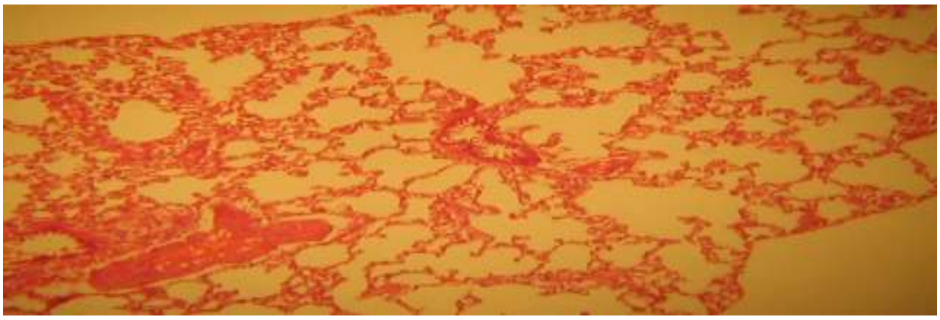

Plate VIII: Histopathologic findings in lungs of rats treated with 250, 500 and 1000 mg /kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90 days showing **normal normal lung architecture (HE x40)**

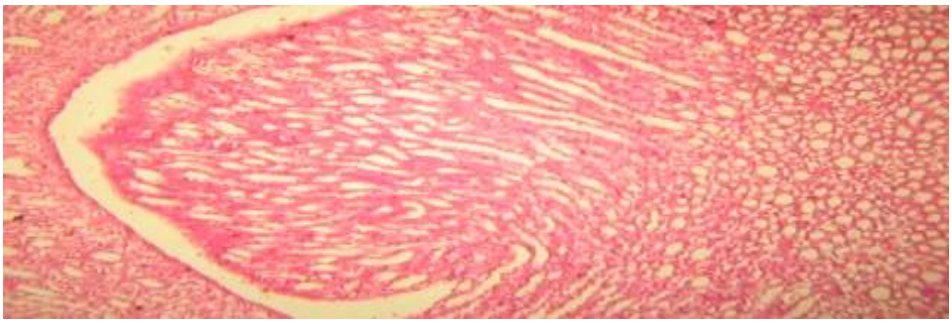

Plate IX: Histopathologic findings in kidney of control rats treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **no abnormality (HE x40)**

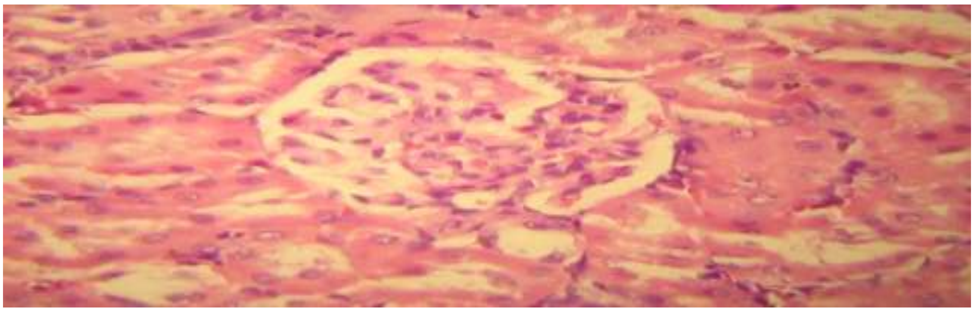

Plate X: Histotologic findings in kidney from groups treated with 250, 500 and 1000 mg/kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90 days **showing no abnormality (HE x40)**

Plate XI: Histologic findings in testes of control group treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **fairly uniform seminiferous tubules and spermatogenesis (HE x40)**

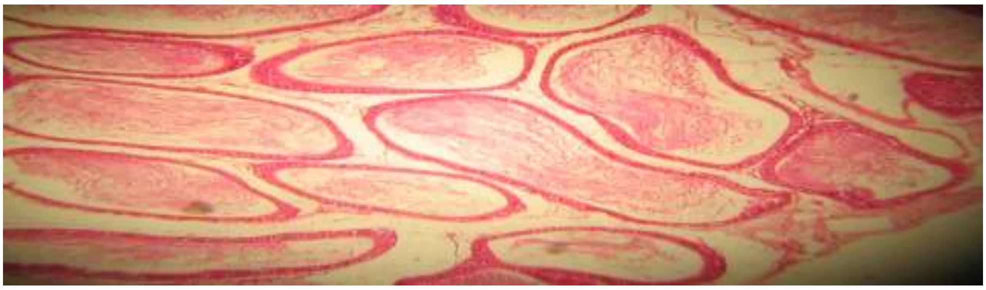

Plate XII: Histologic findings in testes of male rats treated with 250, 500 and 1000 mg/kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90-days **showing normal distribution of seminiferous tubules and spermatogenesis (HE x40)**

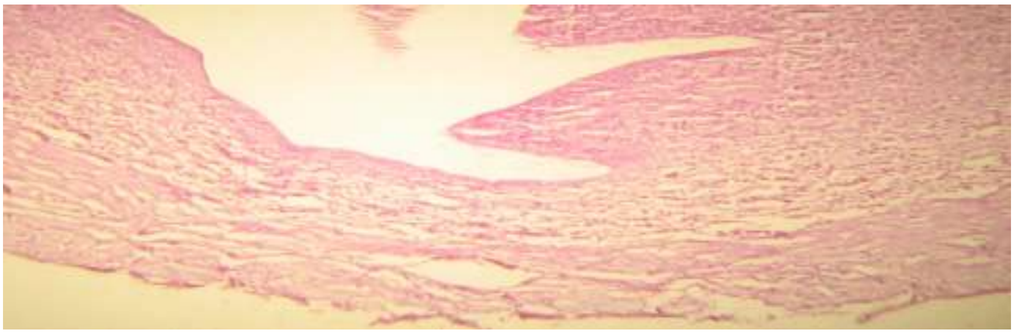

Plate XIII: Histologic findings in ovary from control group (female rats) treated with 10 ml normal saline/kg body weight orally for 42 and 90-days showing **no abnormality (HE x40)**

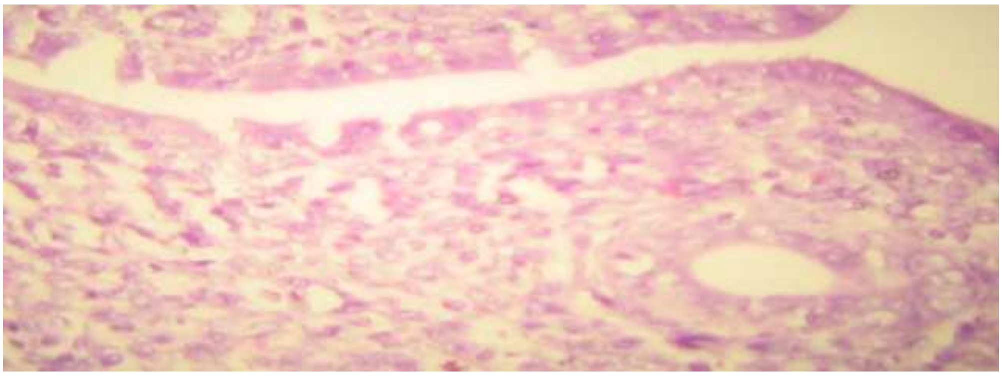

Plate XIV: Histologic findings in ovary from groups (female rats) treated with 250, 500 and 1000 mg/kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90-days **showing no abnormality**

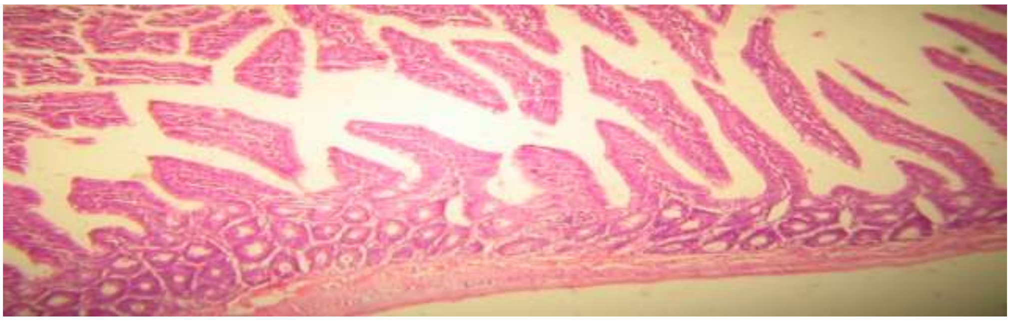

Plate XV: Histotological findings in small intestine of control rats at treated with methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90 days showing **normal mucosa of the intestine (HE X40)**

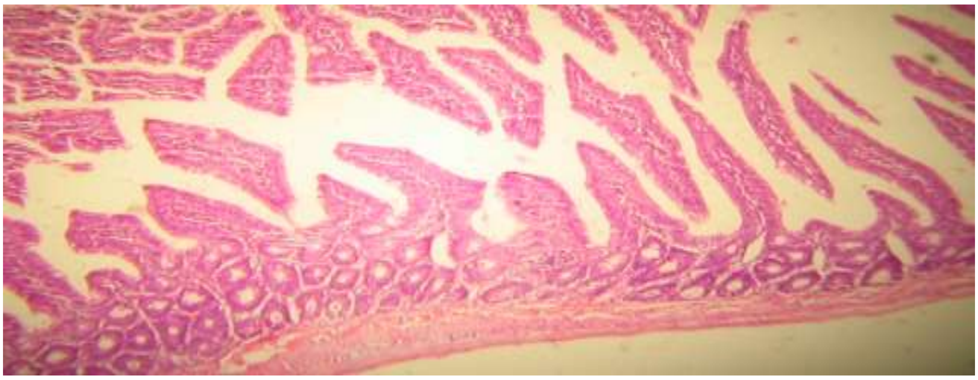

Plate XVI: Histological findings in small intestine of rats treated with 250, 500 and 1000 mg /kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90 days showing **unremarkable changes in the intestine (HE X40)**

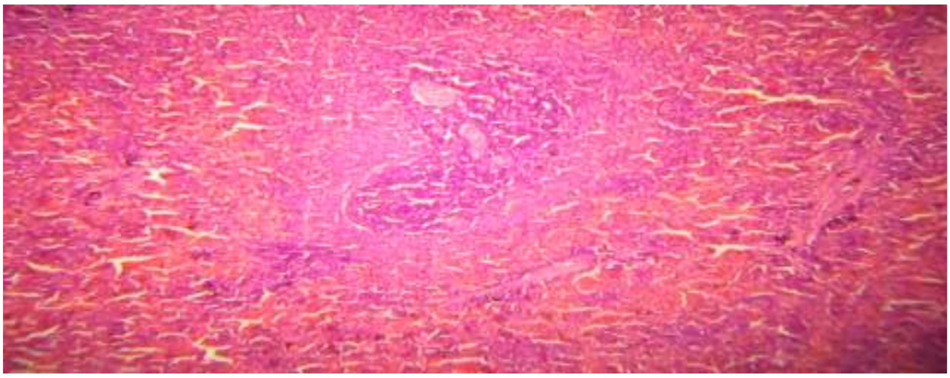

Plate XVII: Histological findings in spleen of controlrats treated with 10 ml normal saline/kg body weight orally for 42 and 90 days showing **no abnormality (HEX40)**

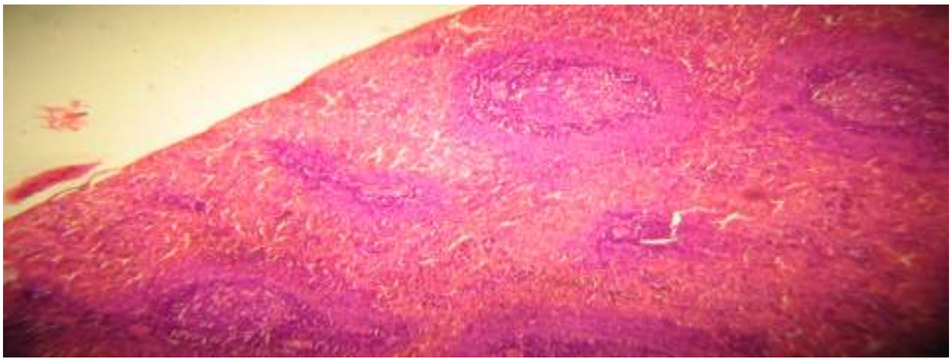

Plate XVIII: Histological findings in spleen of rats treated with 250, 500 and 1000 mg /kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90 days showing **no abnormality (HEX40)**

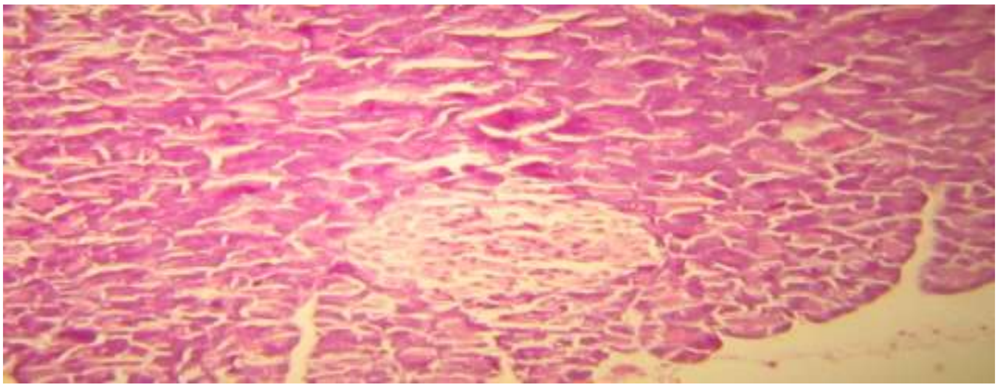

Plate XIX: Histological findings in pancreas of control groups treated with 10 ml normal saline/kg body weight orally for 42 and 90 days post treatment showing **no abnormality (HEX40)**

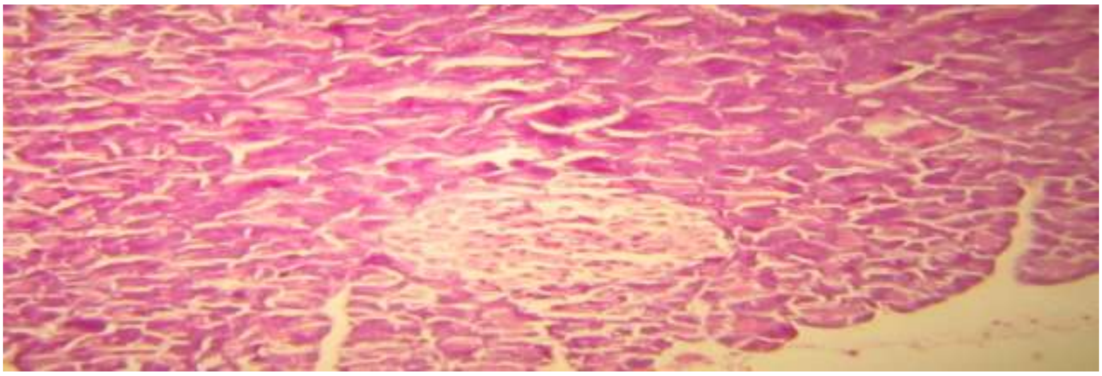

Plate XX: Histological findings in pancreas of groups treated with 250, 500 and 1000 mg /kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90 days **showing normal architecture (HEX40)**

Plate XXI: Histological findings in heart from control group treated with 10 ml normal saline/kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90- days showing **normal architecture (HE x40)**

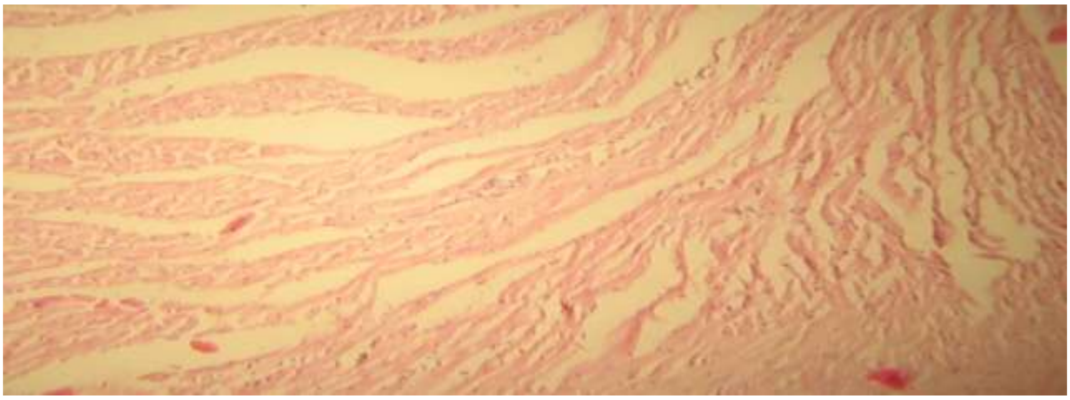

Plate XXII: Histologic findings in heart of groups treated with 250, 500 and 1000 mg /kg body weight of methanolic bulb extract of *Crinum zeylanicum* orally for 42 and 90 day showing **normal architecture of the heart (HEX40)**

CHAPTER FIVE

5.0 DISCUSSION

The choice of aqueous methanol for the extraction of *Crinum zeylanicum* bulb in this study was based on the preliminary screening of the aqueous and methanolic extracts for anti-seizure activity, using pentylenetetrazole-induced seizure model that showed the methanolic extract to be more active and easier to preserve. Methanol is capable of extracting polar and non polar bioactive components from the plant material such that the extract would be rich in chemicals responsible for its biological activities (Chan et al., 2000). The therapeutic benefits of medicinal plant extracts used as traditional remedies are often attributed to a combination of active secondary metabolites (Amos et al., 2001). The methanolic bulb extract of *Crinum zeylanicum* contains sterols, terpenes, resins, tannins, saponins, flavonoids and alkaloids. The saponins have been shown to possess sedative properties, antagonistic effects against amphetamine and decreased spontaneous motor activity in experimental animals (Wagner et al., 1983). Also flavonoids, tannins, alkaloids, sterols, terpenes and resins in medicinal plants were shown by Duke (1992) to possess significant anti-nociceptive, sedative and anti-psychotic effects. The presence of sterols, terpenes , resins, tannins, saponins, flavonoids and alkaloids in the methanolic bulb extract of *Crinum zeylanicum* may thus be responsible for the observed anticonvulsant, sedative and analgesic effects.

The absence of adverse effects and death in animals treated orally with methanolic bulb extract of *Crinum zeylanicum* at over 5000 mg extract/kg body weight and below suggests that the extract is practically non-toxic by this route of administration (Corbett et al., 1984; Salawu et al., 2008) and possesses wide safety margin when used acutely. The behavioural signs of sedation, convulsion and death seen in the mice and rats treated with the extract intraperitoneally suggest the involvement of central nervous system (CNS) in its toxic effect (Salawu et al., 2009). The intraperitoneal median lethal dose of 3525 mg extract/kg body weight obtained showed that the extract is moderately toxic. The lipophilic bioactive components are capable of penetrating the blood brain barrier and may have been responsible for the observed CNS effects.

The human body requires a number of minerals in order to maintain good health. These minerals, absorbed by the plant from the environment for its growth accumulate in different parts of the plants. Plant species that contain trace mineral elements have been reported to display additional beneficial medicinal or therapeutic properties (Mark *et al.,* 2000). Parman *et al.,* (1993) showed that the medicinal values of some plant species used in homeopathic system may be due to the presence of sodium (Na), calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), Magnessium (Mg), potassium (K) and zinc (Zn). The presence of these metals (Na, K, Ca, Mg, Mn, Pb, Fe, Ni and Cr) may be contributing to the biological effects of *Crinum zeylanicum* in CNS disorders. *Crinum zeylanicum* is therefore a source of biologically important elements, which may play a part in its observed therapeutic properties.

Behaviour is the result of coordinated interplay of neuronal inhibition and excitation in the central nervous system and in turn, a function of a number of specific neurotransmitters (Roberts, 1974). Major inhibitory transmitters in the CNS include Gamma amino butyric acid (GABA) in the brain and glycine in the spinal cord and the brain stem, while glutamate and aspartate are major excitatory transmitters. Apart from maintaining normal physiological functions, these neurotransmitters have also been implicated in various CNS disorders including epilepsy, Parkinson’s disease, Huntington’s chorea, anxiety, depression, schizophrenia and Alzheimer’s disease (Meldrum and Garthwaite, 1990; Shigetada, 1992). Elevated levels of GABA in the brain will produce an anticonvulsant effect due to enhanced inhibitory neurotransmission (Wood and Peesker, 1975). Abnormally low levels of glutamate will compromise normal levels of excitation while excessive levels induce seizures and other toxic effects such as excitotoxicity (Farooqul and Horrockes, 1991).

The reduction in motor activity and sedation produced by methanolic bulb extract of *Crinum zeylanicum* are consistent with CNS depressant behavioural effects of anxiolytics, which at higher doses can induce sedation (Irwin, 1968; Treit, 1984). The state of sleep results from depression of neurones of the ascending reticular activating formation with consequent reduction in its activating influence on the cortex. CNS depressant drugs such as sedatives and hypnotics produce their action on CNS through facilitation of GABA-mediated inhibitory transmission, a common pathway for induction of sleep. Pentobarbital sodium produces its biological effects including sedation and reduction of anxiety by enhancing activation of $GABA_A$ receptors thus facilitating the GABA-mediated opening of chloride channels. As potent inducers of hepatic drug-metabolising enzymes especially cytochrome P450 system, pentobarbitone sodium facilitates its own metabolism and that of other drugs co-administered with it. The central depressant activity of the extract was confirmed by its ability to potentiate the pentobarbitone –induced sleep, which may be attributed to an inhibitory effect on the reticular activating system mediated by GABA (N’Gouemo et al., 1994) or an inhibition of pentobarbital metabolism (Kaul and Kulkarni, 1978) through inactivation of the metabolising enzymes thus prolonging the duration of action of pentobarbitone.

Fractionation of the crude methanolic bulb extract yielded three fractions: ethyl acetate, aqueous and solid residue fractions that were evaluated for central depressant effect using the Pentobarbitone- induced sleeping time test. The solid residue fraction was found to be the most active fraction. The ethyl acetate fraction of methanolic bulb extract contains volatile oil, flavonoid and balsam, while the aqueous fraction contains carbohydrate, tannins and saponins. The presence of flavonoids, saponins, alkaloids and sterols in the solid residue fraction which were lacking or present in trace amount in other fractions may be responsible for its being more active than the aqueous soluble and ethyl acetate soluble fractions. The low doses of the SRF that produced biological effects comparable to the high dose of the methanolic bulb extract of *Crinum zeylanicum* illustrate the importance of partial purification on the potency of medicinal plant extracts.

In drug development research, new and potentially neuroleptic, sedative or hypnotic agents are mainly characterized on the basis of their motor effects (Parshad et al., 1997, Ozturk et al., 1996). The spontaneous motor activity (SMA) is a measure of the gross motor activity of the animal, and reflects the integrity of the entire neuromuscular system and its control and regulation by the central nervous system (Parshad et al., 1997). The SMA is used to evaluate gross behavioural effects of drugs in laboratory animals (Carpendo et al., 1994). It measures the level of excitability of the central nervous system (Mansur et al., 1971) which correlates well with drug effect in humans. Any agent that suppresses SMA may possess central sedative properties (Ozturk et al., 1996). Central dopaminergic mechanisms play important roles in the control of motor activity and mental functions (Van Rossum and Hurksmans, 1964; Costal and Naylor, 1976) such that the reduction in SMA produced by the methanolic bulb extract of *Crinum zeylanicum* at the doses used may be due to its inhibitory actions on central dopaminergic systems. Many groups of psychotropic agents including antipsychotics (Baldessarini, 1996), anticonvulsants (McNamara, 1996), antidepressants (Lowe et al., 1978) and narcotic analgesics (Reisine and Pasternak, 1996), can diminish SMA in all species of animals including humans. Saponins obtained from herbs and some

Amaryllidaceae alkaloids have been shown to possess sedative properties and decrease SMA in laboratory animals (Wagner et al., 1983; Dubois et al., 1986). The ability of the extract to suppress SMA, shorten the onset and prolong the duration of pentobarbitone – induced sleep in mice therefore suggests that it contains active principles that are sedative in nature. The hole board test is a measure of exploratory behaviour (File and Wardill, 1975). An agent that decreases this behaviour is considered a sedative (File and Pellow, 1985). Anxiolytics have however been shown to increase the number of head dips in the hole board test (Takeda et al., 1998). The significant reduction in exploratory behaviour in the extract treated mice suggests that the extract possesses sedative properties rather than anxiolytic effects. The lack of inhibitory effect of the extract on motor co-ordination as observed in the tread mill suggests that the extract may not be acting via peripheral neuromuscular blockade but rather centrally thus confirming its central sedative property (Capaso et al., 1996). Apomorphine bind to D2 subtype dopaminergic receptor resulting in inhibition of adenyl cyclase which reduced potassium ion conductance, enhance calcium ion channel activity with resultant hyperactivity of dopamine in the nigro-striatal pathway. Agents that inhibit apomorphine-induced stereotypy can antagonise dopamine receptors in the nigrostriatal system (Tarsy and Baldessarini, 1986). The reduction in apomorphine–induced stereotype behaviour suggests that the extract effect may have been mediated via inhibition of dopaminergic system which may be correlated with its neuroleptic potential.

The PTZ- and PCT-induced seizure tests are used to identify anticonvulsant drugs that are effective against petit mal seizures (Loscher and Schmdidt, 1988) while MES is used to identify anticonvulsant drugs effective against generalized tonic –clonic seizures (Rogawski, 1992; Loscher and Schmidt, 1998). The extract and SRF showed significant anticonvulsant effect against PTZ-, PCT- and MES-induced seizures in mice and day old chicks respectively. The extract and its SRF can thus be useful in the therapeutic management of partial and /or generalized tonic-clonic seizures. *Crinum zeylanicum* bulb methanolic extract protected mice against pentylenetetrazole and significantly delayed the onset of myoclonus jerks and tonic seizures. PTZ, an antagonist of the $GABA_A$ subtype receptor produced seizure via inhibition of GABAergic neurotransmission, thus indirectly facilitating excessive excitatory transmission (De Sarro et al., 1999). Standard antiepileptic drugs such as diazepam and phenobarbitone are known to produce their effects by enhancing GABA-mediated inhibition in the brain (MacDonald and Kelly, 1995). Seizures induced by PTZ can be blocked by drugs such as ethosuximide which acts by reducing T-type Ca^{2+} currents (Meldrum, 1996). Activation of *N*-methyl-D-aspartate (NMDA) receptor system is also involved in the initiation and propagation of PTZ-induced seizures (Velisek et al., 1999, Yudkoff et al., 2006). In this regard, drugs such as felbamate that block glutamatergic excitation mediated by NMDA receptor have demonstrated anticonvulsant activity against PTZ-induced seizures (MacDonald and Kelly, 1995). It is therefore possible that the anticonvulsant effects of *Crinum zeylanicum* bulb methanolic extract and SRF against seizures produced by PTZ might be due to other mechanisms which include inhibition of T-type Ca2+ currents or blockade of glutamatergic neurotransmission mediated by NMDA receptor, in addition to its facilitatory effects on GABAergic transmission.

Despite the diversity of models that could potentially be used to screen for anticonvulsant activity, the MES and the subcutaneous PTZ remain ‘Gold standards’ in the early stages of testing (Rogawski, 2006). MES is used to identify anticonvulsant drugs effective against generalized tonic–clonic seizures (Rogawski et al., 1992; Loscher and Schmidt, 1988). Electroshock stimulates large portions of the brain making the neurons to fire repetitively and synchronously, which is the hall mark of epileptic neurons and epilepsy. Antiepileptic drugs (AEDs) that are clinically effective in the management of generalized tonic–clonic seizures such as carbamazepine, phenytoin, primidone, phenobarbital, valproate and lamotrigine all suppress hind limb tonic extension (HLTE) in MEST (Browning, 1992; Rho and Sankar, 1999). Protection against HLTE also indicates the ability of a testing material to inhibit or prevent seizures discharge within the brainstem seizure substrate (Browning, 1992).

The ability of the SRF to inhibit the HLTE in MEST which was comparable to phenytoin (100% protection) in this model suggests its potential therapeutic value in the management of grand mal epilepsy in man. The anticonvulsant effect of SRF may be mediated through elevation of seizure threshold, inhibition of seizure spread, or both. The plant's bulb extract and its solid residue fraction in addition, were relatively more effective against picrotoxin (PCT) - and pilocarpine-induced seizures in mice. In general, the average onset and latency of tonic hind limb extension were markedly delayed while protection of mice was produced in a dose-dependent manner. Picrotoxin produces convulsant effect by inhibiting and /or attenuating GABAergic neurotransmission via blockade of GABAergic neuronal chloride ion channel (Leonard, 2000). The significant protection of mice by the *Crinum zeylanicum* methanolic bulb extract and SRF against picrotoxin–induced seizure may therefore be due to enhancement or potentiation of GABAergic mechanism via opening of the neuronal chloride ion channel leading to hyperpolarization of the neurons.

The acute administration of a high dose of pilocarpine, a cholinergic agonist, in rodents is an experimental model described by Tursky et al., (1983) to study the pathophysiology of seizures and identify potential therapeutic agents for treatment of temporal lobe epilepsy. Pilocarpine is able to penetrate the blood brain barrier and binds to central muscarinic receptors to activate cholinergic system due to its high lipophilic nature. Activation of muscarinic receptors is the first step in seizure initiation while glutamate, dopamine and GABA systems appear to mediate seizure propagation (Mc Donald et al., 1991; Fritschy et al., 1999). The significant protection of mice against pilocarpine–induced seizure by the extract may therefore be due to its ability to antagonise the effect of glutamate, dopamine and facilitate GABAergic neurotransmission. The SRF significantly prolonged the onset and delayed the latency of tonic hind limb extension in, PCT- and Pilocarpine -induced seizures in mice. There was dose dependent protection of mice given the crude extract and the solid residue fraction orally in PTZ-, PCT-, pilocarpine-induced. The convulsive action of strychnine is due to interference with postsynaptic inhibition mediated by glycine, an important inhibitory transmitter to motor neurons and interneurons in the spinal cord. Glycine receptors are mainly located in the brain stem and spinal cord (Lynch, 2003). Chattipakorn and McMahon (2003), provided evidence of presence of functional glycine receptors (GlyRs) in the dentate gyrus, a brain region known to be highly susceptible to seizure activity (Scharfmann, 2000). They also demonstrated that glycine acting at GlyRs, elicited neuronal inhibition in the dentate gyrus and observed that the GlyR antagonist, strychnine prevented the anti epileptic effect of glycine. Strychnine acts as a selective, competitive antagonist of glycine at all glycine receptors (Kuno and Weakly, 1972).

The crude extract of *Crinum zeylanicum* slightly prolonged the onset of seizures and the latency of tonic hind limb extension in the Strychnine-induced seizure study, while the solid residue fraction protected the mice in a dose-dependent manner. These results suggest that additional mechanisms (possibly via glycine pathways) are involved in the anticonvulsant properties of *Crinum zeylanicum* bulb methanolic extract and SRF thereby contributing to its high potency as an anticonvulsant. The methanolic extract of *Crinum zeylanicum* and its solid residue fraction did not antagonise 4 -amino pyridine-induced seizures in mice. The 4-Aminopyridine (4-AP) is a convulsant which blocks some of the voltage-dependent neuronal potassium channels: the KA channel (or A channel), which regulates the spike frequency in postsynaptic structures, the KV channel (or delayed rectifier), involved in the repolarization phase of the action potential; and the KA Ch receptor-coupled channel (Alexander and Peters, 2000). The effects of 4-AP, therefore, include long presynaptic depolarization and increased transmitter release (Thesleff, 1980). The 4- aminopyridine (4-AP) - induced seizure in mice is a useful model for evaluating drug for drug resistant or refractory epilepsy (Wickenden, A.D., 2002). Antagonists of voltage gated k^+ channel such as 4-aminopyridine, dendrotoxin and charybdotoxin have long been known as powerful convulsants (Zuberi et al., 1999). The inability of the crude extract and SRF to effectively antagonise 4-aminopyridine –induced seizure showed that the extract may not be useful for the management of drug resistant or refractory epileptic seizures. Pathological pain is typically resistant to conventional pharmacological treatments (e.g. opioids, non-steroidal anti-inflammatory (NSAIDs) drugs), and anticonvulsant agents have been used in pain management over the last few decades due to the clinical impression that they are effective in alleviating lancinating and burning pains (McQuay et al., 1995). Specifically, systemically administered carbamazepine (Tegretol®), phenytoin and the newly introduced anticonvulsant agent, gabapentin (Neurontin®) have been widely used in treating various pain disorders (Tremont-Lukats et al., 2000).

The systemic use of anticonvulsants in neuropathic pain is based on the fact that central neuroplasticity plays a prominent role in promoting abnormal pain states, similar to seizure disorders. Therefore, potential analgesic effect of *Crinum zeylanicum* methanolic bulb extract was evaluated. Three different analgesic testing methods were employed with the objective of identifying possible peripheral and central effects of *Crinum zeylanicum* methanolic bulb extract. The acetic acid-induced abdominal constriction and hot-plate methods elucidated peripheral and central activity respectively, while the formalin test investigated both. The extract showed dose-dependent analgesic activity in all the testing methods used signifying that it possesses both centrally- and peripherally- mediated analgesic activities. The acetic acid induced abdominal constriction method is widely used for the evaluation of peripheral analgesic activity (Gene et al., 1998). The test, also called the abdominal constriction response is very sensitive and able to detect analgesic effects of compounds at dose levels that may appear inactive in other methods like the tail-flick test (Collier et al 1968). The method has been associated with the stimulation of cyclooxygenase enzymes COX-1 and COX-2. COX-1 is present in normal undamaged tissues including the stomach and intestine. The second enzyme, COX-2, is expressed only in damaged or inflamed tissue, and it is the therapeutic target for the NSAIDs, both peripherally and possibly also in spinal cord and in brain. The effectiveness of these NSAIDs in inflammatory pains further supports the involvement of prostaglandin biosynthesis in the nociceptive mechanism of acetic acid (Franzotti et al., 2000).

The observed analgesic effect of the extract may therefore be due to inhibition of the synthesis of prostaglandins. Formalin-induced pain has been well established as a valid model for analgesic study. In this model, there is a distinctive biphasic nociceptive response termed early and late phases. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase (Shibata et al., 1989). The early phase showed direct stimulatory effect of formalin on nociceptors in the paw and reflects centrally mediated pain while the late phase is due to inflammation with release of serotonin, histamine, bradykinin and prostaglandins (Tjolsen et al., 1992; Coderre and Melzack, 1992). The significant inhibition of both phases of pain as observed with the extract at 500 and 1000mg/kg orally strongly supported the involvement of both centrally- and peripherally- mediated effects. This suggestion is supported by the significant inhibition of pain responses observed in both the acetic acid abdominal constriction which reveals peripherally-mediated and thermally-induced pain by the hot plate which reveals centrally -mediated analgesic activity. The hot plate method is considered to be selective for screening of compounds acting through the opioid receptors (Mate et al., 2008**).** The significant dose-dependent increase in reaction time to thermal stimulus further supports the presence of centrally-mediated analgesic effect of the plant. The analgesic effect of the extract lasted longer than 60 minutes, probably due to generation of active metabolites following metabolism of the crude extract in the mice. The extract thus possesses potent analgesic activity that can be exploited for the development of phytomedicines useful for the management of mild to moderate and severe pains.

The objective of long term toxicity study in animals is to predict toxicity and provide guidelines for selecting a ‘safe’ dose for human use (Rhiouani et al., 2008). The choice of 90-day for the study is based on the recommendation of World Health Organization (WHO, 1992) which requires that drugs to be used for up to 1-6 months be tested for at least 90 day through the route of intended clinical use. The survival of the rats (males and females) until scheduled euthanasia, coupled with the absence of gross pathological alterations in the organs studied confirms the safety of the methanolic bulb extract of *Crinum zeylanicum* in rats. Significant dose-dependent decrease in body weight of female rats at 500 and 1000 mg extract/kg body weight in weeks 12 and 13 of the study may be due reduced appetite resulting in decrease in feed intake. The extract had no effect on the feed and water intake in male rats and this may explain the absence of significant change in body weight. This difference may be accounted for by hormonal influence in the female rats. According to Raza et al and Teo et al., (2002), decrease in body weight is a useful indicator of adverse effects of drugs and chemicals. Administration of the plant extract for 42-day was not accompanied by decrease in body weight in both male and female rats. Therefore, it may be non-toxic when used for shorter duration than 90-day.

Changes in haematological parameters have a higher predictive value for human toxicity, when data from preclinical animal studies are translated into clinical use (Olson et al., 2000). The exposure of female rats to graded doses (250 – 1000 mg extract/kg body weight) of *Crinum zeylanicum* for 42-day extract produced significant decrease in white blood cells (leukocytes), red blood cells (erythrocytes) and lymphocytes, while levels of platelets and neutrophils were significantly elevated. However, in the male rats, only the red blood cells were significantly decreased while neutrophils and platelets counts were significantly increased. The other haematological parameters were not altered. The changes produced in the haematological parameters by the extract after 42-day in male rats, were insignificant, however these changes showed a tendency for recovery at the end of the 90-day exposure. In the female rats, oral administration of the extract for 90-day produced blood dyscrasia characterized particularly by increase in white blood cells, haematocrits, platelets and lymphocytes. The increase observed in erythrocytes and leukocytes may be due to stimulatory effect of the extract on production of haematopoietic regulatory elements such as colony stimulating factors, erythropoietin and thrombopoiein by the stromal cells and macrophages in the bone marrow (Chang-Gue et al., 2003), thus providing the local environment for haematopoiesis (Udut et al., 2005).

The serum transaminases (ALT and AST) and alkaline phosphatase levels are good indicators of liver function (Martin et al., 1981). Elevated transaminase levels are blood biochemical signs of deep-seated pathological conditions. The AST is normally widely distributed in cardiac, skeletal muscle, liver and kidney tissues with small quantities found in the brain, pancreatic and lung tissues (Rhiouani et al., 2008) such that pathological conditions involving various tissue injuries are usually accompanied by elevated serum AST levels. ALT is a cytoplasmic enzyme found in very high concentration in the liver (Tennekoon et al., 1991; Rhiouani et al., 2008), and increase in serum level of this hepato-specific enzyme suggests hepatocellular damage. The observed increase in liver function parameters in both male and female rats at 90 -day of oral administration suggesting injury to the liver by the extract was supported by histopathological findings. At 1000 mg extract/kg body weight at 90-day lymphocytic infiltration of liver and congestion were seen in female and male rats respectively revealing time-dependent manifestation of toxicity which may be due to repeated exposure to the extract. The normal serum level of the bilirubin suggests that 90-day oral administration of the extract did not result in increased biliary pressure in the liver. The elevated levels of triglyceride, total cholesterol and low density lipoprotein may be due to impairment of lipid homeostasis by the extract in both female and male rats and thus calls for caution in the use of *Crinum zeylanicum* for management of chronic conditions. The extract at all doses used did not affect the serum levels of electrolytes, urea and Creatinine significantly in both male and female rats. The normal values obtained for these renal function parameters (electrolytes, urea and Creatinine) suggest that the extract has no deleterious effect on the kidney.

Evaluation of organ weights in toxicology studies is an integral component in the assessment of pharmaceuticals and phytomedicines for treatment-related toxicities.

Sellers et al (2007) recommended that liver, heart, kidneys, brain, adrenal glands, and testes/ovary (preferably from sexually mature animals) should be weighed in all species in multi-dose general toxicology studies of 7 day to 1 year in duration. Increase or decrease or both in liver weight may suggest treatment-related abnormality including hepatocellular hypertrophy (e.g., enzyme induction or peroxisome proliferation) (Amacher et al., 2006). Liver weights may be elevated in studies of less than 7 day duration for potent hepatic enzyme-inducing compounds. Elevated heart weight may be the only evidence of myocardial hypertrophy that is often macroscopically and microscopically difficult to recognize (Thiedemann, 1991). Changes in kidney weight may reflect renal toxicity, tubular hypertrophy or chronic progressive nephropathy (Greaves, 2000). While organ weights provide useful signals indicating test article-related effects, organ weight data must be interpreted in an integrated fashion with gross pathology and histopathology findings. The absence of significant changes in the relative-organ to body weight of liver, heart, kidney, testes/fallopian tube, brain, lungs, spleen, stomach and intestine observed in this study showed that the extract did not produce toxic changes in these organs. Considering scientific evidence in support of *Crinum zeylanicum* use in folk medicine for the management of epilepsy, pain, psychosis and general debility in children, the liver toxicity and blood dyscracia seen after 90-day treatment in rats suggest that the liver function tests and hematological parameters should be monitored when it is used in the treatment of chronic conditions. These results further showed that the safety of natural products derived from medicinal plants used in folk medicine must be evaluated irrespective of therapeutic benefits that have been and can still be derived from its biological effects. In conclusion, this study provides scientific evidence for the potential values and safety of *Crinum zeylanicum* methanolic bulb extract and its solid residue fraction using animal models of psychosis, sleep, epilepsy and pain. Specifically, it provides scientific rationale for the use of this plant in the Nigerian folk medicine. The central nervous system depressant effects of the extract and its SRF may be mediated via enhancement of GABAergic neurotransmission via inhibition of metabolism of pentobarbitone sodium thus prolonging its effect, agonistic effect at GABA A subtype receptor, neuronal level via enhancement of chloride ion channel opening, central muscarinic receptor antagonist like atropine. The depressant effect on apomorphine-induced hyperactivity may be due to its anti-dopaminergic D2 receptor action at the nigrostriatal path way. The analgesic effect of the extract may have been mediated peripherally via inhibition of cyclo-oxygenase pathway and centrally via activation of endogenous narcotic receptors leading to hyperpolarisation of neuronal membrane with consequent inhibition of pain sensation transmission. These multiple possible mechanisms of action may be due in part to the presence of the phytochemical constituents of the extract and SRF. The study has therefore contributed to the database on African medicinal plants that have been scientifically screened and documented. It is also a contribution to data base on ethnopharmacology of medicinal plants used in the management of central nervous system disorders with emphasis on epilepsy and childhood convulsion. Further investigation into the component phytochemical constituents of the extract and SRF for further purification with a view to isolate, and carry out structural elucidation of a novel lead compound. There is a need for more *in vitro* pharmacological assays such as ^{3}H-GABA uptake in rat cerebral cortex synaptosomes, $GABA_A$ binding assay, GABA uptake and release in rat hippocampal slices, Glutamate receptors: [3H]CPP binding and patch clamp technique to be used to ascertain the exact mechanism of action of bioactive Phytochemical in the plant.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

The results of this study revealed that, the methanolic bulb extract of *Crinum zeylanicum* possesses sedative effect comparable to the standard drug, diazepam used. Prolongation of pentobarbitone-induced sleep, reduction in the mean number of head dips and spontaneous motor-activity strongly suggest CNS depressant effect of *Crinum zeylanicum* bulb. The reduction in apormophine-induced stereotyped behavior by the extract suggests that it may be useful in the management of neuropsychiatric disorders associated with dopaminergic transmission at the nigro-striatal pathway.

The methanolic bulb extract of *Crinum zeylanicum* increased the latency of tonic hind limb extension in maximal electroshock-induced convulsion in chicks. It was shown to be effective against pentylenetetrazole-, picrotoxin- and pilocarpine- induced convulsions. It is however not active against strychnine and 4-aminopyridine–induced convulsion. The solid residue fraction (SRF) possesses anticonvulsant effect against maximal electroshock –induced seizure as well as pentylenetetrazole-, picrotoxin-, pilocarpine- and strychnine-induced convulsion but did not protect against 4-aminopyridine-induced convulsion.

The extract possesses anti-nociceptive effect as reflected in its inhibition of acetic acid-induced writhes in mice, heat (hot plate)-induced pain and formalin-induced paw pain comparable to standard drugs, piroxicam and morphine.

The oral median lethal dose (LD_{50}) of methanolic *Crinum zeylanicum* bulb extract was greater than 5000 mg/kg body weight in mice and rats respectively. The long-term (90 days) oral administration of the crude extract revealed that the extract is safe and did not affect feed and water intake of treated rats adversely. It had no effect on relative organ weight ratio in both male and female treated rats but histopathologically, produced congestion in their livers at the end of 90 days.

The phytochemical tests revealed the presence of balsam, sterols, terpenes, resins, carbohydrate, tannins, phylobatannins, saponins, flavonoids, alkaloids and volatile oils in the methanolic bulb extract of *Crinum zeylanicum*. The solid residue fraction contains sterols, alkaloids and terpenes. The elemental analysis showed presence of sodium, potassium, calcium, magnesium, manganese, lead, iron and nickel in the methanolic extract. These phytochemical and mineral elements may have contributed to the observed sedative, anticonvulsant and analgesic effects.

In conclusion, the efficacy results support the traditional use of *Crinum zeylanicum* bulb in the treatment of neurological disorders especially epilepsy and associated neuropsychiatric disorders (anxiety, psychosis and pain). On short term it is safe in the management of pain, but liver function parameters should however be monitored because of long-term therapeutic management associated with anxiety, psychosis and epilepsy.

Crinum zeylanicum can therefore be further explored in the search for safe and effective new agents for the treatment of epilepsy and epilepsy related psychosis and anxiety.

It is important to carry out further purification of the solid residue fraction with a view to isolate a novel lead compound. There is a need for more *in vitro* pharmacological assays such as ^{3}H-GABA uptake in rat cerebral cortex synaptosomes, $GABA_A$ binding assay, GABA uptake and release in rat hippocampal slices, Glutamate receptors: [3H]CPP binding and patch clamp technique to be used to ascertain the exact mechanism of action of bioactive phytochemicals in the plant.

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